Determination of Seven Neonicotinoids in Surface Water by Liquid Chromatography Triple Quadrupole Mass Spectrometry

1. Scope:

This Section Method (SM) provides a stepwise procedure for the analysis of seven neonicotinoids in surface water. The objective of this standard operating procedure (SOP) is to quantify the concentration of a target list of neonicotinoids in surface water. This solid phase extraction (SPE) method has been developed and validated using prime HLB SPE cartridges followed by liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) analysis.

2. Principle:

Neonicotinoids are extracted from surface water samples with SPE technique. Centrifugation is introduced at the beginning of sample preparation to resolve clogging of the sorbent cartridges. After preconditioning SPE cartridges and sample loading, the samples are eluted and collected with ethyl acetate and hexane/acetone in one glass tube and methanol in another glass tube. The collected extracts are evaporated to 0.5 mL each under a stream of nitrogen and the combined extracts are analyzed by LC-MS/MS.

3. Safety:

3.1 Read the Safety Data Sheet for all materials before use.

3.2 Pesticides are hazardous substances and may have acute and/or chronic toxic effects. Avoid all contact with and inhalation of the materials containing pesticides. Wear a lab coat as well as safety glasses and chemical resistant gloves when handling the samples.

3.3 All general laboratory safety rules for sample preparation and analysis shall be followed.

3.4 All flammable solvents shall be handled with care and used in a fume hood.

3.5 Special storage, use, handling and disposal procedures are necessary to ensure safety when using compressed gases.

4. Interferences:

There were no matrix interferences with the compounds at the time of method development using clean background surface water; however, matrix suppression was detected in real samples with low surrogate recoveries.

5. Apparatus and Equipment:

5.1 A Shimadzu LC system comprising of a system controller, pumps, degasser, autosampler and column oven coupled to an AB Sciex 6500+ QTRAP mass spectrometer with Turbo V-Source, ESI probe, Varian vacuum pump, and Windows 10 Analyst 1.7.2 PC workstation

5.2 Supelco vacuum manifold

5.3 Nitrogen evaporator (Meyer N-EVAP Organomation Model #112 or equivalent)

5.4 Vortex-vibrating mixer

5.5 Centrifuge

5.6 Micropipettes, adjustable, recommended sizes as follows: 10 µL,1000 µL, and 10 mL

6. Reagents and Supplies:

6.1	Acetamiprid	CAS# 135410-20-7
6.2	Clothianidin	CAS# 210880-92-5
6.3	Dinotefuran	CAS# 165252-70-0
6.4	Imidacloprid	CAS# 138261-41-3
6.5	Sulfoxaflor	CAS# 946578-00-3
6.6	Thiacloprid	CAS# 111988-49-9
6.7	Thiamethoxam	CAS# 153719-23-4
6.8	Atrazine-d5 (surrogate)	CAS# 163165-75-1

6.9 Imidacloprid-d4 (surrogate) CAS# 1015855-75-0

6.10 Custom LCMSMS Standards, RESTEK

6.11 Waters Oasis Prime HLB 6cc-500 mg, extraction cartridge

6.12 Water, HPLC grade or higher purity

6.13 Methanol, HPLC grade or higher purity

6.14 Ethyl Acetate, HPLC grade or higher purity

6.15 Acetone, HPLC grade or higher purity

6.16 n-Hexane, high resolution grade

6.17 Formic Acid, HPLC grade

6.18 Ammonium formate, reagent grade or equivalent

6.19 Graduated conical tubes with glass stopper, 15 mL

6.20 Centrifuge tube, pp, cylindrical, 110 mL

6.21 Varian bond Elut reservoir, 75 mL

6.22 Bond Elut SPE cartridge adapter

6.23 Disposable Pasteur pipettes, and other laboratory ware as needed

6.24 Recommended UPLC analytical column: Waters Acquity BEH C18 1.7 µm, 2.1 x 100 mm column or equivalent

7. Standards Preparation:

7.1 Custom standard mixtures of 100 µg/mL are obtained from RESTEK.

7.2 A combination standard of 1.0 µg/mL is prepared from the RESTEK custom mixtures in acetonitrile. The combination standard is also used to dilute to the following concentrations: 0.00125, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1, and 0.25 µg/mL in acetonitrile. These standards are then diluted in half with HPLC grade water immediately before analysis to make the following concentrations: 0.000625, 0.00125, 0.0025, 0.005, 0.0125,

0.025, 0.05, and 0.125 µg/mL for instrument calibration. See standard preparation sheet.

