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)

Department of Environmental Protection
Chemistry Section
Tallahassee Florida

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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-AHERB-AA	USGS Method O-2060-01 analysis, water sample
W-AHB-AA-R	EPA Method 8321B analysis, water sample
S-AHERB-AA	EPA Method 8321B analysis, soil sample
SPLP-AH-AA	EPA Method 8321B analysis, SPLP water sample
TPLP-AH-AA	EPA Method 8321B analysis, TCLP water sample
W-CARB-AA	USGS Method O-2060-01 analysis, water sample
W-CARB-R	EPA Method 8321B analysis, water sample
S-CARB-AA	EPA Method 8321B analysis, soil sample
W-DALAPN-R	EPA Method 8321B analysis, water sample
W-ENDO-AA	EPA Method 8321B analysis, water sample
W-GLYPH	EPA Method 8321B analysis, water sample
W-IND-AA-R	EPA Method 8321B analysis, water sample
S-INDZF-AA	EPA Method 8321B analysis, soil sample
W-PDQUAT	EPA Method 8321B analysis, water sample
W-SUC-AA-R	EPA Method 8321B analysis, water sample
W-UHERB-AA	USGS Method O-2060-01 analysis, water sample
W-UHB-AA-R	EPA Method 8321B analysis, water sample
S-UHERB-AA	EPA Method 8321B analysis, soil sample
SA-LCMSMS	EPA Method 8321B analysis, surface area wipe sample
O-LCMSMS	EPA Method 8321B analysis, unknown screening sample
W-MCYST-AA	EPA Method 8321B analysis, water sample
W-QUAT-NH4	EPA Method 8321B analysis, water sample
W-PYR-AA-R	EPA Method 8321B analysis, water sample
W-LCMS-DIJ	EPA Method 8321B analysis, water sample analysis by direct injection (Endothall, Glyphosate, Malathion, PDquat, Personal Care Products, Herbicides and Sucralose)

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

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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

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 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.

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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	0.002	0.008	6.5	19.5
2,4,5-T	0.002	0.008	6.5	19.5
Silvex	0.002	0.008	6.5	19.5
Acifluorfen	0.002	0.008	6.5	19.5
Dicamba	0.02	0.08	6.5	19.5
Picloram	0.05	0.2	6.5	19.5
Bentazon	0.002	0.008	6.5	19.5
2,4-DB	0.002	0.008	6.5	19.5
MCPP	0.004	0.016	6.5	19.5
MCPA	0.004	0.016	6.5	19.5
Dichlorprop	0.002	0.008	6.5	19.5
Dinoseb	0.02	0.08	6.5	19.5
Triclopyr	0.004	0.016	6.5	19.5
2,4-D-d3	0.002	0.008	6.5	19.5
Benzoic acid	2.000	8.000		
Sucralose	0.01	0.04	1.0	4.0
Sucralose-d6	0.10	0.40	1.0	4.0
Endothall	5	20		
Endothall-d6	5	20		
Glyphosate	10	40		
Glufosinate	10	40		
AMPA	10	40		
Glyphosate C13 15N	50	200		
Positive Ion Mode				
Aldicarb	0.02	0.08	1.0	4.0
Aldicarb Sulfone	0.002	0.008	1.0	4.0
Aldicarb Sulfoxide	0.002	0.008	1.0	4.0
Methomyl	0.002	0.008	1.0	4.0
Oxamyl	0.002	0.008	1.0	4.0
Propoxur	0.002	0.008	1.0	4.0
Methiocarb	0.002	0.008	1.0	4.0
Carbaryl	0.002	0.008	1.0	4.0
Carbofuran	0.002	0.008	1.0	4.0
3-Hydroxycarbofuran	0.002	0.008	1.0	4.0
Bendiocarb	0.02	0.08	1.0	4.0
Carbaryl-d7	0.002	0.01	1.0	4.0
Acetaminophen	0.004	0.016	1	4
Acetaminophen-d4	0.002	0.008	1	4
Carbamazepine	0.0004	0.002	1	4
Carbamazepine-d10	0.002	0.008	1	4
Indaziflam	0.002	0.008	1	4
Indaziflam-N15	0.002	0.008	1	4
Primidone	0.008	0.032	1	4

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Primidone-d5	0.008	0.032	1	4
Diuron	0.002	0.008	1.0	4.0
Fenuron	0.008	0.032	1.0	4.0
Fluometuron	0.004	0.016	1.0	4.0
Fluridone	0.0004	0.002	1	4
Imidacloprid	0.004	0.016	1.0	4.0
Linuron	0.004	0.016	1.0	4.0
Monuron	0.002	0.008	1.0	4.0
Pyraclostrobin	0.02	0.08		
Atrazine-d5	0.002	0.008	1.0	4.0
Diuron-d6	0.002	0.008	1.0	4.0
Didecyldimethylammonium chloride	1	4		
Carbamazepine-d10	0.002	0.008		
Diquat	1	4		
Paraquat	2	8		
Diquat-d4	1	4		
Anatoxin-a	1	4		
Cylindrospermopsin	1	4		
Microcystin LA	1	4		
Microcystin LR	1	4		
Microcystin RR	1	4		
Microcystin YR	1	4		
Nodularin	1	4		
Carbamazepine-d10	0.002	0.008		
Malathion	1	4		
Malathion-d6	1	4		

