Standard Operating Procedure for:

Analysis of Pesticides, Herbicides, and other Chemicals in Water and Sediment/Waste by High Performance Liquid Chromatography Mass Spectrometry

FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION

CHEMISTRY PROGRAM

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1. Scope and Application

1.1 This SOP describes the use of high-performance liquid chromatography (HPLC) coupled with electrospray ionization mass spectrometry (MS) for the analysis of select pesticides, herbicides, and other chemicals in water and sediment/waste samples; analytes are listed in Appendix B. The preparation of these samples is described in SOP LC-011 (FL DEP SOP LC-011. Extraction of Pesticides, Herbisides, and Other Chemicals from Water or Sediment/Waste).

1.2 The laboratory Test IDs and equivalent method associations are provided in Table 1. These methods comply with the requirements of EPA method 8321B and USGS method O-2060-01 for the parameters in Table 5.1 of the laboratory's quality manual (EPA Method 8321B. Solvent-Extractable Nonvolatile Compounds by High-Performance Liquid Chromatography/Thernospray/Mass Spectrometry (HPLC/TS/MS) or Ultraviolet (UV) Detection, Revision 2, 2007), (USGS O-2060-01. Determination of Pesticides in Water by Graphited Carbon-Based Solid-Phase Extraction and HIgh-Performance Liquid Chromatography/Mass Spectrometry, 2001), and (FL DEP Quality Manual). The USGS Method O-2060-01 is an approved test procedure of the National Pollutant Discharge Elimination System under the Clean Water Act. Method 8321B is listed in the SW-846 publication under Subtitle C of the Resource Conservation and Recovery Act.

Table 1. FDEP Laboratory Test IDs and Equivalent Methods.

Lab Test ID	Test Description
W-CAB-AA-R	EPA Method 8321B carbamate analysis/EPA Method 3535A preparation, water matrix
W-CARB-AA	USGS Method O-2060-01 carbamate analysis/EPA Method 3535A preparation, water matrix
W-E8321-21	EPA Method 8321B analysis, water matrix analysis by direct injection, 21 days holding time
W-E8321-DI	EPA Method 8321B analysis, water matrix analysis by direct injection, 7 days holding time
W-E8321-MS	EPA Method 8321B analysis/EPA Method 3535A preparation, water matrix
W-U2060-MS	USGS Method O-2060-01 analysis/EPA Method 3535A preparation, water matrix
SPLP-AH-AA	EPA Method 8321B analysis/EPA Method 3010/3535A preparation, SPLP water matrix
TPLP-AH-AA	EPA Method 8321B analysis/EPA Method 3011/3535A preparation, TCLP water matrix
W-MCYST-AA	EPA Method 8321B analysis of microcystins, water matrix
W-SAXTN-MS	EPA Method 8321B analysis of saxitoxin, water matrix
W-PDQUAT	EPA Method 8321B analysis of diquat/paraquat by Direct Injection, water matrix
W-PFAS-MS	EPA Method 8321B analysis of PFAS compounds, water matrix
W-QUAT-NH4	EPA Method 8321B quanternary ammonium analysis, water sample
S-AHERB-AA	EPA Method 8321B analysis of acid herbicides, soil matrix
S-E8321-MS	EPA Method 8321B analysis/EPA Method 3570 preparation, soil matrix
S-PFAS-MS2	EPA Method 8321B analysis of PFAS compounds, soil matrix
SA-LCMSMS	EPA Method 8321B analysis, surface area wipe matrix
O-LCMSMS	EPA Method 8321B analysis, unknown screening sample

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1.3 The acceptance limits, as provided in EPA Method 8321B and USGS Method O-2060-01, are programmed into the 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 (FL DEP Quality Manual).

1.4 Limits of detection (LOD) and Demonstration of Capability (DOC)

LOD and DOC 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 where there is evidence that the method performance is not affected by sub-matrix distinctions within these groups. Specifically, LOD and DOC shall be determined separately for the system matrix sub-groups listed in section 1.4.1. Where a suitable matrix cannot be found another similar matrix type may be used, or samples may need to be prepared in the absence of a matrix blank. 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, or no reference materials are available for a matrix. Refer to SOP LB-007 and SOP LB-031 for specific procedural details (FL DEP SOP LB-007. Procedure and Policy for Demonstration of Capability for Methods, Instruments, and Laboratory Staff.) (FL DEP SOP LB-031. Limit of Detection Verification.). LOD verifications are performed quarterly for all the analytes for each matrix type at 1 to 4 times to method detection limit but not higher than the PQL.

1.4.1 System Matrix Sub-Groups

- Aqueous – all aqueous samples excluding drinking water and salt/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 of the extent of effect this matrix has on the LOD and DOC is left to the discretion of the HPLC lab supervisor.

