Determination of Atrazine, Bromacil, Diuron, Hexazinone, Metribuzin, Norflurazon, Prometon, Prometryn, Simazine, Desethylatrazine, Norflurazon Desmethyl, Desisopropylatrazine, Diaminochlorotriazine, Tebuthiuron and Clothianidin in Groundwater by Liquid Chromatography Triple Quadrupole Mass Spectrometry

1. Scope:

This Section Method provides a stepwise procedure for the analysis of Atrazine, Bromacil, Diuron, Hexazinone, Metribuzin, Norflurazon, Prometon, Prometryn, Simazine, Desethylatrazine (DEA), Norflurazon Desmethyl (DSMN), Desisopropylatrazine (ACET), Diaminochlorotriazine (DACT), Tebuthiuron and Clothianidin in groundwater. The objective of this standard operating procedure is to quantify the concentration of these analytes using liquid chromatography-triple quadrupole mass spectroscopy (LC/MS/MS). The reporting limit for all analytes is 0.02 ppb except for DSMN and Hexazinone. The reporting limit for DSMN and Hexazinone is 0.01 ppb.

2. Principle:

A Water Oasis ® MCX Cartridge (500 mg) is used to retain the analytes from groundwater samples. The cartridges are placed under vacuum to eliminate any remaining water. The analytes are eluted with 5% ammonium hydroxide in methanol. The eluant is concentrated, reconstituted in 1:3 methanol/water and analyzed by Liquid Chromatography Triple Quadrupole Mass Spectroscopy.

3. Safety:

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

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

3.3 All flammable solvents should be used and handled with care in a ventilated area.

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

4. Interference:

There were no known matrix interferences that caused quantitative problems during method development and validation.

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 Triple Quad 6500 mass spectrometer with Turbo V-Source, ESI probe, Varian vacuum pump, and Windows 10 Analyst 1.6.2 PC workstation or equivalent

5.2 LC/MS/MS Column: Waters Acquity UPLC HSS T3 1.8 µm, 2.1 x 100mm column (part # 186003539) or equivalent

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

5.4 Balance (Mettler PC 4400) or equivalent.

5.5 Vortex-vibrating mixer

5.6 Solid phase extraction manifold, Sapelo Visiprep TM24 or equivalent

5.7 Solid phase extraction manifold accessories: vacuum source, vacuum chamber, vacuum controller, cartridge fittings (tube adapters) and connectors, sample delivery tubing with stainless steel weight, sample collection tubes and rack

6. Reagents and Supplies:

6.1 Methanol (Fisher MS grade) or equivalent

6.2 Water (Fisher MS grade) or equivalent

6.3 Acetic acid (HPLC grade)

6.4 Ammonium hydroxide (reagent grade) or equivalent

6.5 Elution reagent: 5% ammonium hydroxide in methanol

6.6 Hydrochloric acid 25%

6.7 DI water

6.8 Reconstitution reagent: 1:3 methanol/water

6.9 Diluent for calibration standard: 1:1 methanol/water

6.10 Solid phase extraction cartridges: Waters Oasis® MCX 6 cc, (500 mg) cartridge

6.11 Graduated test tube, 15 mL (calibrated at 0.5 mL with methanol)

6.12 Glass beakers, 400 mL

7. Standards Preparation:

7.1 Standards

7.1.1	Diaminochlorotriazine (DACT)	CAS#3397-62-4
7.1.2	Desisopropylatrazine (ACET)	CAS#11007-28-9
7.1.3	Desethylatrazine (DEA)	CAS#6190-65-4
7.1.4	Metribuzin	CAS#21087-64-9
7.1.5	Bromacil	CAS#314-40-9
7.1.6	Atrazine	CAS#1912-24-9
7.1.7	Norflurazon	CAS#27314-13-2
7.1.8	Simazine	CAS#122-34-9
7.1.9	Hexazinone	CAS#51235-04-2
7.1.10	Diuron	CAS#330-54-1
7.1.11	Prometon	CAS#1610-18-0
7.1.12	Prometryn	CAS#7287-19-6
7.1.13	Propazine (Surrogate)	CAS#139-40-2
7.1.14	Norflurazon desmethyl (DSMN)	CAS#23576-24-1

7.1.15 Tebuthiuron CAS#34014-18-1

7.1.16 Clothianidin CAS#210880-92-5

7.2 DACT stock standard is purchased from Restek at a concentration of 100 µg/mL in methanol and diluted to 10.0 µg/mL (intermediate standard solution) with methanol.

7.3 Triazine stock standard mix of ACET, DEA, Prometon, Hexazinone, Prometryn, Simazine, Metribuzin, DSMN, Atrazine, Norflurazon, and Propazine is purchased from Restek at a concentration of 100 µg/mL in acetonitrile and diluted to 10.0 µg/mL (intermediate standard solution) with acetonitrile.