7.3 The combination standard at 0.01 µg/mL is prepared by dilution of 1.0 µg/mL in Acetonitrile. A 500 µL of this standard with final concentration of 0.05 ng/mL is used for matrix spike. See standard preparation sheet.

7.4 The combination surrogate standard at 0.01 µg/mL is prepared by dilution of 0.1 µg/mL in Acetonitrile. A 500 µL of this standard with final concentration of 0.05 ng/mL is used for all surface water samples. See standard preparation sheet.

7.5 Standards are kept in the designated freezer for storage.

7.6 The expiration date of each standard is six months from the preparation date. The standards prepared with HPLC grade water are prepared fresh for each analysis.

Mobile Phase Preparation:

7.7 Aqueous Solution: For 500 mL, mix 470 ± 2 mL HPLC grade water, 25 ± 0.5 mL methanol, 4.50 ± 0.25 mL 1 M ammonium formate and 0.5 ± 0.05 mL formic acid.

7.8 Organic Solution: For 500 mL, mix 450 ± 2 mL methanol and 45 ± 0.5 mL HPLC grade water with 4.50 ± 0.25 mL 1 M ammonium formate and 0.5 ± 0.05 mL formic acid.

8. Sample Preservation and Storage:

Store all samples prior to extraction in a refrigerator (4 ± 3 °C). Based on a 28-day storage study, holding time for extraction of surface water samples is 7 days from the date of sample collection.

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provides clean background surface water for matrix blank and spikes.

9.2 Preparation of Blank and Spike

9.2.1 Reagent Blank: Transfer a mixture of 1:1 ACN:H_2O (v:v) into a glass vial and analyze it in a sample batch.

9.2.2 Matrix Blank: Transfer 100 mL of background surface water into a 110 mL centrifuge tube, shake and centrifuge it. Then, follow the same extraction procedure as described in Sections 9.3.3 - 9.3.9.

9.2.3 Matrix Spike: Transfer 100 mL of background surface water into a 110 mL centrifuge tube and spike to 0.05 ng/mL with combination standard, shake and centrifuge it. Then, follow the same extraction procedure as described in Sections 9.3.3 - 9.3.9.

9.3 Test Sample Extraction

9.3.1 Remove samples from the refrigerator and allow them to reach ambient temperature.

9.3.2 Transfer 100 mL of water samples into a 110 mL centrifuge tube, spike with surrogates, shake and centrifuge it at 4000 rpm for 20 min.

9.3.3 Attach SPE cartridges to vacuum manifold.

9.3.4 Precondition the SPE cartridges with 5mL Ethyl Acetate, 5 mL Hexane, 5 mL Acetone, 5 mL Methanol and 5 mL HPLC Grade Water sequentially in a slow dropwise fashion (2-3 mL/min).

9.3.5 Attach Bond Elut adapters on top of the SPE cartridges and connect Bond Elut reservoirs to the SPE cartridges. Load 100 mL of sample into each reservoir.

9.3.6 After loading samples, rinse the cartridges with 5 mL HPLC Grade Water and dry them under vacuum condition for 60 minutes.

9.3.7 Elute the cartridge with 5 mL Ethyl Acetate followed by 6 mL of 3:1 Hexane:Acetone (v:v) in a calibrated glass tube and, separately, 5 mL Methanol in another glass tube.

9.3.8 Evaporate the sample extracts to 0.5 mL in a water bath at 40 ± 2 °C under a gentle stream of nitrogen. For each sample, combine these two extracts to a 1 mL final volume in an auto sampler vial.