1.5 Method Detection Limit (MDL) and Practical Quantitation Limit (PQL)

MDLs are established using procedures given in EPA Chapter 40 Part 136, Appendix B of the Federal Register. Also, refer to DEP SOP LC-011 (FL DEP SOP LC-011. Extraction of Pesticides, Herbisides, and Other Chemicals from Water or Sediment/Waste). In summary, seven replicate samples are analyzed near the target MDL. The results of the seven replicates are tabulated and statistical calculations are performed to determine the concentration of analyte where there is analyte present with 99% confidence. The PQL is four times the MDL. MDLs and PQLs are updated on an annual basis for each analyte and matrix. A list of MDLs and PQLs can be found in Table 5 of the laboratory's quality manual (FL DEP Quality Manual). The actual detection limits reported may vary depending on, for example, the amount of water or sediment/waste sample extracted and the final extract volumes.

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1.6 The methods detailed in this SOP are comparable to EPA method 8321B and USGS method O-2060-01 with the main difference in the mass spectrometry technique used. Our MS systems utilize polypropylene glycols (PPGs) for calibration of the mass spectrometer and an electrospray ionization source for sample introduction. All the MS techniques utilized are well established by the manufacturer and widely used for similar compounds.

2. Acronyms and Abbreviations

Table 2. Acronyms

Acronym	**Meaning**
NELAP	Nation Environmental Laboratory Accreditation Program
DOC	Demonstration of capability
HPLC	High performance liquid chromatography
PQL	Practical quantitation limit
LC-MS/MS	Liquid chromatography with mass spectral fragmentation
MCA	Multi-channel acquisition (signal averaging)
CCV	Continuing calibration verification
PFAS	Per- and polyfluoroalkyl substances
LOD	Limit of detection
LIMS	Laboratory information management system
MS	Mass spectrometry
MDL	Method detection limit
EICP	Extracted ion current profile
ICAL	Initial calibration
NIPI	Negative ion/ positive ion
MRM	Multiple reaction monitoring

Table 3. Compound Abbreviations

Abbreviation	**Compound**
4:2 FTS	1H, 1H, 2H, 2H- Perfluorohexanesulfonic acid
6:2 FTS	1H, 1H, 2H, 2H- Perfluorooctanesulfonic acid
8:2 FTS	1H, 1H, 2H, 2H- Perfluorodecanesulfonic acid
HFPO-DA	Hexafluoropropylene oxide dimer acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
N-EtFOSAA	N-Ethylperfluoro-1-octanesulfonamidoacetic acid
N-MeFOSAA	N-Methylperfluoro-1-octanesulfonamidoacetic acid
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanesulfonic acid
PFDA	Perfluorodecanoic acid
PFDoA	Perfluorododecanoic acid
PFDS	Perfluorodecane sulfonic acid
PFHpA	Perfluoroheptanoic acid
PFHpS	Perfluoroheptane sulfonic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFNS	Perfluorononane sulfonic acid

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PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFPeA	Perfluoropentanoic acid
PFPeS	Perfluoropentane sulfonic acid
PFTeDA	Perfluorotetradecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFUnA	Perfluoroundecanoic acid
AMPA	1-Aminomethylphosphonic acid
Glyphosate	N-(Phosphonomethyl)glycine

3. Method Summary

3.1 An aliquot of the sample or sample extract is analyzed by LC-MS/MS (FL DEP SOP LC-011. Extraction of Pesticides, Herbisides, and Other Chemicals from Water or Sediment/Waste). Each analyte is identified and quantified based on known precursor ions (both in standard and unknown samples) that are fragmented into at least two daughter ions, each with a particular retention time. The LC column and gradient program, and MS method are optimized for each analyte. Analytes with similar parameters can grouped into the same program.

4. Apparatus and Equipment

4.1 2-mL crimp top glass and polypropylene auto sampler vials with crimp caps.

4.2 Syringe filters:

- Millipore (SLCR013NK) PTFE membrane, hydrophilic, 0.45 µm, 13 mm diameter, or equivalent.
- PTFE membrane, hydrophilic, 0.45 µm, 15 mm diameter
- Titan3, 17mm, 0.45 µm polypropylene membrane

4.3 Disposable luer-tip syringes, 3 mL.

4.4 Guard cartridge, reversed phase (Whatman), or equivalent.

4.5 AB Sciex 6500+ LC-MSMS Mass spectrometer with heated electrospray ionization source and coupled Shimadzu Nexera HPLC system that contains an autosampler, column oven, degassing unit, and communications bus module. The column used for each analysis can be found in Table 2 in Appendix A. The electrospray ionization source can ionize a mass range sufficient for the calibration range of the target analytes at the required concentration. The source can generate both positive and negative ions.

4.9 AB Sciex Analyst Software - A computer system interface for continuous data acquisition and storage of machine-readable mass spectra are obtained throughout the duration of the chromatographic program. Software for operation of the LC and MS includes an ion search function and EICP graph function. The software is capable of integration of an individual ion abundance in an EICP given a pre-designated retention time.