7.4 Triazine stock standard mix of Tebuthiuron and Diuron is purchased from Restek at a concentration of 100 µg/mL in methanol and diluted to 10.0 µg/mL (intermediate standard solution) with methanol.

7.5 Bromacil stock standard is purchased from Restek at a concentration of 100 µg/mL in acetonitrile and diluted to 10.0 µg/mL (intermediate standard solution) with acetonitrile.

7.6 Clothianidin stock standard is purchased from AcuuStandard at a concentration of 1000 µg/mL in Acetonitrile and diluted to 10.0 µg/mL (intermediate standard solution) with acetonitrile.

7.7 Propazine is purchased at a concentration of 1 mg/mL and then diluted to 1.0 ug/mL in methanol for spiking as a surrogate.

7.8 A combination standard of 1.0 µg/mL is prepared with 1:3 methanol/water from the combination of all the intermediate standard solutions of 10.0 µg/ml. The combination working standard is diluted from 1.0 µg/ml to the following concentrations: 0.5, 0.25, 0.1, 0.05, 0.025, 0.0125, 0.00625 µg/mL with 1:3 methanol/water.

7.9 The working standards of 1.0, 0.5, 0.25, 0.1, 0.05, 0.025, 0.0125, 0.00625 µg/ml are diluted with 1:1 methanol/water to prepare the following concentrations: 0.1, 0.05, 0.025, 0.01, 0.005, 0.0025, 0.00125, 0.000625 µg/mL for instrument calibration. The calibration standards should be prepared freshly every time before use.

7.10 Keep all standards in the designated refrigerator for storage.

7.11 The expiration date of each standard is one year from the preparation date or the expiration date of the stock standards whichever comes first.

8. Mobile Phase Preparation:

8.1 Aqueous Solution: 0.04% acetic acid in water: For 1000 mL water add 400 µL acetic acid.

8.2 Organic Solution: 0.04% acetic acid in methanol: For 1000 mL methanol, add 400 µL acetic acid.

9. Sample Preservation and Storage:

Store all samples waiting for extraction in a separate refrigerator (32-40 °F). The holding time for extraction of groundwater samples is 28 days from the date of sample collection. The holding time is based on a 28-day storage stability study where no degradation was observed through day 28. See Appendix III.

10. Test Sample Preparation:

10.1 Background Preparation

The Department of Pesticide Regulation provides background groundwater to be used in method validation and quality control (QC) samples.

10.2 Preparation of Matrix Blank and Matrix Spike

10.2.1 Matrix Blank: Weigh out 250 g of background water and follow the test sample extraction procedure (see Section 10.3).

10.2.2 Matrix Spike: Weigh out 250 g of background water. Spike 50 µL of 1.0 ug/mL of spiking solution into the background water and let it stand for 1 minute. Follow the test sample extraction procedure (see Section 10.3).

10.3 Test Sample Extraction

10.3.1 Remove sample from refrigerator and allow the sample to come to ambient temperature.

10.3.2 Weigh 250 ± 0.25 g of sample into a 400 mL glass beaker.

10.3.3 Add 50 µL of 1.0 ug/mL spiking solution of Propazine surrogate to each sample except the matrix blank. Note: the volume of methanol in spiking solution added to the sample should be 0.1% or less of the sample volume.

10.3.4 Adjust pH to 2.5 – 3.0 with 25% hydrochloric acid.

10.3.5 An MCX cartridge (500 mg) is connected to the vacuum manifold.

10.3.6 Condition the cartridge with total ~15 mL of methanol at a flow rate ~ 8 mL/minutes followed by ~ 15 mL of DI water by applying vacuum.

10.3.7 Turn off the vacuum when the DI water has just passed through the cartridge. Refill MCX cartridge with DI water. Attach the sample delivery tubes to the cartridge and attach the weighted tube ends.

10.3.8 Allow the sample to pass through the conditioned cartridges by applying vacuum. Adjust the flow rate to ~8 mL/minutes.

10.3.9 After all the sample has passed through the cartridges, increase the vacuum to ~ 20 psi for about 2 minutes. Detach the sample delivery tube from MCX cartridge. Shake out any excess water in the cartridge reservoir.

10.3.10 Place the graduated test tubes into the vacuum manifold under each corresponding SPE cartridge.

10.3.11 Elute and collect all chemicals with 15 ± 0.5 mL of 7-8% ammonium hydroxide in methanol at a flow rate of ~8 mL/minutes.