1.6 Table 3 lists the LOD check for all the tests performed

Table 3: LOD Check – Amount of Spike Standard Required

Method	Reported MDL in LIMS In PPB	On Column Conc. (µg/L) Required for MDL	Lowest ICAL Standard (µg/L) Used for Analysis	LOD Check Conc. on Column (µg/L)	Routine LFB's (µg/L)	LOD Check Spike Amount (mL)	LOD Spike Conc.
W-AHERB-AA	0.0020	0.5	0.5	2.5	25	0.025	0.10 ppm
S-AHERB-AA	6.5	20	0.5	100	100	0.1	10 ppm
W-CARB-AA	0.0020	0.5	0.5	2.5	25	0.025	0.10 ppm
S-CARB-AA	1.0	1	0.50	5	25	0.05	1 ppm
W-GLYPH	10	10	10	50	100	0.5	1 ppm
W-PDQUAT	1.0	50	100	200	1000	0.20	5 ppm
W-MCYST-AA	1	1	1	5	10	0.5	200 ppb
W-SUC-AA-R	0.010	10	10	50	250	0.05	500 ppb
W-UHERB-AA	0.0020	0.5	0.5	2.5	25	0.025	0.10 ppm
S-UHERB-AA	1.0	1	0.5	5	25	0.05	1 ppm

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.

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2 SUMMARY OF THE METHOD

2.1 An aliquot of the samples extract (prepared in DEP SOP LC-011) are analyzed by LC-MSMS analysis.

2.2 In MS/MS analysis, for each compound the identification and quantitation is performed based on the known precursor ion (in standard and unknown sample) to its two main intense fragment ions, that is, already known MRM (Multiple Reaction Monitoring) transition combined 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).

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 50 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 API 3200 and AB Sciex 6500+ LC-MSMS system. A triple quadrupole mass spectrometer capable of scanning from 1 to 1800 amu and 6500+ Triple Quad 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 reglulators. 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.

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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 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 and Mobile Phase B: Acetonitrile for Sucralose analysis only

4.5 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.

4.6 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 Acid Herbicides, Carbamates and Malathion 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. For Carbamates and Sucralose analyze within 21 days. For direct injection analysis for Carbamazepine, Fluridone, Indaziflam, Pyraclostrobin, Endothall, Glyphosate, Glufosinate, AMPA, Diquat and Paraquat samples must be analyzed within 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. One line gauge set at 100 psi for Gas 1 and 2 and Sheath Gas, and second gauge 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.

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6.1.2 Follow the API 3200 and 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, QI 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: PPG POSITIVE PARAMETERS:

Scan Type (Q1)	Center (amu)	Width (amu)	Time (Sec.)
MS/MS	59.050	6.000	0.6100
	175.133	6.000	0.6100
	616.464	6.000	0.6100
	906.673	6.000	0.6100
	1254.925	6.000	0.6100
	1545.134	6.000	0.6100
Q1 Reserpine	609.200	6.000	0.6100
Reserpine MS/MS Product of 609.200	174.100	6.000	0.6100

PPG NEGATIVE PARAMETERS:

MS/MS	44.998	6.000	0.6100
	585.385	6.000	0.6100
	933.636	6.000	0.6100
	1223.845	6.000	0.6100
	1572.097	6.000	0.6100

MS/MS	POSITIVE	NEGATIVE
Curtain Gas (CUR)	10	10
Ion Spray Voltage (IS)	5500	-4500
Temperature (TEM)	0.0	0.0
Ion Source Gas 1 (GS1)	12	10
Ion Source Gas 2 (GS2)	0	0
Interface Heater (ihe)	On	On
Declustering Potential (DP)	97.0	-35
Entrance Potential (EP)	10.0	-10
Focusing Lens 1 (/Q1)	-11	
Collision Cell Rod Offset (RO2)	-100.0	

Reserpine MS/MS

Collision Gas (CAD)	5
Declustering Potential (DP)	80.0
Collision Energy (CE)	45.0
Collision Cell Exit Potential (CXP)	8.0

Advance MS:

Scan Mode	Profile
Step Size	0.1 amu
Resolution Q1	Unit
Intensity Threshold	0
Setting Time	0 (ms)
Range between Mass	5.007(ms)

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MCA ON

6.1.4 Instead of manually performing a calibration for all polarity, quad and resolution combinations (8 possible), automatic Resolution Optimization can be used.

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 0.20 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 – 2.5 amu; Resolution – Unit; Polarity Positive. Product Ion – Auto Search – Criteria – 3 peaks; 2 most intense peaks; exclude 20; amu 30; 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.

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 Table lists the recommended default values for each parameter if FIA Compound Optimization is not used (for API3200).

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Parameters	Initial Tune	1 mL	0.5 mL	0.2 mL	Acid Herbicides by LC-MSMS
Curtain Gas CUR	25	25	25	25	25
Collision Gas CAD	4	4	4	4	5
Ion Spray Voltage IS	4800	4800	4800	4800	-4500
Temperature TEM	0	650	550	500	550
Ion Source Gas 1 GS1	25	60	50	50	50
Ion Source Gas 2 GS2	5	75	60	50	60
Interface Heater Ihe	on	on	on	on	On

6.2.14 Curtain Gas – Ramp 0 to 50 maximum; CAD Gas pressure in Q2 0 to 12 (API 3200); IS – 5500 maximum; TEM – 0 to 750 maximum; GS1 and GS2 – low heat – high GS1; high heat – high GS2

6.2.15 APCI Probe – 1 mL/min UPLC flow rate and no GS2

6.2.16 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 of Pesticides is accomplished by the external calibration method. The order of the calibration fit used is linear through zero, linear, linear weighted (1/x) 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

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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. Re-quantify PQL level by adding PQL level from the same curve as quality control sample and the target compounds recovery must be within ≤20%. Otherwise, a new curve must be regenerated until the requirement is met. To add the PQL level, copy the data file in D\ drive to different place and re-name the file and add it back to the same D\ drive.