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5. Reagents and Chemicals

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

5.1 Solvents

- Acetonitrile (Fisher Scientific, HPLC grade)
- Methanol (Fisher Scientific, HPLC grade)
- Deionized water (in-house, HPLC grade, tested to ensure free of analytes)

5.2 Chemicals

- Formic acid (Acros, reagent grade)
- Ammonium acetate (Fisher, HPLC grade)
- Ammonium fluoride (Fisher, ACS Grade)
- Ammonium formate (Acros, reagent grade)
- Heptafluorobutyric acid

5.3 In-house liquid nitrogen gas is used and split with two additional regulators prior to the instruments. Each gas pressure is set to deliver the required volumes.

5.4 Mobile phases are dependent on the compounds being analyzed (prepared in-house). See table 2 in Appendix A for details.

5.5 See DEP SOP LC-039 for required standards. (FL DEP SOP LC-039. HPLC Standard Preparation.).

6. Sample Preparation Procedure

6.1 Refer to DEP SOP LC-011 for details on sample preparation for water and sediment/waste matrices (FL DEP SOP LC-011. Extraction of Pesticides, Herbisides, and Other Chemicals from Water or Sediment/Waste).

6.2 Typically, sample extracts must be analyzed within 40 days of extraction. The exceptions are carbamates, which must be analyzed within 21 days of extraction; direct injection analyses, which must be done within 7 days of collection; and PFAS which must be analyzed within 28 days from extraction. Refer to the reference methods and analytical tests for applicable holding times.

7. Data Acquisition and Analysis

7.1 Calibration and Optimization

7.1.1 MS acquisition information for PPGs calibration can be found in Table 4.

Table 4. Target Mass, Intensity, and Width of PPGs from 10 MCA scans.

	Target Mass (Da)	**Intensity (cps)**	**Width (Da)**
+Q1	59.05	4.98E+07	0.626
	175.133	2.66E+07	0.738
	500.38	8.39E+07	0.742

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	Target Mass (Da)	Intensity (cps)	Width (Da)
	616.464	6.08E+07	0.755
	906.673	1.80E+08	0.713
+Q3	59.05	4.93E+07	0.741
	175.133	2.28E+07	0.723
	500.38	9.50E+07	0.648
	616.464	5.84E+07	0.778
	906.673	2.06E+08	0.75
-Q1	44.998	1.05E+09	0.643
	411.259	9.02E+05	0.771
	585.385	3.96E+06	0.688
	933.636	2.01E+07	0.716
-Q3	44.998	7.43E+08	0.707
	411.259	3.17E+05	0.714
	585.385	3.18E+06	0.709
	933.636	1.97E+07	0.729

7.1.2 Tuning parameters are instrument dependent and vary with the type of experiment. Analyte response is optimized the for the specific analysis by manual, semiautomatic, and/or automatic tuning procedures. Perform a tune when the mass calibration changes or every six months as part of the preventative maintenance done by a service engineer.

7.1.3 Automatic optimization for MS/MS analysis optimizes certain compound-dependent parameters for MRM transitions. In an automatic optimization experiment, parameters can be optimized for two transitions from the specified precursor ion for each compound. The software determines the optimal conditions for the compound's precursor ion and selected fragment ions used in MRM analysis. Optimization is stored under the acquisition method.

7.1.4 For LC-MS/MS, inject compounds individually or as a mix at 0.50 PPM in 1:1 methanol:water with a flow rate of 10 µL/minute.

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

7.1.6 In manual optimization, the compound dependent parameters (DP, EP, CE, and CXP) for the known MRMs are standardized.

7.1.7 Optimize each MRM transition for curtain gas, collision gas, ion spray voltage, temperature, gas 1, and gas. Set mobile phase A & B (1:1 ratio) at 0.5 mL/min and make 10 µL injection of standard mix and set a run time of 2 minutes.

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7.3 Acquisition Method and Batch

7.3.1 Setup all the HPLC conditions in the method file which includes divert valve settings, gradient program, flow rate, injection volume, and equilibration time. Use the suggested gradient program and column for each method.

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

7.3.3 Each extraction batch must contain one extraction method blank, LFB, and duplicate matrix spikes. A batch is not to exceed 20 samples. If there is insufficient sample for matrix spikes, prepare duplicate LFBs.

7.3.4 First evaluate the instrument blank by injecting mobile phase followed by an initial calibration curve. Quantitative analysis is accomplished by an external calibration method. The calibration fit options are linear through zero, linear, linear weighted (1/x or 1/x^2), or quadratic. For building an initial calibration curve a minimum of 5 calibration levels must be used. The linearity through the 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 that the target and surrogate compound recovery is within ≤ 20% by analyzing mid-level ICAL standard. If the curve is not satisfactory, a new curve must be generated until the requirements are met.

7.3.5 Each run has the following run sequence: mobile phase, CCV, and calibration standards (minimum 5 calibration levels are required) with second source check standard (if the CCV is not within ± 30% accuracy based on the current curve). If the check standard passes, then proceed with the rest of the run sequence by injecting five to eight samples followed by a 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 the MDL. CCV standard recoveries must be within ± 30% of the expected concentration. If at any time the continuing calibration check standard fails to fall within this criterion, any samples analyzed after it must be re-analyzed after ensuring a CCV passes. Otherwise, run a new five-point calibration curve.