10.3.12 Concentrate the eluant to ~0.5 mL in a water bath at 38 ± 2 °C under a gentle stream of nitrogen. Bring to a final volume of 1.0 mL with reconstitution reagent (1:3, water/methanol). Vortex for 30 seconds. Transfer the extract into an autosampler vial for analysis by ESI/LC/MS/MS.

11. Instrument Analysis:

11.1 Instrument Calibration

11.1.1 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 0.1, 0.05, 0.025, 0.01, 0.005, 0.0025, 0.00125 and 0.000625 µg/ml.

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

11.2 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
- Matrix spike
- A set of up to 12 test samples
- A reagent blank; and
- A set of calibration standards (8 levels)

11.3 Instrument Conditions

11.3.1 LC/MS/MS Operating Conditions

11.3.1.1 LC Instrument: Shimadzu LC30

11.3.1.2 Column: Waters Acquity UPLC HSS T3 1.8 µm, 2.1 x 100 mm column

11.3.1.3 Column Temperature: 40 °C

11.3.1.4 Mobile Phase: Gradient

11.3.1.5 Solvent 1: 0.04% acetic acid in water

11.3.1.6 Solvent 2: 0.04% acetic acid in methanol

11.3.1.7 Injection Volume: 3.0 µL

Table 1. LC Gradient

Time (min)	Flow Rate (mL/min)	Mobile Phase A	Mobile Phase B
0.5	0.40	95.0	5.0
2.0	0.40	40.0	60.0
8.50	0.40	5.0	95.0
9.50	0.40	5.0	95.0
9.51	0.40	95.0	5.0

11.3.2 Mass Spectrometer Conditions

To achieve a mass spectrum, an AB Sciex Triple Quad 6500 with an ESI interface is used. See Table 2 for mass spectrometer parameters in positive mode. The mass spectrometer operates in positive scheduled Multiple Reaction Monitoring (MRM) mode as described in Table 3.

Table 2. Mass Spectrometer Operating Parameters

Parameter	Setting
Ion Mode	Positive (ES+)
Source Temp	150°C
Curtain Gas	25.0
Ion Spray Voltage	4500
Temp	450
Ion Source Gas 1	50
Ion Source Gas 2	50
Collision Gas	Medium

Table 3. MRM Parameters for Detection of Triazines and Clothianidin

Analyte	RT	Precursor Ion	Product Ion[1]	Declustering Potential	Collision Energy	Entrance Potential	Exit Potential
ACET	3.66	173.9	**95.9**	61	25	10	16
		173.9	103.8	61	29	10	16
Atrazine	5.12	215.8	**174**	56	23	10	22
		215.8	95.9	56	31	10	16
Bromacil	4.58	261	**205**	41	17	10	26
		261	187.9	41	37	10	20
DACT	2.50	145.8	**78.96**	51	23	10	18
		145.8	67.9	51	27	10	16
		145.8	103.9	51	25	10	16
DEA	4.02	187.9	**145.9**	46	23	10	20
		187.9	103.8	46	31	10	18
DSMN	4.91	289.9	**269.9**	176	31	10	16
		289.9	145	176	53	10	10
Diuron	5.25	233	**72.0**	56	21	10	12
		233	45.9	56	43	10	12
Hexazinone	4.57	252.9	**171**	46	21	10	22
		252.9	71	46	39	10	18
Metribuzin	4.60	214.9	**187.1**	76	23	10	26
		214.9	48.9	76	47	10	12
Norflurazon	5.22	303.8	**283.9**	101	31	10	36
		303.8	159.9	101	39	10	18
Prometon	5.05	225.9	**184**	81	25	10	24
		225.9	142	81	29	10	18
Prometryn	6.05	241.9	**157.9**	101	31	10	18
		241.9	200	101	25	10	30
Propazine	5.71	229.9	**188**	66	23	10	24
(Surrogate)		229.9	145.9	66	31	10	18
Simazine	4.59	201.9	**124**	66	23	10	16
		201.9	103.9	66	31	10	12
Tebuthiuron	4.66	228.9	**171.9**	56	23	10	20
		228.9	116	56	35	10	14

Analyte	RT	Precursor Ion	Product Ion[1]	Declustering Potential	Collision Energy	Entrance Potential	Exit Potential
Clothianidin	3.69	249.9	**168.9**	36	15	10	16
		249.9	131.9	36	19	10	16

[1] Quantitation ions are in bold

12. Quality Control:

12.1 Method Detection Limits

Method Detection Limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, seven groundwater samples are spiked at 0.01 ppb and processed through the entire method along with a matrix blank (see Section 10.3). The standard deviation derived from the spiked sample recoveries was used to calculate the MDL for each analyte using the following equation:

$$MDL = tS$$

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. Trace will be reported when results fall within the MDL and the reporting limit. The results for the standard deviations and MDL are in Appendix I.