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-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 3200/UPLC Negative and Positive Ion Mode Acquisition Methods

Negative Ion Mode Parameter Table

Component	Q1 Mass	Q3 Mass	DP	EP	CEP	CE	CXP
Dalapon	140.9	96.9	-25	-9.5	-10	-12	-2
	140.9	104.8	-25	-9.5	-10	-10	-2
Picloram	240.6	196.7	-25	-3.5	-14	-12	-4.0
	238.8	194.7	-20	-3.0	-16	-12	-4.0
Imazapyr	260.0	173.1	-20	-1.0	-20	-26	-4.0
	260.0	216.2	-20	-1.0	-20	-14	-14.0
Dicamba	218.9	174.9	-15	-2.0	-12	-8	-4.0
	220.6	176.8	-15	-3.0	-14	-8	-4
Bentazon	239.0	132.0	-40	-5.5	-14	-34	-2
	239.0	175.0	-40	-5.5	-14	-26	-4
2, 4-D-d3	221.9	183.8	-25	-3.5	-16	-20	-4
	223.5	165.8	-25	-3.5	-14	-20	-4
2, 4-D	218.8	160.9	-20	-8.5	-12	-18	-4
	220.6	162.9	-20	-8.0	-12	-18	-4
MCPA	198.9	140.9	-25	-6.5	-10	-18	-2
	200.9	143.0	-25	-4.0	-12	-18	-4
Triclopyr	253.7	195.8	-20	-7.0	-14	-14	-4
	255.5	197.9	-40	-9.0	-12	-12	-4

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Dichlorprop	232.9	160.8	-20	-7.0	-12	-18	-4
	232.9	124.9	-20	-7.0	-12	-36	0
MCPP	213.0	141.0	-25	-5.0	-12	-18	-4
	213.0	71.1	-25	-5.0	-12	-16	0
2, 4, 5-T	252.9	194.7	-20	-8.0	-14	-18	-4
	254.5	196.8	-20	-8.0	-12	-18	-4
2, 4-DB	246.9	160.9	-15	-3.0	-12	-12	-4
	248.6	162.6	-20	-4.0	-12	-12	-10
Silvex	266.7	194.9	-20	-8.0	-12	-24	-4
	268.8	196.8	-20	-8.0	-12	-24	-4
Acifluorfen	359.9	316.0	-20	-7.5	-14	-12	-22
	361.5	196.7	-20	-7.5	-14	-34	-4
Dinoseb	239.0	193.2	-40	-3.0	-12	-32	-12
	239.0	134.0	-40	-3.0	-12	-54	-0
Benzoic acid	120.8	76.9	-35	-3.5	-12	-16	0
Triclosan	286.8	35	-15	-5	-16	-25	-50
	288.8	35	-20	-6	-16	-25	-44
Acesulfame-d4	166.0	86.0	-35	-3.5	-12	-20	0
Acesulfame	161.9	81.8	-35	-3.5	-12	-20	0
	161.9	77.9	-35	-3.5	-12	-42	-4
Sucralose-d6	403.0	367.0	-40	-8.0	-16	-15	-28
	403.0	365.0	-30	-8.0	-27	-12	-26
Sucralose	395.0	359.0	-40	-10.5	-27	-14	-10
	397.0	359.0	-40	-10.5	-27	-13	-28
Endothall-d6	191	147	-30	-7	-14	-22	-4
	191	46.2	-30	-7	-14	-42	-4
Endothall	185	141	-25	-12	-12	-20	-4
	185	42.8	-25	-12	-12	-42	-2
Glyphosate	167.887	62.9	-25	-4.5	-10.0	-32	0
	167.887	78.8	-25	-4.5	-10.0	-50	0
Glyp. 13C 15N	169.885	62.9	-25	-7	-10.0	-32	0
Glyp. 13C 15N	169.87	78.8	-30	-2.5	-12	-56	0
AMPA	110	63	-50	-10.5	-13.9	-24	0
	110	79	-50	-10.5	-13.9	-32	0

Positive Ion Mode Parameter Table

Component	**Q1 Mass**	**Q3 Mass**	**DP**	**EP**	**CEP**	**CE**	**CXP**
Acetaminophen-d4	156.1	114.0	31	6	12.9	15	4