7.3.6 Review the current injection or run to see if the data is collecting properly. Check all the MRM transitions by opening their EICP. Review all the injections for proper collection of data before proceeding to quantitation.

7.4 Parameters for acquisition can be found in Table 1 and 2 in Appendix A

7.5 When processing data, verify the retention time half window for each peak is set to 30 seconds. Also, the Gaussian smooth width should be set at 1.0 points, noise percentage is set at 90%, and the peak splitting is set to 6 points.

7.6 Reporting

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7.6.1 In MS/MS analysis, identification and quantitation of each compound is based on the known precursor ion fragmenting into known daughter ions. MRM transitions from the known precursor ion can be used for quantitation, confirmation, and reporting. For analyte concentrations above the MDL, ion ratios are used for further confirmation.

7.6.2 The surrogate, lab fortified blank, and matrix spike recoveries in each batch are used to validate the data and must be calculated.

7.6.3 If the surrogate recovery is outside of established limits of the analysis, then the results must be flagged and an explanation offered. For surrogate failures, re-extraction of the sample must be performed, even after expiration. Follow DEP SOP LB-027 for reporting (FL DEP SOP LB-027. Standard Operating Procedure for Reporting Qualified Data and Correcting Quality Control Problems.).

7.6.4 The lab fortified blank and matrix spike recoveries must be within the acceptance range with a RSD of <30%. This range is evaluated once a year after initial method development. 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 can be observed to recover low due to matrix interferences.

7.6.5 A comment must be added to qualified data in the final report explaining any deviation from the routine limits (FL DEP SOP LB-027. Standard Operating Procedure for Reporting Qualified Data and Correcting Quality Control Problems.).

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

7.7 Calculations

Spike recoveries

$$\%\text{Rec} = \frac{\left(\text{amount detected}, \frac{\mu g}{L}\right)(\text{final extract volume, mL})}{(\text{spike volume, mL})\left(\text{spike concentration}, \frac{\mu g}{L}\right)} * 100$$

Concentration in water samples

$$\text{Conc}\left(\frac{\mu g}{L}\right) = \frac{\left(\text{detected conc}, \frac{\mu g}{L}\right)(\text{final extract volume, mL})}{(\text{volume of sample extracted, mL})}$$

Concentration in sediment samples

$$\text{Conc}\left(\frac{\mu g}{kg}\right) = \frac{\left(\text{detected conc}, \frac{\mu g}{L}\right)(\text{final extract volume, mL})}{(\text{dry weight of sample, g})}$$

Example: If 25 µg/L was detected in a sample extracted from 30 g of sediment with a final volume of 10 mL then,

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$$\text{Conc}\left(\frac{\mu g}{kg}\right) = \frac{\left(25\frac{\mu g}{L}\right)(10\text{ mL})}{(30\text{ g})} = 8.3\frac{\mu g}{kg}$$

Dilution factor for soil samples

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

Example: if the dry weight is 8.15%, then

$$\text{Dilution } \textit{factor} = \frac{1}{0}.0815 = 12.27$$

If performing dilutions, show all calculations and include copies of the dilution/sample prep logbook entry in results folder. When entering the dilution factor for soil samples in QC Manager, make sure that the dilution factor is correct.

8. Data Reporting and Archival

8.1 When analysis is complete, transfer the results as a text file into the LIMS QC Manager. If a sample has been diluted or has a dry weight less than 100 %, ensure that the information was entered properly into the QC Manager.

8.2 Add the appropriate qualifiers and comments after performing a complete review of the sample data.

8.3 Ensure that the results are consistent with the type of each sample. For example, equipment blanks should not contain hits while an effluent sample could. If anything looks suspicious, further analysis is necessary before posting results.

8.4 Include cover page, photocopy of preparation logbook page, run sequence, the results table, printout of QC results, dilutions page if any, and printout of analytes above MDL in the results folder. Submit folder to the section supervisor for review, authorization, and archival.

8.5 Original data files are archived in X:\Labs\Lab3\Chem_Data\Organic\LC.

9. Quality Control

See DEP SOP LB-027 for guidelines for qualifying data when quality control results are outside established control limits and re-analysis is not a viable or desired option. Definitions of the available qualifying codes are listed (FL DEP SOP LB-027. Standard Operating Procedure for Reporting Qualified Data and Correcting Quality Control Problems.).

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10. Safety / Hazardous Waste Management

See SOP OG-001 for specific details on safety and hazardous waste management (FL DEP SOP OG-001. Safety and Waste Management Practices in the Organic Analysis Groups (Volatiles, Semi-Volatile, and Pesticide Laboratories).).