9.3.9 Dilute 1 to 2 times all samples and QCs using acetonitrile/water (1:1). Submit the diluted extract for LC-MS/MS analysis.

10. Instrumental Analysis:

10.1 Instrument Calibration

10.1.1 The calibration standard curve consists of a minimum of five levels for a quadratic curve or three levels for a linear curve. The lowest level must be at or below the corresponding reporting limit. The current working standard levels are 0.000625, 0.00125, 0.0025, 0.005, 0.0125, 0.025, 0.05, 0.125 µg/mL.

10.1.2 Calibration is obtained using a quadratic or linear regression with the correlation coefficient (r) equal to or greater than 0.995.

10.2 Sequence Arrangement

The LC-MS/MS needs to be conditioned with standards or sample extracts 2 to 3 times before running the following recommended sequence:

- A set of calibration standards (8 levels)
- Reagent blank
- Matrix blank
- Reagent blank
- Matrix spike
- A set of up to 12 test samples
- A set of 2 reagent blanks; and
- A set of 3 calibration standards

10.3 Instrument Conditions

10.3.1 LC Separation Conditions

A Shimadzu LC30 liquid chromatograph is equipped with Waters Acquity BEH C18 1.7 µm, 2.1 x 100 mm column. Samples are eluted using a gradient system at a flow rate of 0.4 mL/min throughout the 18 min run-time at 50 °C with injection volume of 3 µL. See Table 1.

Table 1. LC Gradient

Step	Time (Minute)	Mobile Phase A (%)	Mobile Phase B (%)
0	0.01	98	2
1	0.25	70	30
2	10.00	0	100
3	15.00	0	100
4	15.10	98	2
5	18.00	0	0

10.3.2 Mass Spectrometer Conditions

To achieve a mass spectrum, an AB Sciex Triple Quad 6500+ mass spectrometer with an ESI interface is used. See Table 2 for mass spectrometer parameters in positive mode. The mass spectrometer operates in positive scheduled MRM (Multiple Reaction Monitoring) mode described in Table 3, by monitoring 2 transitions for each compound. Quantitation ions are bolded.

Table 2. Mass Spectrometer Operating Parameters

Parameter	Setting
Ion Mode	Positive
Curtain Gas	30
Ion Spray Voltage	4500
Temperature	250
Ion Source Gas 1	50
Ion Source Gas 2	50
Collision Gas	7
Electron Multiplier	1500
MRM Detection Window (sec)	60

Table 3. MRM Parameters for Neonicotinoid Detection in Positive Mode

Compound	RT (Minute)	Precursor Ion	Product Ion [1]	Declustering Potential	Collision Energy	Entrance Potential	Exit Potential
Acetamiprid	2.70	223.0 223.0	**125.9** 89.9	56 56	25 43	10 10	18 14
Clothianidin	2.36	249.9 249.9	**168.9** 131.9	36 36	15 19	10 10	16 16
Dinotefuran	1.55	203.0 203.0	**129.1** 114.0	43 43	14 16	10 10	16 17
Imidacloprid	2.31	256.0 256.0	**208.9** 175.1	30 30	21 25	10 10	26 16
Sulfoxaflor	2.95	278.1 278.1	**174.1** 154.1	66 66	13 38	10 10	22 18
Thiacloprid	3.08	252.9 252.9	**125.8** 90.0	76 76	27 51	10 10	20 12
Thiamethoxam	1.85	291.9 291.9	**211.0** 180.9	41 41	19 29	10 10	30 24
Atrazine-d5 (surrogate)	5.48	220.9 220.9	**179.0** 101.0	61 61	25 31	10 10	24 16
Imidacloprid-d4 (surrogate)	2.29	259.9 259.9	**213.0** 179.0	56 56	21 23	10 10	26 22

[1] Quantitation ions are bolded

11. Quality Control:

11.1 Method Detection Limit

Method Detection Limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 surface water samples are spiked at 0.01 ppb and processed through the entire method (Section 9.3) along with a matrix blank. The standard deviation derived from the spiked sample recoveries is used to calculate the MDL using the following equation:

$$\mathrm{MDL} = \mathrm{tS}$$

Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses.