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Acetaminophen	152.1	110.1	36	5	12.8	21	4
	152.1	65	36	5	12.8	39	4
Aldicarb Sulfoxide	207.1	132.0	26	10	14	11	10
	207.1	89.2	26	10	14	19	8
Oxamyl	237.1	72.2	21	3	14	23	4
	237.1	90.1	21	3	14	13	6
Aldicarb Sulfone	240.1	86.1	21	10	14	27	8
	240.1	76.1	21	10	14	19	6
Methomyl	163.1	106.0	30	10	10	20	15
	163.1	88.0	30	10	10	20	15
Primidone-d5	224.2	167.1	41	7	15.2	15	4
Primidone	219.1	91.1	36	2	15	35	4
	219.1	162	41	9	15	15	4
3-Hydroxycarbofuran	238.1	163.0	26	10	14	19	14
	238.1	107.1	26	10	14	39	8
Fenuron	165.1	72.1	31	10	12	29	4
	165.1	46.2	31	10	12	27	4
Imidacloprid	256.1	209.0	31	10	16	23	16
	256.1	175.0	31	10	16	23	14
Aldicarb	208.1	116.1	21	2	12	13	10
	208.1	89.1	21	2	12	25	8
Carbamazepine-d10	247.2	204.2	56	8	15.9	27	4
Carbamazepine	237.1	194.1	46	6.5	15.6	29	4
	237.1	193.1	46	6.5	15.6	43	4
Monuron	199.1	72.2	36	10	12	31	4
	199.1	46.2	36	10	12	27	4
Propoxur	210.1	111.0	26	10	14	19	10
	210.1	93.1	26	10	14	33	8
Bendiocarb	224.1	109.0	26	10	14	23	10
	224.1	81.0	26	10	14	45	6
Carbofuran	222.1	165.1	31	10	14	17	14
	222.1	123.0	31	10	14	29	10
Fluometuron	233.0	72.1	46	4	12	39	4
	233.0	46.1	46	4	12	35	4
Atrazine-d5	221.2	179.1	46	4	14	23	16
	223.1	181.0	46	4	16	27	16
Carbaryl-d7	209.3	152.1	26	11	14	13	4
	209.3	124.2	26	11	14	35	4
Carbaryl	202.1	144.9	26	10	14	15	12
	202.1	127.1	26	10	14	37	10
Diuron-d6	241.0	78.2	46	4.5	14	27	4
	241.0	52.3	46	4.5	14	39	4

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Diuron	233.0	72.2	41	10	14	35	4
	233.0	46.2	41	10	14	31	4
Indaziflam-N15	306.1	162.1	56	3	17.9	21	4
	306.1	142.1	56	3	17.9	35	4
Indaziflam	302.2	158	61	4	17.7	21	6
	302.2	138.1	61	4	17.7	33	4
Methiocarb	226.1	169.0	26	10	14	13	14
	226.1	121.1	26	10	14	25	10
Linuron	249.1	160.0	36	10	16	23	14
	249.1	182.0	36	10	16	21	14
Fluridone	330.093	309.3	76	6	18	45	24
	330.093	77.2	76	6	18	97	4
Pyraclostrobin	388.1	194.0	26	7.5	14	29	4
	388.1	163.1	26	7.5	14	17	4
Didecyldimethyl	326	43	76	7	18	59	4
ammonium chloride	326	58	76	7	18	71	4
	326	186	76	7	18	37	4
Diquat	183	157	35	10	10	32	3
	183	168	35	10	10	35	3
Paraquat	185	170	40	10	10	30	3
	185	169	40	10	10	35	3
Diquat-d4	186	158	35	10	10	32	3
	186	78	26	12	22	51	4
Anatoxin-a	166.133	131.1	36	3	10	23	4
	166.133	43.0	36	3	10	37	4
	166.133	91.0	36	3	10	25	4
Cylindrospermopsin	416.157	194.3	71	10.5	46	103	12
	416.157	193.9	71	10.5	46	127	10
Microcystin LA	910.421	135.2	61	10	100	81	10
	910.421	107.4	61	10	100	129	8
Microcystin LR	995.472	135.2	81	10.5	74	103	12
	995.472	70.2	81	10.5	74	125	4
Microcystin RR	519.854	135.1	46	10	48	43	12
	519.854	103.1	46	10	48	91	8
Microcystin YR	1045.426	135.2	71	10.5	46	103	12
	1045.426	127.1	71	10.5	46	127	10
Nodularin	825.43	135.1	86	10.5	42	79	10
	825.43	70.2	86	10.5	42	117	6
Microcystin LF	986.5	135.2	81	10.5	74	103	12
	986.5	212.8	81	10.5	74	125	4

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Microcystin LF-d5	991.5	135.2	81	10.5	74	103	12
	991.5	212.8	81	10.5	74	125	4
Microcystin LY	1002.5	135.2	81	10.5	74	103	12
	1002.5	212.8	81	10.5	74	125	14
Malathion	331.003	127.1	26	9	28	19	6
	331.003	99.10	26	9	28	29	4
Malathion-d6	337.059	127.2	21	12	32	19	6
	337.059	99.1	21	12	32	33	4

Negative Ion Mode Methods:

Acid Herbicides:

Synchronization Mode – Lc Sync, Auto-Equilibration – Off, Accu Duration – 28 min, Ion Source – Turbo Ion Spray, Polarity - Negative, 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 – 20, Collision Gas CAD – 5, Ion Spray Voltage IS - -3000, Temperature TEM – 650, Ion Source Gas 1 GS1 – 60, Ion Source Gas 2 GS2 – 70, Interface Heater Ihe – On, Integrated Valco Valve – Position Name for Step 0 – B not in use, Time 4.0 min – B not in use, Time 4.1 min – A, Time 24 min – A, Time 24.1 min – B not in use

Column: Phenomenex – Luna, 5 microns, Phenyl-Hexyl, 4.6 x 150 mm, S/N 490638 – 8.
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 – 25 µL.