11. References

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Appendix-A

Table 1. MS Acquisition Parameters

Analysis	Acquisition (mins)	Polarity	Time (ms)	Source Gas	Collision Gas	Ion Spray Volatage	Temperature (°C)	Ion Source Gas 1	Ion Source Gas 2	Interface Heater	Valve Time (min)
NIPI	27	-/+	100	30	10	-/+ 3000	400	55	60	on	1-27
Acrylamide/Benzoic acid	11	-/+	10/20	30	10	-/+ 3000	400	55	60	on	1-8
Sucralose/ Endothall	15	-	15	40	9	- 3000	550	60	70	on	2.5-14
Glyphosates	4	-		40	5	-3000	550	60	70	on	1-3
Microcystins	16	+		30	10	5250	500	55	60	on	2-13
Didecyldimethyl-ammonium chloride	11	+		30	10	3000	400	55	60	on	1-10
Diquat/ Paraquat	13	+		20	2	5500	700	70	60	on	6.3-7.5
PFAS	23	-	25	30	12	-4500	400	30	30	on	6-15

Table 2. LC Acquisition Parameters

Analysis	Column	Mobile Phase A	Mobile Phase B	Gradient Program
NIPI	RESTEK Luna 2.7 µm biphenyl, 4.6 x 150 mm	5 mmol ammonium acetate and 0.1% formic acid in HLPC water	0.1% formic acid in acetonitrile	Start 95:5 A:B 0-13 mins shift to 70:30 A:B 13-22 mins shift to 20:80 A:B 22-23 mins shift to 10:90 A:B 23-24 min hold 24-24.9 min 2:98 A:B 24.9-29.5 min hold 29.5-30 95:5 A:B 30-34 min hold
Acrylamide/Benzoic acid	RESTEK Luna 2.7 µm biphenyl, 4.6 x 150 mm	5 mmol ammonium acetate and 0.1% formic acid in HLPC water	0.1% formic acid in acetonitrile	start 95:5 A:B 0.1-5 mins shift to 70:30 A:B 5-6.3 mins shift to 0:100 A:B 6.3-6.4 mins shift to 95:5 A:B 6.4-10.9 min hold 11.0 mins stop

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Analysis	Column	Mobile Phase A	Mobile Phase B	Gradient Program
Sucralose/ Endothall		0.25 mmol ammonium fluoride in HPLC water	acetonitrile	start 95:5 A:B 0-4 mins shift to 70:30 A:B 4-5 mins shift to 10:90 A:B 5-9.2 mins shift to 1:99 A:B 9.2-12.5 min hold 12.5-12.9 mins shift to 95:5 A:B 12.9-15 mins hold
Endothall		5 mmol ammonium acetate and 0.1% formic acid in HPLC water	0.1% formic acid in acetonitrile	start 95:5 A:B 0-4 mins shift to 70:30 A:B 4-5 mins shift to 10:90 A:B 5-9.2 mins shift to 1:99 A:B 9.2-12.5 min hold 12.5-12.9 mins shift to 95:5 A:B 12.9-15 mins hold
Glyphosates	Waters SummetryShield RP8 150 x 3.9 mm	5 mmol ammonium acetate and 0.1% formic acid in HLPC water	0.1% formic acid in acetonitrile	start-3 mins 100:0 A:B 3-4.9 shift mins 2:98 A:B 4.9-7 mins hold 7-7.5 shift to 100:0 A:B 7.5-10 mins hold
Microcystins	RESTEK Luna 2.7 µm biphenyl, 4.6 x 150 mm	5 mmol ammonium acetate and 0.1% formic acid in HLPC water	0.1% formic acid in acetonitrile	start-1.5 mins 95:5 A:B 1.5-3 mins shift to 75:25 A:B 3-8 mins shift to 45:55 A:B 8-8.9 shift to mins 20:80 A:B 8.9-9 shift to mins 2:98 A:B 9-14.0 mins hold 14.0-16.0 mins shift to 95:5 A:B
Saxitoxin	TOSOH TSKgel Amide-80 3 µm 4.6 x 150 mm	5 mmol ammonium acetate and 0.1% formic acid in HLPC water	0.1% formic acid in acetonitrile	start-1 min 80:20 A:B 1-9 mins shift to 50:50 A:B 9-10 mins shift to 50:50 A:B 10-10.5 shift to mins 60:40 A:B 10.5-12 shift to mins 60:40 A:B 12.5 mins hold at 20:80 A:B

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Analysis	Column	Mobile Phase A	Mobile Phase B	Gradient Program
Didecyldimethylammonium chloride	Phenomenex Synergy 4 µm funsion RP 80A 50 x 2 mm	5 mmol ammonium acetate and 0.1% formic acid in HLPC water	0.1% formic acid in acetonitrile	start 95:5 A:B 0-5 mins shift to 30:70 A:B 5-6.3 mins shift to 0:100 A:B 6.3-6.4 shift to 95:5 A:B 6.4-11 hold
Diquat/ Paraquat		5 mmol heptafluorobutyric acid in HPLC water	0.1% formic acid in acetonitrile	start 95:5 A:B 0-8 mins shift to 70:30 A:B 8-9 mins shift to 10:90 A:B 9-13 mins shift to 95:5 A:B
PFAS	Restek Biphenyl 2.7 µm, 150 m x 4.6 mm ID Waters, CORTECS T3 2.7 µm, 150 mm x 3 mm ID Cat. #186008490	20 mmol ammonion acetate in HPLC water (1.54 grams in 1 Liter Water)	acetonitrile	start 85:15 A:B 0-4 mins shfit to 60:40 A:B 4-13 mins shift to 20:80 A:B 13-14 shift to 1:99 A:B 14-15 mins shift to 1:99 A:B 15.1-18.9 mins 85:15 19.0 Stop Total Flow – 0.7 mL/min Injection Volume – 5 uL