For the n=7 replicates used to determine the MDL, t=3.143. A set MDL of 0.004 ppb was established for this method. Trace will be reported when results fall within this MDL and the Reporting Limit. The results for the standard deviations and MDL are in Appendix I.

11.2 Reporting Limit

Reporting limit (RL) refers to a level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. The RL is chosen as a range 1-5 times the MDL, as per client agreement. The RL for this method is 0.02 ppb for all compounds except Imidacloprid, which is 0.01 ppb.

11.3 Method Validation

Initially, these seven neonicotinoids along with other 60 pesticides were validated and analyzed using EMON-SM-05-049. Centrifugation was later added at the beginning of sample preparation to resolve clogging of the sorbent cartridges. This current method provides protocols including the validation and method detection limit performed for neonicotinoids in this standard operating procedure.

The method validation consisted of three sample sets. Each set included three levels of fortification and a matrix blank. All spikes and matrix blanks were processed through the entire analytical method (Section 9.3). Spike levels and recoveries for the analytes are shown in Appendix II.

Based on client request, a separate standard operating procedure for neonicotinoids was prepared including 0.02 ppb for Acetamiprid, Clothianidin, Dinotefuran, Sulfoxaflor, Thiacloprid, Thiamethoxam and 0.01 ppb for Imidacloprid. In addition, this method shows a set MDL of 0.004 ppb for all compounds. All these results are in agreement with the client’s need and request.

11.4 Control Charts and Limits

A control chart was generated using the data from the method validation. The upper and lower control limits are set at ± 3 standard deviations of the percent recovery, shown in Appendix II.

11.5 Acceptance Criteria

11.5.1 Each set of samples will have one matrix blank sample and one matrix spike sample.

11.5.2 The retention time should be within ± 0.1 minute of that of the standards.

11.5.3 The recovery of the matrix spike shall be within the control limits. See Appendix II.

11.5.4 The relative abundances of structurally significant ions used for confirmation must be within ± 30% when compared to a standard injection during the same run.

11.5.5 The sample shall be diluted if results fall outside the calibration curve.

11.5.6 Matrix suppression was detected in real samples with low surrogate recoveries. Originally a dilution of 1-10 with acetonitrile/water (1:1) was used but it was determined that a dilution of 1-2 times was sufficient. Diluting samples and QCs improves peak resolution and has a better match with the calibration curve; diluting does not change the reporting limit or method detection limit. It also reduces matrix interferences in samples with a high matrix effect.

11.6 Measurement Uncertainty:

Measurement uncertainties may be calculated for each compound or compound group. A minimum of 30 data points is required. Sample volume, matrix variability, SPE cartridge, accuracy of standards and spiking solutions are critical parameters that contribute to the measurement uncertainty.

12. Calculations:

Quantitation is based on an external standard calculation using either the peak area or height. The Linear Ion Trap Quadruple LC-MS/MS software uses quadratic or linear curve fits. Alternatively, at the chemist's discretion, concentrations may be calculated using the response factor for the standard whose value is < 30% to the level in the sample.

$$\text{ppb} = \frac{(\text{sample peak area or ht})(\text{STD conc})(\text{STD vol. injected})(\text{final vol. of sample})(1000\ \mu\text{L/mL})}{(\text{STD peak area or ht})(\text{sample vol. injected})(\text{sample weight } g)}$$

13. Reporting Procedure:

Sample results are reported according to the client's analytical laboratory specification sheets.

14. Discussion:

14.1 The Department of Pesticide Regulations requested a SPE method for seven neonicotinoids in surface water. Prime HLB cartridge was selected for extraction process and pesticides were determined by LC/MS-MS.