Time Program: 0.10 min – Pumps – Pump B Conc. – 5%; 13 min – Pumps – Pump B Conc. – 30%; 23 min – Pumps – Pump B Conc. – 90%; 23.01 min – Pumps – Pump B Flow Rate 0.8 mL/min; 23.02 min - Pumps – Pump B Flow Rate 1.5 mL/min; 25 min Pumps – Pump B Conc. – 90%; 25.01 min – Pumps – Pump B.Conc – 5%; 27.8 min - Flow Rate 1.5 mL/min; 27.9 min – Pumps – Pump B Flow Rate – 0.8 mL/min; 28.0 min – Controller – Stop.

Calibration levels: 250, 100, 50, 25, 10, 5, 2.5 and 0.5 µg/L; Continuing calibration verification standard (CCV) level: 25 µg/L; Surrogate spike level: 100 µg/L and lab fortified blanks and Matrix spike level: 100 µg/L. For low level Surrogate spike level: 50 µg/L and lab fortified blanks and Matrix spike level: 25 µg/L.

Calibration levels for Benzoic acid/2,4-D-d3: 10/1, 5/0.5, 2.5/0.25, 1/0.1, 0.5/0.05 and 0.25/0.025 µg/mL; Continuing calibration verification standard (CCV) level for Benzoic acid/2,4-D-d3: 1/0.1 µg/mL; Surrogate spike level: 50 µg/L and lab fortified blanks and Matrix spike level: 1000 µg/L.

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Sucralose:

Accu Duration – 19 min, Polarity - Negative, Time – 200 msec, Edit Parameters – Source Gas CUR – 20, Collision Gas CAD – 5, Ion Spray Voltage IS - -3000, Temperature TEM – 650, Ion Source Gas 1 GS1 – 60, Ion Source Gas 2 GS2 – 70, Interface Heater Ihe – On, Integrated Valco Valve – Position Name for Step 0 – B not in use, Time 4.0 min – B not in use, Time 4.1 min – A, Time 14.0 min – A, Time 14.1 min – B not in use.

Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam, Primidone, Pyraclostrobin and Sucralose) and Urea Herbicides Calibration levels: 100/400, 50/200, 25/100, 10/40, 5/20, 2.5/10 and 0.5/2 µg/L; Continuing calibration verification standard (CCV) level: 25/100 µg/L; Surrogate spike level: 50/200 µg/L and lab fortified blanks and Matrix spike level: 25/100 µg/L. Mobile phase A: HPLC water and Mobile Phase B: Acetonitrile.

Time Program: 0.10 min – Pumps – Pump B Conc. – 5%; 13 min – Pumps – Pump B Conc. – 30%; 13.01 min – Pumps – Flow Rate – 0.8 mL/min; 13.02 min – Pumps – Flow Rate – 1.5 mL/min; 13.03 min – Pump B Conc. – 90%; 15 min – Pumps – Pump B Conc. – 90%; 15.01 min – Pumps – Pump B Conc. – 5%; 18.8 min - Pumps – Flow Rate – 1.5 mL/min; 18.9 min – Pumps – Flow Rate – 0.8 mL/min; 19 min – Controller – Stop. Injection Volume – 25 µL.

Endothall:

Accu Duration – 19 min, Polarity - Negative, Time – 200 msec, Edit Parameters – Source Gas CUR – 25, Collision Gas CAD – 4, Ion Spray Voltage IS - -3000, Temperature TEM – 650, Ion Source Gas 1 GS1 – 60, Ion Source Gas 2 GS2 – 70, Interface Heater Ihe – On, Integrated Valco Valve – Position Name for Step 0 – B not in use, Time 4.0 min – B not in use, Time 4.1 min – A, Time 14.0 min – A, Time 14.1 min – B not in use.

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.

Time Program: 0.10 min – Pumps – Pump B Conc. – 5%; 13 min – Pumps – Pump B Conc. – 30%; 13.01 min – Pumps – Flow Rate – 0.8 mL/min; 13.02 min – Pumps – Flow Rate – 1.5 mL/min; 13.03 min – Pump B Conc. – 90%; 15 min – Pumps – Pump B Conc. – 90%; 15.01 min – Pumps – Pump B Conc. – 5%; 18.8 min - Pumps – Flow Rate – 1.5 mL/min; 18.9 min – Pumps – Flow Rate – 0.8 mL/min; 19 min – Controller – Stop. Injection Volume – 25 µL.

Glyphosate:

Accu Duration – 12 min, Polarity - Negative, Time – 200 msec, Edit Parameters – Source Gas CUR – 25, Collision Gas CAD – 4, Ion Spray Voltage IS - -4500, Temperature TEM – 650, Ion Source Gas 1 GS1 – 60, Ion Source Gas 2 GS2 – 75, Interface Heater Ihe – On, Integrated Valco Valve – Position Name for Step 0 – B not in use, Time 1.4 min – B not in use, Time 1.5 min – A, Time 2.9 min – A, Time 3.0 min – B not in use.

Calibration levels (Glyphosate, AMPA/Glyphosate C13) – 250/1250, 100/500, 50/250, 25/125 and 10/50 µg/L; Continuing calibration verification standard (CCV) level: 50 µg/L; Surrogate spike level: 250 µg/L and lab fortified blanks and Matrix spike level: 50 µg/L and surrogate at 250 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. Time Program: 0.10 min – Pumps – Pump B Conc. – 0%, hold for 6 min; 6.01 min – Pump B Conc. – 90%; 8 min – Pumps – Pump B

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Conc. – 90%; 8.01 min – Pumps – Pump B Conc. – 0%; 11.9 min - Pumps – Pump B Conc. – 0%; 12 min – Controller – Stop. Injection Volume – 40 µL.