Table 3. QC Solutions

Analysis	Target to Surrogate Ratio	Calibration Levels (µg/L)	CCV/LFB/MSPK Level (µg/L)	SUR Level (µg/L)
NIPI	1:2	100, 50, 25, 10, 5, 2.5, 1, 0.5, 0.25	2.5/10/10	20
Acrylamide/Benzoic acid	25:1	100, 75, 50, 25, 10, 5	50/50/50	2
Sucralose/ Endothall	1:02	25, 10, 5, 2.5, 1, 0.25, 0.1, 0.05, 0.01 Endothall: 100, 75, 50, 25, 10, 5	2.5/2/2 Endothall: 25	4 Endothall: 25
Glyphosates	1:05	25, 10, 2.5, 1, 0.25, 0.1	1	
Microcystins/Saxitoxin	1:100	50, 25, 10, 2.5, 1, 0.25	10	
Didecyldimethylammonium chloride	1:50	100, 50, 25, 10, 5, 2.5, 0.5	25/10/10	
Diquat/ Paraquat		50, 25, 10, 5, 2, 1	10	10
PFAS	1:01	100, 25, 5, 1, 0.5, 0.25, 0.05	1/2	2

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Table 4. Acquisition Parameters for Target Analytes

Analyte	CAS #	Ion-Mode	Q1 Mass	Q3 Mass	Duration	DP	EP	CE	CXP
2,4,5-T	93-76-5	N	252.9/ 254.9	194.9/ 196.9	10	-35	-10	-20	-10
2,4-D	94-75-7	N	218.9/ 220.9	160.9/ 162.9	10	-25	-10	-18	-10
2,4-DB	94-82-6	N	246.9/ 248.9	160.9/ 162.9	10	-20	-10	-16	-10
3-Hydroxycarbofuran	16655-82-6	P	238.2	163/ 107.1	10	45	10	20/ 40	10
Acesulfame K	55589-62-3	N	161.881	78/ 82/ 64.1	10	-30/ -30/ -15	-10	-48/ -20/ -68	-9/ -9/ -3
Acetaminophen	103-90-2	P	152.1	110.1/ 65	10	50	10	22/ 39	10
Acetamiprid	135410-20-7	P	223.027	126.1/99.1	10	51	10	29/51	14/12
Acifluorfen	50594-66-6	N	360	316/ 195	10	-25	-10	-13/ 35	-10
Acrylamide	79-06-1	P	72	55/44	8	35/76	10	15/29	10/6
Afidopyropen	915972-17-7	P	594.2	202.2/148	10	31	10	45/59	10/4
Aldicarb	116-06-3	P	208.2/ 208.1	116.1/ 89.1	10	31	10	10.5/ 23	10
Aldicarb Sulfone	1646-88-4	P	240.1	86.1/ 76.1	10	40	10	25.5/ 16.5	10
Aldicarb Sulfoxide	1646-87-3	P	207.1	132/ 89.2	10	45	10	9/ 19	10
AMPA	1066-51-9	N	109.882	63/ 79/ 81	50	-50/ -30/ -30	-10	-24/ -36/ -18	-7/ -11/ -9
Anatoxin-a	64285-06-09	P	166.1	131.1/ 91	20	50	10	23/ 30	12
Bendiocarb	22781-23-3	P	223.939	167/ 81	50	30	10	13/ 43	20/ 10
Bentazon	25057-89-0	N	239	132/ 175	10	-70	-10	-35/ -26	-10
Benzoic acid	65-85-0	N	121	77/ 77.1	10	-35	-10	-16	-10/ -36
Benzovindiflupyr	1072957-71-1	P	398	378/342	10	71	10	21/25	12/22
Carbamazepine	298-46-4	P	237.1	194.1/ 193.1	10	45	10	28/ 46	10
Carbaryl	63-65-2	P	202.1	144.9/ 127.1	10	45	10	15/ 38	10
Carbofuran	1563-66-2	P	222.1	165.1/ 123	10	45	10	17/ 30	10
Clothianidin	210880-92-5	P	250	132/169	10	66/26	10	21/27	18/8
Cylindrospermopsin	143545-90-8	P	416.1/ 416.2/ 416.1	194.1/ 176.1/ 336.1	20	100	10	47/ 49/ 31	12
Dalapon	75-99-0	N	140.819	96.9/ 104.9	10	-5	-7	-12/ -10	-13
Dicamba	1918-00-9	N	218.85/ 220.9	174.911/ 176.9	10	-20	-10	-10/ -11	-11/ -10
Dichlorprop	120-36-5	N	232.9/ 232.8	160.9/ 124.902	10	-20	-10	-18/ -40	-10/ -15
Didecyldimethylammonium chloride	7173-51-5	P	326.3	186.2/ 41/ 43	25	80	10	39/ 92/ 72	12/ 10/ 10
Dinotefuran	165252-70-0	P	203.08	114.1/129	10	1/11	10	25/17	14/16