12.2 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 in a range 1-5 times the MDL, as per client agreement. The RL for all analytes is 0.02 ppb except for DSMN and Hexazinone. The RL for DSMN and Hexazinone is 0.01 ppb.

12.3 Method Validation

The method validation consisted of 5 sample sets. Each set included 5 levels of fortification (0.01, 0.02, 0.05, 0.25, and 0.5 ppb). All spikes and matrix blanks were processed through the entire analytical method (see Section 10.3). Spike levels and recovery data is shown in Appendix II.

12.4 Control Charts and Limits

Control charts were generated using the data from the method validation for each analyte. The upper and lower warning and control limits are set at ± 2 and 3 standard deviations of the % recovery, respectively, as shown in Appendix II.

12.5 Acceptance Criteria

12.5.1 Each set of samples will have a matrix blank and a matrix spike sample.

12.5.2 The retention time of the analyte must be within ± 0.1 minute of each analyte within the same sequence.

12.5.3 The recoveries of the matrix spikes shall be within the control limits. When spike recoveries fall outside the limits, the analyst must investigate the cause. Samples within any bracket that contains non-conforming matrix spikes are re-analyzed. If the spike fails again, then the bracketed samples must be re-extracted and re-analyzed. If the spike recoveries fall within the control limits, then the results from the re-analyzed samples can be reported.

12.5.4 The sample shall be diluted if results fall outside of the calibration curve. Calculated concentrations must be less than the highest-level standard concentration. Dilution does not change the reporting limit or method detection limit. It also reduces matrix interferences in samples with a high matrix effect.

12.5.5 Bracketing standard curves should have a percent change ≤ 20%.

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

12.5.7 Analyze a reagent blank to demonstrate that the system is clean and free of interferences.

13. Calculations:

Quantitation is based on an external standard calculation using either the peak area or height. The LC/MS/MS software used a quadratic curve fit, with all levels weighted equally. 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.

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

14. Reporting Procedure:

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

15. Discussion:

15.1 The Department of Pesticide Regulations requested to add Clothianidin.

15.2 This method is updated to add Clothianidin and reduce the sample volume from 500 mL to 250 mL. Method validation consisted of 5 sample sets. Each set included 5 levels of fortification and a method blank. The MDL spikes consisted of 7 replicates. All spikes, matrix blanks, and MDL spikes were processed through the extraction method and analyzed. See Appendix II.

15.3 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 glass bottles containing background groundwater were spiked and stored in the refrigerator until analyzed on day 0, 2, 4, 8, 14, 21 and 28. A matrix blank and a matrix spike were also extracted. The storage stability study showed no degradation for these analytes until day 28. The results are shown in Appendix III.

15.4 Propazine is used as a surrogate. Spike propazine to each sample and process through the entire analytical method. This allows the extraction steps to be monitored.

15.5 The segment durations in the mass spectrometer settings determine the retention time windows for each analyte. As the LC 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 analytical column may also necessitate adjustments of retention time windows. The retention time windows should be verified before each sequence and adjusted as necessary.

16. References:

Determination of Atrazine, Bromacil, Cyanazine, Diuron, Hexazinone, Metribuzin, Norflurazon, Prometon, Prometryn, Simazine, Desethyl Atrazine (DEA), Deisopropyl Atrazine (ACET), Diamino Chlorotraizine (DACT), Tebuthiuron and the metabolites Tebuthiuron-104, Tebuthiuron-106, Tebuthiuron-107 and Tebuthiuron-108 in Well Water by MCX extraction and Liquid Chromatography- triple quadrupole mass spectrometry, EM 62.9 Revision: 5 of 5/4/2020.