14.2 All RESTEK custom standard mixtures were stored in the freezer and combination standards were stored in the refrigerator. After six months, several compounds in the combination standards had degraded, some by 50 percent when compared to freshly prepared standards, while the standards in the freezer showed no degradation. Therefore, all standards will be stored in the freezer. Prior to use, working standards will be diluted with HPLC grade water for analysis.

14.3 No substantial matrix suppression occurred during the method validation process using clean background surface water (American River surface water) provided by DPR. However, matrix suppression was observed in real samples analyzed previously.

14.4 A storage stability study was done with this project. The storage stability study consisted of a 0.5 ppb spike level and 3 replicates over a 28-day period in amber glass bottles. Three bottles containing background surface water were spiked and stored in the refrigerator until analyzed on 0, 2, 4, 7, 10, 14, 21 and 28 days. Along with the storage spikes, a matrix blank and a method control spike were also extracted. This storage study showed no degradation for all compounds until day 10, so the hold time for extraction of the surface water samples is 7 days from sampling. The results are shown in Appendix III.

14.5 Imidacloprid-d4 and Atrazine-d5 are used as surrogates. Extraction efficiency is monitored throughout the entire analytical method using a 0.05 ppb surrogate level in each sample.

14.6 The segment durations in the mass spectrometer settings determine the retention time windows for each analyte. As the HPLC column performance may change over time because of irreversible contamination, phase stripping, etc., it may be necessary to adjust these windows before beginning a sequence for the observed retention times of the analytes.

Installation of a new guard column or analytical column may also necessitate adjustments of window times. The retention time windows should be verified before each sequence and adjusted as necessary.

15. References:

15.1 Vanderford, B. J., Snyder, S. A., Analysis of Pharmaceuticals in Water by Isotope Dilution Liquid chromatography/Tandem Mass Spectrometry, Environ. Sci. Technol. 2006, 40, 7312-7320.

15.2 Donato, F. F, Martins, M. L., Munaretto, J. S., Prestes, O. D., Adaimem M. B., Zanella, R., Development of a Multiresidue Method for Pesticide Analysis in Drinking Water by Solid Phase Extraction and Determination by Gas and Liquid Chromatography with Triple Quadrupole Tandem Mass Spectrometry, J. Braz. Chem. Soc. 2015, 26, 2077-2087.

15.3 Carvalho, J. J., Jeronimo, P. C. A., Goncalves, C., Alpendurada, M. F., Evaluation of a Multiresidue Method for Measuring Fourteen Chemical Groups of Pesticides in Water by Use of LC-MS-MS, Anal. Bioanal. Chem. 2008, 392, 955-968.

Appendix I

MDL Study for Seven Neonicotinoids in Surface Water

Compound	Spike (ng/mL)	Spike 1	Spike 2	Spike 3	Spike 4	Spike 5	Spike 6	Spike 7	SD	MDL
Acetamiprid	0.01	0.00998	0.01026	0.00996	0.01078	0.01179	0.01032	0.01115	0.00068	0.00213
Clothianidin	0.01	0.00989	0.01001	0.00993	0.01012	0.00996	0.009415	0.01000	0.00023	0.00071
Dinotefuran	0.01	0.00765	0.00781	0.00736	0.00809	0.00758	0.00750	0.00769	0.00023	0.00074
Imidacloprid	0.01	0.01049	0.01039	0.00973	0.01120	0.01088	0.00955	0.01044	0.00058	0.00184
Sulfoxaflor	0.01	0.01018	0.01078	0.01023	0.01129	0.01075	0.01035	0.01111	0.00043	0.00137
Thiacloprid	0.01	0.01014	0.01074	0.01000	0.01152	0.01039	0.01074	0.01077	0.00051	0.00159
Thiamethoxam	0.01	0.00932	0.00944	0.00811	0.00996	0.00923	0.00900	0.00840	0.00063	0.00198
Atrazine-d5	0.01	0.00954	0.01015	0.00945	0.01054	0.01032	0.00972	0.00994	0.00041	0.00128
Imidacloprid-d4	0.01	0.01052	0.01121	0.01015	0.01145	0.01069	0.00983	0.00994	0.00062	0.00195