Positive Ion Mode Methods:

Carbamates, Urea Herbicides and Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam, Primidone and Pyraclostrobin):

Accu Duration – 28 min, Polarity - Positive, Time – 100 msec, Edit Parameters – Source Gas CUR – 20, Collision Gas CAD – 5, Ion Spray Voltage IS - 3000, Temperature TEM – 650, Ion Source Gas 1 GS1 – 50, Ion Source Gas 2 GS2 – 70, Interface Heater Ihe – On, Integrated Valco Valve – Position Name for Step 0 – B not in use, Time 4.0 min – B not in use, Time 4.1 min – A, Time 24 min – A, Time 24.1 min – B not in use.

Calibration levels: 100, 50, 25, 10, 5, 2.5 and 0.5 µg/L; Continuing calibration verification standard (CCV) level: 25 µg/L; Surrogate spike level: 50 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.

Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam, Primidone, Pyraclostrobin and Sucralose) and Urea Herbicides Calibration levels: 100/400, 50/200, 25/100, 10/40, 5/20, 2.5/10 and 0.5/2 µg/L; Continuing calibration verification standard (CCV) level: 25/100 µg/L; Surrogate spike level: 50/200 µg/L and lab fortified blanks and Matrix spike level: 25/100 µg/L.

For Direct Injection Analysis for Carbamazepine, Fluridone, Indaziflam and Pyraclostrobin: Calibration levels: 10, 5, 2.5, 0.5, 0.1, 0.01 and 0.002 µg/L; Continuing calibration verification standard (CCV) level: 2.5 µg/L; Surrogate spike level: 2.5 µg/L and lab fortified blanks and Matrix spike level: 0.1 µg/L and Injection volume of 50 µL.

Time Program: 0.10 min – Pumps – Pump B Conc. – 5%; 13 min – Pumps – Pump B Conc. – 30%; 23 min – Pumps – Pump B Conc. – 90%; 23.01 min – Pumps – Pump B Flow Rate 0.8 mL/min; 23.02 min - Pumps – Pump B Flow Rate 1.5 mL/min; 25 min Pumps – Pump B Conc. – 90%; 25.01 min – Pumps – Pump B.Conc – 5%; 27.8 min - Flow Rate 1.5 mL/min; 27.9 min – Pumps – Pump B Flow Rate – 0.8 mL/min; 28.0 min – Controller – Stop. Injection Volume – 25 µL.

Microcystins:

Accu Duration – 16 min, Polarity - Positive, Time – 200 msec, Edit Parameters – Source Gas CUR – 25, Collision Gas CAD – 6, Ion Spray Voltage IS - 3000, Temperature TEM – 550, Ion Source Gas 1 GS1 – 50, Ion Source Gas 2 GS2 – 70, Interface Heater Ihe – On, Integrated Valco Valve – Position Name for Step 0 – B not in use, Time 5.0 min – B not in use, Time 5.1 min – A, Time 15.0 min – A, Time 15.1 min – B not in use.

Calibration levels: 50, 25, 10, 5, 2 and 1 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. Time Program: 0.10 min – Pumps – Pump B Conc. – 5%; 10 min – Pumps – Pump B Conc. – 60%; 10.01 min – Pumps – Pump B Conc. – 90%; 11 min – Pumps – Pump B Conc. – 90%; 11.01 min – Pumps – Pump B Conc. – 5%; Flow Rate – 0.8 mL/min; 16.0 min – Controller – Stop. Injection Volume – 50 µL.

Didecyldimethylammonium chloride:

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Accu Duration – 10 min, Polarity - Positive, Time – 150 msec, Edit Parameters – Source Gas CUR – 25, Collision Gas CAD – 6, Ion Spray Voltage IS - 3000, Temperature TEM – 550, Ion Source Gas 1 GS1 – 50, Ion Source Gas 2 GS2 – 70, Interface Heater Ihe – On, Integrated Valco Valve – Position Name for Step 0 – B not in use, Time 2.0 min – B not in , Time 2.1 min – A, Time 8.0 min – A, Time 8.1 min – B not in use.

Calibration levels: 100, 50, 25, 10, 5, 2 and 1 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. Time Program: 0.10 min – Pumps – Pump B Conc. – 5%; 5 min – Pumps – Pump B Conc. – 100%; 7 min – Pumps – Pump B Conc. – 100%; 10 min – Pumps – Pump B Conc. – 5%; 10.1 min – Controller – Stop. Injection Volume – 10 µL. Column used - Phenomenex – Synergy 4-micron Fusion-RP 80A, 50 x 2 mm, S/N 653877-15

Diquat and Paraquat:

Accu Duration – 13 min, Polarity - Positive, Time – 200 msec, Edit Parameters – Source Gas CUR – 15, Collision Gas CAD – Medium, 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 – Position Name for Step 0 – B not in use, Time 6.2 min – B not in use, Time 6.3 min – A, Time 7.5 min – A, Time 7.6 min – B not in use.

Calibration levels (Diquat/Diquat-d4/Paraquat): 500, 250, 100, 25, 5, 2 and 1 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 Heptafluorobutyric acid. Mobile phase B: Acetonitrile containing 0.1 % Formic acid. Time Program: 0.01 min – Pumps – Pump B Conc. – 5%; 8 min – Pumps – Pump B Conc. – 30%; 8.01 min – Pump B Conc. – 90%; 9.00 min – Pumps – Pump B Conc. – 90%; 9.01 min – Pumps – Pump B Conc. – 5%; Flow Rate – 0.8 mL/min; 13 min – Controller. Injection Volume – 25 µL.