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Analyte	CAS #	Ion-Mode	Q1 Mass	Q3 Mass	Duration	DP	EP	CE	CXP
Dinoseb	88-85-7	N	239	193/ 134	10	-50	-10	-34/ -58	-10
Diquat	6385-62-2	P	183	157/ 168	25	136	10	33/ 35	11/ 18
Diuron	330-54-1	P	233	72.2/ 46.2	10	40	10	36/ 31	10
Duloxetine HCl		N	298	154/ 44	10	81	10	9/ 35	16/ 20
Endothall	145-73-3	N	184.915	141/ 43.2	10/ 50	-20/ -30	-10/ -3	-20/ -48	-17/ -19
Fenuron	101-42-8	P	165.1	72.1/ 46.2	10	40	10	21/ 19	10
Fluometuron		P							
Fluridone	59756-60-4	P	330.037	310.1/ 259	10	150	10	47	10
4:2 FTS	757124-72-4	N	327	307/81	25	-60	-10	-28/-50	-12
6:2 FTS	27619-97-2	N	427	407/81	25	-80	-10	-32/-72	-12
8:2 FTS	39108-34-4	N	527	507/81	25	-60/-111	-10	-40/-82	-12/-9
Glufosinate	77182-82-2	N	179.915	95/ 85	50	-35	-10	-24/ -26	-11
Glyphosate	1071-83-6	N	167.887	62.9/ 78.8/ 149.9	50	-5/ -50	-10	-26/ -52/ -17.16	-9/ -11/ -7
HFPO-DA	13252-13-6	N	285	169/185	25	-20/-70	-10	-7/-24	-13/-11
HFPO-DA 13C	DEP-SURR-108	N	287	169	25	-20	-10	-7	-13
Hydrocodone	125-29-1	N	300/ 300/ 300.001	199/ 171/ 128	10	4/ 1/ 4	10	41/ 51/ 73	12/ 20/ 34
Ibuprofen	15687-27-1	P	205/ 206/ 205.001/ 206.001	161/ 162/ 161.001/ 162.001	10	-30/ -35/ -13.92/ -22.76	-10/ -10/ -7.64/ -8.07	-10/ -10/ -9.34/ -9.73	-10/ -5/ -13/ -7.08
Imazapyr	81334-34-1	N	259.978	216/ 173	10	-25/ -40	-10	-18/ -28	-13/ -19
Imidacloprid	138261-41-3	P	256.1	209/ 175	10	35	10	23/ 26	10
Indaziflam	950782-86-2	P	302.2	158/ 138.1	10	45	10	23/ 35	10
Iohexol	66108-95-0								
Linuron	330-55-2	P	249.1	160.1/ 182	10	35	10	24/ 21	10
Malathion		P							
Mandestrobin	173662-97-0	P	314.1	192.1/160.1	10	101	10	15/27	14/12
MCPA	94-74-6	N	198.9/ 200.9	140.9/ 143	10	-35	-10	-20	-10
MCPP	93-65-2	N	213/ 215	141/ 143	10	-20	-10	-20	-10

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Analyte	CAS #	Ion-Mode	Q1 Mass	Q3 Mass	Duration	DP	EP	CE	CXP
Metformin HCl	1115-70-4	N	130/ 130/ 130.001/ 130.001/ 130.001/ 130/ 130.01	71/ 43/ 60.001/ 71.001/ 43.001/ 60/ 71.01	10	4/ 4/ 32.03/ 32.03/ 161/ 161/ 161	10/ 10/ 9.93/ 9.93/ 10/ 10/ 10	41/ 43/ 19.15/ 32/ 65/ 17/ 29	36/ 4/ 8.06/ 6.61/ 20/ 8/ 8
Methiocarb	2032-65-7	P	226.1	169/ 121.1	10	40	10	13/ 25	10
Methomyl	16752-77-5	P	163.1	106/ 88	10	40	10	15	10
Desmethyl microcystin LR	120011-66-7	P	981.6	103/599.3	20	111	10	127/63	14/6
Microcystin LA	96180-79-9	P	910.5	402.3/ 776.4/ 375.2	20	90	10	35/ 27.5/ 43	12
Microcystin LF	154037-70-4	P	986.5	478.2/ 375.1/ 852.5	20	95	10	37/ 45/ 30	12
Microcystin LR	101043-37-2	P	995.5	105/ 213.2/ 135.1	20	120	10	150/ 75/ 117	12
Microcystin LW	157622-02-1	P	1025.5	135.2/517.2	20	70/176	10	106/37	14/20
Microcystin LY	123304-10-9	P	1002.5	494.2/ 265/ 868.4	20	90	10	38/ 62/ 30	12
Microcystin RR	111755-37-4	P	519.9	103.1/ 135.1/ 213.1	20	90	10	91/ 36/ 45	12
Microcystin WR	138234-58-9	P	1068.6	103/213.1	20	70/66	10	165/77	9/16
Microcystin YR	101064-48-6	P	1045.5/ 1045.4	375.2/ 135.1	20	160	10	70/ 130	12
Monuron		P	198.885	72/ 126	10	30	10	21/ 35	10/ 14
Naproxen	22204-53-1	P	228.9	169/ 170/ 185	10	-20	-10	-38/ -20/ -10	-21/ -9/ -11
N-EtFOSAA	2991-50-6	P	583.8	418.9/ 482.9/ 526	25	-60	-10	-28/ -22/ -28	-13/ -15/ -17
N-MeFOSAA	2355-31-9	P	569.8	419/ 482.9/ 511.9	25	-70	-10	-28/ -22/ -30	-13/ -15/ -17
Nodularin		P	825.4	227/ 135.1/ 70.2	20	160	10	65/ 99/ 170	12
Oxamyl	23135-22-0	P	237.1	72.1/ 90.1	10	40	10	27/ 12	10
Paraquat	1910-42-5	P	185	170/ 169	25	156	10	29/ 43	22/ 8
PFBA	375-22-4	N	212.9	169	25	-25	-10	-14	-15
PFBS	29420-49-3	N	298.8	79.9/ 98.9	25	-70	-10	-62/ -36	-9/ -11
PFDA	335-76-2	N	512.8	468.9/ 218.9	25	-35	-10	-16/ -24	-15/ -25