Appendix II

Method Validation Study for Seven Neonicotinoids in Surface Water

Compound	Spike Level (ng/mL)				Control Limit (%)	
Acetamiprid	**Set #**	**0.02**	**0.1**	**0.5**	**Mean**	103.8
	1	103.8	106.3	112.9	**SD**	8.75
	2	87.3	93.1	113.9	**UCL**	130.1
	3	109.6	103.2	104.5	**LCL**	77.6
Clothianidin	**Set #**	**0.02**	**0.1**	**0.5**	**Mean**	95.5
	1	105.9	96.1	109.6	**SD**	9.82
	2	77.0	86.3	91.2	**UCL**	125.0
	3	98.3	96.9	98.6	**LCL**	66.1
Dinotefuran	**Set #**	**0.02**	**0.1**	**0.5**	**Mean**	83.5
	1	91.0	72.3	100.7	**SD**	11.8
	2	74.0	69.7	85.9	**UCL**	119.0
	3	92.6	71.0	94.2	**LCL**	48.0
Imidacloprid	**Set #**	**0.02**	**0.1**	**0.5**	**Mean**	99.3
	1	113.2	96.1	108.6	**SD**	9.77
	2	82.0	95.6	92.2	**UCL**	128.6
	3	106.9	94.6	104.6	**LCL**	70.0
Sulfoxaflor	**Set #**	**0.02**	**0.1**	**0.5**	**Mean**	102.8
	1	103.8	108.9	114.5	**SD**	8.17
	2	96.9	94.7	115.2	**UCL**	127.3
	3	96.5	95.9	98.9	**LCL**	78.3

Compound	Spike Level (ng/mL)				Control Limit (%)	
Thiacloprid	**Set #**	**0.02**	**0.1**	**0.5**	**Mean**	104.2
	1	106.0	109.7	118.6	**SD**	8.33
	2	100.0	92.3	109.4	**UCL**	129.2
	3	107.5	94.0	100.5	**LCL**	79.2
Thiamethoxam	**Set #**	**0.02**	**0.1**	**0.5**	**Mean**	96.0
	1	102.3	101.0	106.8	**SD**	8.56
	2	78.4	100.8	97.0	**UCL**	121.6
	3	90.6	89.6	97.1	**LCL**	70.3
Atrazine-d5 (Surrogate)	**Set #**	**0.02**	**0.1**	**0.5**	**Mean**	101.2
	1	104.9	110.1	114.4	**SD**	9.18
	2	98.7	95.5	111.0	**UCL**	128.7
	3	91.3	89.4	95.3	**LCL**	73.7
Imidacloprid-d4 (Surrogate)	**Set #**	**0.02**	**0.1**	**0.5**	**Mean**	100.7
	1	109.5	101.8	109.3	**SD**	10.8
	2	78.0	95.6	102.4	**UCL**	133.1
	3	108.3	91.5	110.1	**LCL**	68.4

Appendix III

Storage Stability Study for Seven Neonicotinoids in Surface Water

Compound	Recovery (%)								
Acetamiprid	**Spike**	**Day 0**	**Day 2**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
	blk								
	QC Spk	103.8	104.4	100.1	103.7	105.4	104.3	111.8	114.3
	Spk 1	102.2	101.8	102.1	100.1	100.6	101.1	105.1	100.6
	Spk 2	106.6	103.3	104.5	103.0	103.7	98.4	101.7	105.6
	Spk 3	102.1	104.0	104.7	104.1	102.6	100.1	100.9	101.9
Clothianidin	**Spike**	**Day 0**	**Day 2**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
	blk								
	QC Spk	91.8	99.0	94.8	93.4	99.3	90.9	94.3	95.6
	Spk 1	80.7	85.1	98.5	95.6	91.4	90.4	96.0	88.3
	Spk 2	94.7	96.4	88.6	96.0	95.0	95.8	84.8	84.9
	Spk 3	99.3	95.0	96.5	99.2	95.8	86.1	82.0	80.2
Dinotefuran	**Spike**	**Day 0**	**Day 2**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
	blk								
	QC Spk	99.0	103.0	95.3	92.3	85.5	86.2	96.6	97.6
	Spk 1	99.0	103.1	104.0	97.8	77.6	76.8	79.7	80.0
	Spk 2	105.9	102.4	102.9	102.8	77.5	78.8	77.7	72.3
	Spk 3	105.6	106.3	100.5	105.5	75.8	74.9	74.1	69.8