Malathion:

Accu Duration – 11 min, Polarity - Positive, Time – 200 msec, Edit Parameters – Source Gas CUR – 25, Collision Gas CAD – Medium, Ion Spray Voltage IS - 3000, Temperature TEM – 550, Ion Source Gas 1 GS1 – 50, Ion Source Gas 2 GS2 – 70, Interface Heater – On, Integrated Valco Valve – Position Name for Step 0 – B not in use, Time 4.0 min – B not in use, Time 4.1 min – A, Time 8.0 min – A, Time 8.1 min – B not in use.

Calibration levels (Malathion/Malathio-d6) in MCAA Water: 250, 100, 50, 25, 10, 5 and 1 µg/L. Continuing calibration verification standard (CCV) level in MCAA Water: 25 µg/L; Surrogate spike level: 25 µg/L and lab fortified blanks and Matrix spike level: 10 µg/L. Mobile phase A: HPLC water containing 0.1% Formic acid. Mobile phase B: Acetonitrile containing 0.1 % Formic acid. Time Program: 0.01 min – Pumps – Pump B Conc. – 40%; 6 min – Pumps – Pump B Conc. – 90%; 7 min – Pump B Conc. – 90%; 7.1 min – Pumps – Pump B Conc. – 40%; 10.9 min – Pumps – Pump B Conc. – 40%; Flow Rate – 0.8 mL/min; 11 min – Controller - Stop. Injection Volume – 20 µL.

Positive / Negative / Positive Ion Mode Method:

Acetaminophen/Sucralose/ Urea Herbicides and Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam, Primidone and Pyraclostrobin):

Accu Duration Total– 28 min, Period 1 – 8.5 min, Polarity – Positive for Acetaminophen, Period 2 – 2.5 min, Polarity – Negative for Sucralose, Period 3 – 17 min, Polarity – Positive for the rest of the Positive Ion Mode analytes, Time – 100 msec, Edit Parameters – Source Gas CUR – 20, Collision Gas CAD – 5, Ion Spray Voltage IS - + and -3000, Temperature TEM – 650, Ion Source

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Gas 1 GS1 – 50, Ion Source Gas 2 GS2 – 70, Interface Heater Ihe – On, Integrated Valco Valve – Position Name for Step 0 – B not in use, Time 4.0 min – B not in use, Time 4.1 min – A, Time 24 min – A, Time 24.1 min – B not in use.

Personal Care Products (Acetaminophen, Carbamazepine, Indaziflam, Primidone, Pyraclostrobin and Sucralose) and Urea herbicides Calibration levels: 100/400, 50/200, 25/100, 10/40, 5/20, 2.5/10 and 0.5/2 μg/L; Continuing calibration verification standard (CCV) level: 25/100 μg/L; Surrogate spike level: 50/200 μg/L and lab fortified blanks and Matrix spike level: 25/100 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.

For Direct Injection Analysis for Carbamazepine, Fluridone, Indaziflam and Pyraclostrobin: Calibration levels: 10, 5, 2.5, 0.5, 0.1, 0.01 and 0.002 μg/L; Continuing calibration verification standard (CCV) level: 2.5 μg/L; Surrogate spike level: 2.5 μg/L and lab fortified blanks and Matrix spike level: 0.1 μg/L and Injection volume of 50 μL.

Time Program: 0.10 min – Pumps – Pump B Conc. – 5%; 13 min – Pumps – Pump B Conc. – 30%; 23 min – Pumps – Pump B Conc. – 90%; 23.01 min – Pumps – Pump B Flow Rate 0.8 mL/min; 23.02 min - Pumps – Pump B Flow Rate 1.5 mL/min; 25 min Pumps – Pump B Conc. – 90%; 25.01 min – Pumps – Pump B.Conc – 5%; 27.8 min - Flow Rate 1.5 mL/min; 27.9 min – Pumps – Pump B Flow Rate – 0.8 mL/min; 28.0 min – Controller – Stop. Injection Volume – 25 μL.

API 6500+ Triple Quad/UPLC Acquisition Method

Parameter Table

Component	Q1 Mass	Q3 Mass	DP	EP	CE	CXP
Sucralose-d6	401.1	35.1	-45	-10.0	-66	-15
	403.0	35.1	-40	-10.0	-66	-15
Sucralose	395.0	35.0	-40	-10.0	-60	-15
	395.0	359.0	-30	-10.0	-16	-11

Sucralose:

Synchronization Mode – Lc Sync, Auto-Equilibration – Off, Accu Duration – 14 min, Ion Source – Turbo Ion Spray, Polarity - Negative, Scan Type – MRM(MRM), Resolution Q1 – Unit, Resolution – Q3 – Unit, Intensity Threshold – 0.00 cps, Setting Time – 50 msec, 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 – Position Name for Step 0 – A not in use, Time 4.0 min – A not in use, Time 4.1 min – B, Time 8.0 min – B, Time 8.1 min – A not in use.

Column: Phenomenex – Luna, 5 microns, Phenyl-Hexyl, 4.6 x 150 mm, S/N 490638 – 8. Mobile phase A: HPLC Water. Mobile phase B: Acetonitrile.

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

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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 – 50 µL.