Appendix I

Method Detection Limit and Reporting Limit

Analyte	Spike (ppb)	Spike 1	Spike 2	Spike 3	Spike 4	Spike 5	Spike 6	Spike 7	SD	MDL	RL
ACET	0.01	0.00688	0.00682	0.00829	0.00787	0.00748	0.00768	0.00822	0.000589	0.00185	0.02
Atrazine	0.01	0.00703	0.00769	0.00846	0.00725	0.00779	0.00867	0.00913	0.000773	0.00243	0.02
Bromacil	0.01	0.00732	0.00742	0.00855	0.00790	0.00777	0.00836	0.00934	0.000710	0.00223	0.02
DACT	0.01	0.00745	0.00865	0.00871	0.00775	0.00880	0.00886	0.00934	0.000665	0.00209	0.02
DEA	0.01	0.00711	0.00730	0.00770	0.00707	0.00743	0.00760	0.00838	0.000448	0.00141	0.02
DSMN	0.01	0.00738	0.00711	0.00877	0.00776	0.00775	0.00819	0.00973	0.000899	0.00283	0.01
Diuron	0.01	0.00754	0.00722	0.00801	0.00785	0.00788	0.00819	0.00893	0.000538	0.00169	0.02
Hexazinone	0.01	0.00744	0.00736	0.00837	0.00743	0.00791	0.00825	0.00878	0.000553	0.00174	0.01
Metribuzin	0.01	0.00703	0.00777	0.00802	0.00819	0.00974	0.00834	0.00974	0.001005	0.00316	0.02
Norflurazon	0.01	0.00740	0.00760	0.00882	0.00802	0.00838	0.00873	0.00950	0.000737	0.00232	0.02
Prometon	0.01	0.00712	0.00702	0.00811	0.00766	0.00773	0.00810	0.00935	0.000780	0.00245	0.02
Prometryn	0.01	0.00753	0.00872	0.00818	0.00715	0.00804	0.00843	0.00959	0.000798	0.00251	0.02
Propazine (Surrogate)	0.01	0.00735	0.00733	0.00870	0.00761	0.00798	0.00855	0.00948	0.000802	0.00252	0.02
Simazine	0.01	0.00680	0.00684	0.00897	0.00747	0.00769	0.00791	0.00875	0.000849	0.00267	0.02
Tebuthiuron	0.01	0.00767	0.00780	0.00881	0.00785	0.00816	0.00847	0.00922	0.000578	0.00182	0.02
Clothianidin	0.01	0.00756	0.00736	0.00844	0.00785	0.00703	0.00753	0.00780	0.000443	0.00139	0.02

Appendix II

Method Validation Data and Control Limits

Analyte	Spike Level (ppb)						Control Limit	%
	Set #	0.01	0.02	0.05	0.25	0.5		
ACET	1	70.27	75.60	84.91	79.46	74.26	**Mean**	81.8
	2	93.92	87.04	90.96	86.03	87.47	**SD**	6.63
	3	79.18	84.44	90.41	74.15	84.96	**RSD**	8.11
	4	73.89	73.05	77.14	80.25	71.06	**UCL**	102
	5	82.41	86.10	85.67	84.02	87.35	**LCL**	61.9
Atrazine	1	75.01	78.16	83.30	78.43	71.55	**Mean**	82.1
	2	92.73	82.93	84.53	83.47	86.90	**SD**	4.96
	3	84.46	78.78	85.15	75.95	84.95	**RSD**	6.05
	4	84.51	78.43	81.09	83.62	73.18	**UCL**	97.0
	5	89.12	84.52	81.18	85.05	86.14	**LCL**	67.2
Bromacil	1	74.17	76.76	88.39	84.57	78.31	**Mean**	85.2
	2	95.73	83.09	87.66	84.47	90.31	**SD**	5.04
	3	84.03	87.27	91.19	83.68	88.36	**RSD**	5.92
	4	79.81	82.77	86.25	87.81	77.26	**UCL**	100
	5	89.01	86.11	84.21	87.26	90.78	**LCL**	70.0
DACT	1	84.54	79.01	86.72	92.42	82.60	**Mean**	90.7
	2	95.92	91.70	95.38	92.34	97.70	**SD**	5.55
	3	90.51	88.84	98.96	90.95	93.61	**RSD**	6.12
	4	93.99	90.34	96.12	96.03	84.31	**UCL**	107
	5	85.71	94.06	79.04	94.06	93.15	**LCL**	74.1
DEA	1	73.51	72.19	78.27	79.06	72.33	**Mean**	79.8
	2	89.36	85.18	85.54	86.12	85.74	**SD**	5.68
	3	80.42	76.41	83.59	74.33	79.27	**RSD**	7.12
	4	74.32	71.77	75.70	79.67	70.98	**UCL**	96.9
	5	88.56	82.54	80.85	85.14	85.34	**LCL**	62.8