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Analyte	CAS #	Ion-Mode	Q1 Mass	Q3 Mass	Duration	DP	EP	CE	CXP
PFDoA	307-55-1	N	612.8	568.9/ 168.9	25	-40	-10	-18/ -34	-19
PFDS	2806-15-7	N	599	80/99	25	-85	-10	-118/-84	-12
PFHpA	375-85-9.	N	362.9	318.9/ 168.9	25	-30	-10	-14/ -24	-27/ -19
PFHpS	375-92-8	N	449	80/99	25	-65	-10	-88/-50	-12
PFHxA	307-24-4	N	312.9	268.9/ 118.9	25	-25	-10	-12/ -26	-23/ -13
PFHxS	355-46-4	N	398.8	79.9/ 98.9	25	-95	-10	-84/ -72	-9/ -11
PFNA	375-95-1	N	462.8	418.9/ 218.9	25	-35	-10	-14/ -26	-13/ -19
PFNS	98789-57-2	N	549	80/99	25	-70	-10	-110/-70	-12
PFOA	335-67-1	N	412.8	368.9/ 168.9	25	-30	-10	-14/ -24	-13/ -19
PFOS	1763-23-1	N	498.8	79.9/ 98.9	25	-135/ -60	-10	-110/ -88	-9/ -11
PFPeA	2706-90-3	N	262.9	219	25	-20	-10	-14	-15
PFPeS	630402-22-1	N	349	80/99	25	-60	-10	-66/-45	-12
PFTeDA	376-06-7	N	712.7	668.9/ 168.9	25	-50	-10	-20/ -36	-21/ -17
PFTrDA	72629-94-8	N	662.7	618.9/ 168.9	25	-50	-10	-18/ -36	-19
PFUnA	2058-94-8	N	562.8	518.9/ 168.9/ 268.9	25	-40	-10	-16/ -32/ -26	-17/ -11/ -23
Picloram	1918-02-1	N	240.8/ 238.9	196.81/ 194.91	10	-30	-10	-14	-11/ -9
Primidone	125-33-7	P	219.1/ 218.981	162/ 91	10	40	10	17/ 31	10/ 11
Propoxur	114-26-1	P	210.1	111/ 93.1	10	40	10	20.5/ 35	10
Pyraclostrobin	175013-18-0	P	388.1	194/ 163.1	10	35	10	17/ 31	10
Saxitoxin	35523-89-8	P	300	282.2/179	20	146	10	25/33	10/16
Silvex	93-72-1	N	266.9/ 268.9	194.9/ 196.9	10	-20	-10	-18	-10
Simvastatin		N	436.33/ 419.1/ 436.2/ 436.2	199/ 243/ 199/ 419	10	21/ 1/ 1/ 1	3.5/ 10/ 10/ 10	23/ 21/ 27/ 11	4/ 22/ 22/ 22
Sucralose	56038-13-2	N	395.063/ 395	35	10	-45	-10	-60/ -16	-15/ -11
Thiamethoxam	153719-23-4	P	291.9	211/181	10	51	10	17/31	12
Tolfenpyrad	129558-76-5	P	384.1	197.1/154.1	10	81	10	35/55	12/10
Triclopyr	55335-06-3	N	253.1/ 254.9	194.9/ 196.902	10	-25	-10	-18/ -16	-11

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Appendix-B

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 HPLC 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
10/02/2018	Added per- and polyfluoroalkyl substances
02/06/2016	General edits regarding grammar, spelling, and document organization. Removed unnecessary information.
02/12/2020	Added new analytes

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