Compound	Recovery (%)								
Imidacloprid	**Spike**	**Day 0**	**Day 2**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
	blk								
	QC Spk	102.3	107.0	102.7	100.9	106.7	107.1	102.3	108.7
	Spk 1	102.1	104.0	105.4	103.3	102.0	100.0	100.3	103.0
	Spk 2	112.8	103.5	106.7	105.3	102.3	101.3	93.8	96.1
	Spk 3	105.4	102.6	104.2	104.6	99.9	101.5	93.9	94.2
Sulfoxaflor	**Spike**	**Day 0**	**Day 2**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
	blk								
	QC Spk	103.0	106.4	100.7	106.7	112.3	103.0	107.1	109.0
	Spk 1	103.3	100.5	100.1	103.0	103.9	103.7	101.6	97.8
	Spk 2	114.9	104.1	106.5	102.4	107.2	105.4	98.1	99.5
	Spk 3	107.8	102.8	102.1	106.8	102.8	101.8	98.3	99.6
Thiacloprid	**Spike**	**Day 0**	**Day 2**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
	blk								
	QC Spk	108.2	107.2	105.1	105.2	111.3	107.9	110.1	102.2
	Spk 1	104.8	107.7	108.3	109.3	106.0	99.6	99.8	101.5
	Spk 2	111.4	111.9	113.5	105.6	104.8	101.2	100.8	102.5
	Spk 3	113.3	106.9	103.6	108.0	99.0	100.9	96.0	98.2
Thiamethoxam	**Spike**	**Day 0**	**Day 2**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
	blk								
	QC Spk	94.6	85.8	97.2	95.3	98.8	88.9	90.4	91.8
	Spk 1	87.4	87.3	96.3	97.6	96.0	91.3	91.8	90.1
	Spk 2	98.5	90.8	97.8	97.6	94.9	92.1	90.3	91.6
	Spk 3	97.9	97.2	96.9	99.5	89.6	92.6	80.2	80.8

Compound	Recovery (%)								
Atrazine-d5	**Spike**	**Day 0**	**Day 2**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
	blk								
	QC Spk	105.3	111.0	101.5	103.0	107.3	100.4	105.4	107.9
	Spk 1	99.9	110.0	110.2	104.5	93.9	92.8	101.3	95.5
	Spk 2	109.6	103.9	108.6	101.6	92.2	90.4	95.0	96.5
	Spk 3	106.1	107.2	105.6	106.0	89.9	91.6	91.9	93.3
Imidacloprid-d4	**Spike**	**Day 0**	**Day 2**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
	blk								
	QC Spk	104.2	105.2	101.7	103.7	106.7	100.3	102.1	103.9
	Spk 1	103.8	102.3	105.8	103.7	102.8	101.5	88.7	84.5
	Spk 2	109.7	100.9	104.0	99.9	103.7	95.5	83.7	84.4
	Spk 3	106.8	99.7	98.9	104.6	99.0	103.5	84.2	84.9

Written By:

Original signed by **8/22/2022**

Bahar Nakhjavan Date
Sr. Environmental Scientist (Specialist)

Approved By:

Original signed by **8/22/2022**

Jacob Oaxaca Date
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Approved By:

Original signed by **8/22/2022**

Maryam Khosravifard Date
Environmental Program Manager I

Approved By:

Original signed by **8/24/2022**

Sarva Balachandra Date
Quality Assurance Officer

Date	What was Revised? Why?