Analyte	Spike Level (ppb)						Control Limit	%
	Set #	0.01	0.02	0.05	0.25	0.5		
DSMN	1	77.98	77.96	83.91	82.34	77.01	**Mean**	85.1
	2	98.82	89.24	89.68	87.77	91.60	**SD**	5.22
	3	85.68	82.67	84.92	80.02	84.33	**RSD**	6.13
	4	87.59	79.31	84.01	88.63	77.8	**UCL**	101
	5	89.76	86.94	82.66	87.87	90.01	**LCL**	69.5
Diuron	1	76.34	81.54	85.24	84.34	75.83	**Mean**	84.1
	2	92.96	86.22	86.08	85.49	89.43	**SD**	5.19
	3	83.51	84.26	89.71	80.31	82.03	**RSD**	6.18
	4	78.25	77.28	80.73	83.07	73.91	**UCL**	99.7
	5	90.65	87.72	86.11	90.05	91.23	**LCL**	68.5
Hexazinone	1	77.95	78.55	85.82	83.22	76.63	**Mean**	82.1
	2	92.12	84.51	85.12	84.05	88.73	**SD**	4.43
	3	85.92	81.86	85.21	79.15	85.32	**RSD**	5.39
	4	76.32	76.59	80.77	83.42	72.07	**UCL**	95.3
	5	83.18	81.23	78.18	81.71	83.99	**LCL**	68.8
Metribuzin	1	76.70	79.36	83.52	81.74	74.66	**Mean**	84.2
	2	103.03	88.35	87.79	84.66	87.43	**SD**	7.52
	3	82.27	81.19	86.56	77.30	84.83	**RSD**	8.94
	4	77.54	78.02	86.78	86.42	73.01	**UCL**	107
	5	106.37	85.57	85.57	82.59	82.98	**LCL**	61.6
Norflurazon	1	75.37	81.32	87.78	84.37	78.72	**Mean**	85.3
	2	95.51	88.88	88.51	87.50	91.30	**SD**	4.92
	3	89.83	85.84	86.26	79.41	83.45	**RSD**	5.77
	4	82.26	81.4	81.93	86.35	77.23	**UCL**	100
	5	90.28	87.38	82.51	87.59	91.96	**LCL**	70.6
Prometon	1	73.59	79.42	80.35	81.13	71.61	**Mean**	81.3
	2	86.79	82.85	83.72	82.95	86.89	**SD**	4.01
	3	83.09	79.77	86.39	75.93	80.96	**RSD**	4.93
	4	82.85	79.05	81.44	83.7	74.68	**UCL**	93.3
	5	83.17	83.95	79.94	82.67	86.17	**LCL**	69.3

Analyte	Spike Level (ppb)						Control Limit	%
	Set #	0.01	0.02	0.05	0.25	0.5		
Prometryn	1	75.02	94.46	91.64	80.39	72.65	**Mean**	82.9
	2	87.77	81.69	81.94	82.91	86.29	**SD**	5.25
	3	83.65	78.52	83.28	75.58	81.24	**RSD**	6.33
	4	85.36	80.34	83.83	87.96	75.36	**UCL**	98.6
	5	90.11	81.95	80.67	84.03	85.89	**LCL**	67.2
Propazine (Surrogate)	1	75.06	78.17	83.15	81.35	71.84	**Mean**	83.6
	2	94.35	85.11	85.77	84.00	87.26	**SD**	5.20
	3	85.81	82.06	88.38	77.64	84.40	**RSD**	6.21
	4	82.93	79.17	84.1	86.48	76.22	**UCL**	99.2
	5	91.91	87.50	83.02	86.76	88.29	**LCL**	68.0
Simazine	1	74.41	77.44	81.22	82.82	74.84	**Mean**	80.7
	2	88.93	79.25	80.60	86.22	89.29	**SD**	4.73
	3	78.81	77.65	83.17	76.34	84.69	**RSD**	5.86
	4	71.59	81.12	83.88	84.2	72.52	**UCL**	94.9
	5	80.26	77.21	81.53	84.13	85.47	**LCL**	66.5
Tebuthiuron	1	78.96	80.58	86.32	84.77	78.23	**Mean**	85.1
	2	94.84	88.53	89.02	83.64	90.75	**SD**	4.53
	3	84.78	87.89	88.16	81.74	85.47	**RSD**	5.33
	4	83.69	80.16	81.1	84.99	74.93	**UCL**	98.7
	5	88.82	85.45	84.91	89.49	89.87	**LCL**	71.5
Clothianidin	1	75.85	72.96	77.88	76.80	70.52	**Mean**	78.0
	2	87.41	81.63	82.27	83.37	86.30	**SD**	5.32
	3	79.14	78.06	81.32	74.51	77.35	**RSD**	6.82
	4	73.76	67.98	72.74	75.07	68.46	**UCL**	94.0
	5	85.99	82.86	77.70	79.54	81.77	**LCL**	62.1

Definitions

SD = Standard deviation
RSD=Relative Standard deviation
UCL=Upper control limit
LCL=Lower control limit