Time Program: 0.10 min – Pumps – Pump B Conc. – 5%; 8.00 min – Pumps – Pump B Conc. – 60%; 8.01 min – Pumps – Total Flow – 0.8 mL/min; 8.02 min – Pumps – Total Flow – 1.5 mL/min; 8.03 min - Pumps – Pump B Conc. 90%; 10.00 min – Pumps – Pump B Conc. – 90%; 10.01 min – Pumps – Pump B Conc. – 5%; 13.80 min – Pumps – Total Flow – 1.5 mL/min; 13.90 min – Pumps – Total Flow – 0.8 mL/min; 14.0 min – Controller – Stop.

Calibration levels: 20, 10, 5, 1, 0.4, 0.2, 0.05 and 0.01 µg/L; Continuing calibration verification standard (CCV) level: 1 µg/L; Surrogate spike level: 2 µg/L and lab fortified blanks and Matrix spike level: 1.0 µg/L and surrogate spike level: 2.0 µg/L.

6.4 QUANTITATIVE DATA PROCESSING

6.4.1 **Quantitate** - Quantitation Wizard - Open the data files to Quantitate from the list of data files in the batch and add them to the selected sample list – Next – select to use proper Results Table Setting – Next – create new method (give same name) – next – replace "yes". Select data file (Mid-level calibration standard) – Next – select peak name to match the ions – Next – check proper peak selected (RT and Integration). Click advanced and check the followings (units, smooth width 3), OK – Next – Linear – Finish.

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 with xls extension for printing.

6.4.3 **Exporting Results Table for QCManager**: Export visible columns in Results Table (order of columns – Sample Name/Analyte Peak Name/Calculated Concentration (ug/L)/ Analyte Units/Acquisition Date) with xls extension to Excel and give the same file name as Run Sequence and Results Table (AH080407QCM.xls). Open the file in Excel to edit the rows and press save to keep the Tab Delimited Settings. Close the file and click "Don't save" to not to lose the settings. Transfer file to QCManager folder to do further calculations and reporting the results into LIMs.

6.5 REPORTING

6.5.1 In MS/MS analysis, for each compound the identification and Quantitation is performed by based on the known precursor ion (in standard and unknown sample) to its two main intense fragment ions, that is, already known MRM transition combined with retention time. Both MRM transitions from the known precursor ion can be used for Quantitation, confirmation and reporting. For positives, both 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.

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Table: Recovery Limits Table

Compound	% Rec Water	% Rec Soil
Negative Ion Mode		
2,4-D	40 - 140	50 - 140
2,4,5-T	40 - 140	50 - 140
Silvex	40 - 140	50 - 140
Acifluorfen	40 - 140	50 - 140
Dicamba	40 - 140	50 - 140
Picloram	40 - 140	50 - 140
Bentazon	40 - 140	50 - 140
2,4-DB	40 - 140	50 - 140
MCPP	40 - 140	50 - 140
MCPA	40 - 140	50 - 140
Dichlorprop	40 - 140	50 - 140
Dinoseb	40 - 140	50 - 140
Triclopyr	40 - 140	50 - 140
2,4-D-d3	40 - 150	40 - 150
Benzoic acid	40 - 150	
Sucralose	40 - 140	
Sucralose-d6	40 - 140	
Endothall	40 - 140	
Endothall-d6	40 - 140	
Glyphosate	60-140	
AMPA	60-140	
Glyphosate C13 15N	50-140	
Positive Ion Mode		
Aldicarb	40 - 140	50 - 140
Aldicarb Sulfone	40 - 140	50 - 140
Aldicarb Sulfoxide	40 - 140	50 - 140
Methomyl	40 - 140	50 - 140
Oxamyl	40 - 140	50 - 140
Propoxur	40 - 140	50 - 140
Methiocarb	40 - 140	50 - 140
Carbaryl	40 - 140	50 - 140
Carbofuran	40 - 140	50 - 140
3-Hydroxycarbofuran	40 - 140	50 - 140
Bendiocarb	40 - 140	50 - 140
Carbaryl-d7	40 - 140	50 - 140
Acetaminophen	40 - 140	50 - 140
Acetaminophen-d4	30 - 140	50 - 140
Carbamazepine	40 - 140	50 - 140
Carbamazepine-d10	40 - 140	50 - 140
Indaziflam	40 - 140	50 - 140
Indaziflam-N15	40 - 140	50 - 140
Primidone	40 - 140	50 - 140
Primidone-d5	40 - 140	50 - 140

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Diuron	40 - 140	50 - 140
Fenuron	40 - 140	50 - 140
Fluometuron	40 - 140	50 - 140
Fluridone	40 - 140	50 - 140
Imidacloprid	40 - 160	50 - 140
Monuron	40 - 140	50 - 140
Pyraclostrobin	40 -140	
Linuron	40 - 140	50 - 140
Diuron-d6	40 - 140	50 - 140
Didecyldimethylammonium chloride	40-140	
Carbamazepine-d10	40-140	
Diquat	40-140	
Paraquat	40-140	
Diquat-d4	40-140	
Anatoxin-a	50 - 150	
Cylindrospermopsin	50 - 150	
Microcystin LA	50 - 150	
Microcystin LR	50 - 150	
Microcystin RR	50 - 150	
Microcystin YR	50 - 150	
Nodularin	50 - 150	
Carbamazepine-d10	50 - 150	
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.

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:

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

6.6.2 Calculation of positives in water samples:

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

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.

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. 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.

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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. Make a copy of results page, positive detection page, results table and run sequence, and file it in the results notebook. 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.

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*.

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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.

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

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