Appendix III

Storage Stability Study Data

Analyte	Sample type / Spike level	% Recovery						
		Day 0	Day 2	Day 4	Day 8	Day 14	Day 21	Day 28
ACET	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	82.6	85.2	83.5	87.2	78.8	79.8	83.5
	Spk 1 @ 0.5 ng/ml	91.0	86.6	78.4	81.7	77.8	77.8	75.2
	Spk 2 @ 0.5 ng/ml	88.1	85.7	81.4	88.0	79.5	85.3	81.9
	Spk 3 @ 0.5 ng/ml	88.0	86.6	83.6	87.9	81.3	81.4	84.1
Atrazine	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	82.4	86.1	87.1	88.0	80.0	86.1	83.7
	Spk 1 @ 0.5 ng/ml	90.8	88.1	76.9	80.9	79.1	78.1	73.5
	Spk 2 @ 0.5 ng/ml	88.2	86.1	82.6	83.8	80.4	87.8	81.1
	Spk 3 @ 0.5 ng/ml	86.7	88.8	84.9	91.5	82.8	83.3	81.2
Bromacil	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	82.9	87.1	81.4	85.8	83.8	86.7	91.1
	Spk 1 @ 0.5 ng/ml	90.0	86.0	76.1	79.5	79.7	82.0	80.0
	Spk 2 @ 0.5 ng/ml	88.7	86.6	80.6	82.7	84.7	94.5	87.6
	Spk 3 @ 0.5 ng/ml	86.2	85.8	83.1	84.8	86.0	86.5	92.6
DACT	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	71.95	73.05	69.88	71.04	69.82	71.74	72.3
	Spk 1 @ 0.5 ng/ml	79.98	72.92	65.7	65.45	69.01	68.1	68.0
	Spk 2 @ 0.5 ng/ml	76.74	71.52	67.79	70.28	70.57	77.45	75.6
	Spk 3 @ 0.5 ng/ml	75.09	72.32	72.19	71.4	72.56	68.23	77.3
DEA	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	80.7	82.9	83.1	85.3	77.8	80.2	83.3
	Spk 1 @ 0.5 ng/ml	88.9	86.1	78.3	80.1	78.7	77.1	74.7
	Spk 2 @ 0.5 ng/ml	86.9	83.0	84.8	85.7	79.8	86.8	79.5
	Spk 3 @ 0.5 ng/ml	84.4	86.4	84.9	86.3	81.0	83.2	85.1
DSMN	QC Spk @ 0.5 ng/ml	85.3	85.4	86.7	89.6	85.8	84.6	90.3
	Spk 1 @ 0.5 ng/ml	96.2	87.5	79.7	81.5	80.3	77.9	81.7
	Spk 2 @ 0.5 ng/ml	94.6	88.5	85.2	88.7	88.5	90.4	87.7
	Spk 3 @ 0.5 ng/ml	93.3	87.7	88.2	89.5	89.6	84.8	89.6

Analyte	Sample type / Spike level	% Recovery						
		Day 0	Day 2	Day 4	Day 8	Day 14	Day 21	Day 28
Diuron	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	89.2	84.1	84.6	84.8	83.0	87.3	84.3
	Spk 1 @ 0.5 ng/ml	97.0	84.9	79.5	80.6	81.2	79.6	79.1
	Spk 2 @ 0.5 ng/ml	93.3	82.8	83.7	86.1	86.4	90.7	85.9
	Spk 3 @ 0.5 ng/ml	92.1	83.6	87.1	88.6	87.6	82.8	85.8
Hexazinone	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	83.9	83.9	82.7	83.9	81.9	86.4	88.7
	Spk 1 @ 0.5 ng/ml	85.9	85.7	77.2	79.0	76.7	80.5	78.8
	Spk 2 @ 0.5 ng/ml	85.7	83.7	80.9	83.0	82.1	90.9	83.9
	Spk 3 @ 0.5 ng/ml	86.9	83.8	84.6	85.6	82.6	85.5	90.7
Metribuzin	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	80.9	87.0	82.1	82.3	85.5	80.7	85.4
	Spk 1 @ 0.5 ng/ml	89.8	86.6	76.0	79.6	81.8	77.1	73.9
	Spk 2 @ 0.5 ng/ml	87.8	89.0	80.6	86.3	83.6	87.1	87.5
	Spk 3 @ 0.5 ng/ml	87.0	89.2	84.6	83.8	84.3	83.0	86.7
Norflurazon	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	87.4	89.0	87.6	89.6	87.1	89.0	91.5
	Spk 1 @ 0.5 ng/ml	97.5	89.1	80.4	81.6	82.5	79.6	81.9
	Spk 2 @ 0.5 ng/ml	94.2	87.1	84.7	87.8	85.4	90.7	88.7
	Spk 3 @ 0.5 ng/ml	92.4	88.8	87.3	88.8	87.3	84.0	89.1
Prometon	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	82.9	86.0	82.0	80.5	83.3	92.2	89.4
	Spk 1 @ 0.5 ng/ml	87.6	86.7	75.7	78.0	79.1	82.8	76.3
	Spk 2 @ 0.5 ng/ml	85.6	86.0	80.1	82.8	82.1	96.0	89.2
	Spk 3 @ 0.5 ng/ml	83.2	89.5	81.0	82.7	83.7	90.0	86.8
Prometryn	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	83.9	90.0	85.7	89.3	84.2	90.2	86.0
	Spk 1 @ 0.5 ng/ml	87.8	90.1	80.3	77.8	74.7	73.1	74.4
	Spk 2 @ 0.5 ng/ml	89.8	87.3	83.8	86.5	80.9	89.9	85.2
	Spk 3 @ 0.5 ng/ml	86.4	90.5	85.7	88.3	79.8	81.2	83.5

Analyte	Sample type / Spike level	% Recovery						
		Day 0	Day 2	Day 4	Day 8	Day 14	Day 21	Day 28
Propazine (Surrogate)	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	84.5	89.4	84.0	85.0	81.5	86.2	85.5
	Spk 1 @ 0.5 ng/ml	93.4	91.0	77.6	77.8	79.1	77.0	74.3
	Spk 2 @ 0.5 ng/ml	91.0	86.8	82.0	83.6	81.5	88.7	83.7
	Spk 3 @ 0.5 ng/ml	88.8	89.4	84.3	85.2	83.0	82.5	83.0
Simazine	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	82.6	86.6	83.0	84.7	81.5	81.7	85.5
	Spk 1 @ 0.5 ng/ml	90.9	87.2	76.9	77.1	81.4	77.4	73.9
	Spk 2 @ 0.5 ng/ml	89.6	88.3	81.5	84.5	81.8	89.4	82.9
	Spk 3 @ 0.5 ng/ml	87.0	87.5	84.0	85.5	83.5	84.3	81.9
Tebuthiuron	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	86.2	83.0	82.1	84.8	79.1	85.4	86.6
	Spk 1 @ 0.5 ng/ml	92.3	85.7	77.5	78.8	79.4	81.9	78.5
	Spk 2 @ 0.5 ng/ml	91.0	85.2	80.2	85.5	82.7	89.9	85.2
	Spk 3 @ 0.5 ng/ml	87.4	84.8	83.3	87.8	83.3	86.3	86.2
Clothianidin	Blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk @ 0.5 ng/ml	75.4	75.7	76.8	84.8	77.9	82.5	77.5
	Spk 1 @ 0.5 ng/ml	84.3	76.8	72.6	76.6	76.5	80.4	70.3
	Spk 2 @ 0.5 ng/ml	82.2	75.8	78.2	80.5	80.7	87.9	76.0
	Spk 3 @ 0.5 ng/ml	79.9	77.7	81.3	81.7	79.6	83.4	77.7

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Revision Log:

Date	What was revised? Why?
9/10/07	Add data for Desmethyl Norflurazon in groundwater.
1/09/09	Add data for Tebuthiuron and metabolites in groundwater.
1/27/20	Removed Waters and Finnigan validation results.
	Removed Section 11.2 Waters and Finnigan LCMS Instrument conditions Adde ABSciex Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer.
	Added Sciex 6500 validation results.
5/13/20	Added Sciex MDL results.
2/01/23	Tile name has been updated to include new analyte and removal of Tebuthiuron metabolites.
	Updated scope section on reporting limit.
	Well water wording replaced with groundwater.
	Included more details in principle section.
	Safety section was updated to include more information.
	Section 5, added type of LC system and column ID.
	Section 6, changed B & J to Fisher brand. Hydrochloric acid changed to percentage. Added diluent for calibration standard. Removed filtration steps. Add glass beaker 400mL.
	Section 7, removed stock standard obtained from CDFA/CAC standard repository. 7.2-7.9 added more details on new vendor and standard preparation.
	Added mobile phase preparation.
	Section 9, 28 day storage stability study added.
	Section 10, updated 500g to 250g. Also included spike amount and new acceptance criteria for weigh amount. 10.3.5, mcx cartridge updated from 150mg to 500mg. 10.3.7, removed type I to DI water. Also added “attach the weighted tube ends”. 10.3.10, more clarification was added. 10.3.11, removed 5% and updated to 7-8% ammonium hydroxide. 10.3.12, wording changed.
	Section 11, minimum changed from 3 to 5 levels for calibration curve and add its correlation coefficient value. 11.2, added sequence arrangement. 11.3, added LC/MS/MS operating conditions section.
	Updated section 12 in quality control. Acceptance criteria and reagent blank has also been added.
	Added new analyte of Clothianidin, removed Tebuthiuron metabolites, reduced sample volume from 500 mL to 250 mL, added new validation, MDL and storage stability study data results.