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Determination of Selected Rice Herbicides in Groundwater

1. Scope:

This section method (SM) is applicable to the analysis of Bensulfuron-methyl, Bispyribac sodium, Clomazone, Halosulfuron methyl, MCPA, Molinate, Orthosulfamuron, Penoxsulam, Propanil, Propiconazole, Thiobencarb and Triclopyr in groundwater. It is followed by all authorized EMON personnel.

2. Principle:

Groundwater sample (500 mL) is acidified with 3 N Hydrochloric acid. A solid phase extraction HLB cartridge is used to retain the selected rice herbicides from acidified water samples. The analytes are eluted with acetonitrile/methanol (50:50) solution. The eluant is concentrated and analyzed by Liquid Chromatography coupled to a Linear Ion Trap Quadrupole LC/MS/MS. The reporting limit is 0.05 ppb for all compounds.

3. Safety:

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

4. Interferences:

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

5. Apparatus and Equipment:

5.1 Nitrogen Evaporator (Meyer N-EVAP Organomation Model #112 or equivalent)
5.2 Balance (Mettler PC 4400 or equivalent)
5.3 Vortex-vibrating mixer
5.4 Solid phase extraction manifold, Supelco Visiprep TM24 or equivalent
5.5 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.
5.6 pH meter Mettler-Toledo or equivalent
5.7 Ultra High-Performance Liquid Chromatography (UHPLC) equipped with a linear ion trap quadrupole (MS/MS)

6. Reagents and Supplies:

6.1	Bensulfuron-methyl	CAS# 83055-99-6
6.2	Bispyribac sodium	CAS# 125401-92-5
6.3	Clomazone	CAS# 81777-89-1
6.4	Halosulfuron-methyl	CAS#100784-20-1
6.5	MCPA	CAS#94-74-6
6.6	Molinate	CAS#2212-67-1
6.7	Orthosulfamuron	CAS#213464-77-8
6.8	Penoxsulam	CAS#219714-96-2
6.9	Propanil	CAS#709-98-8
6.10	Propiconazole	CAS#60207-90-1
6.11	Thiobencarb	CAS#28249-77-6
6.12	Triclopyr	CAS#55335-06-3
6.13	2,4,5 T	CAS#93-76-5
6.14	Simazine-d10	CAS#220621-39-6
6.15	Diclofenac-d4 (IS)	CAS#153466-65-0

6.16 Methanol, MS grade, Fisher Optima LC/MS or equivalent
6.17 Water, MS grade, Fisher Optima LC/MS or equivalent
6.18 Acetonitrile, Fisher Optima or equivalent
6.19 Hydrochloric acid 3 N
6.20 Pipettes; air cushioned and positive displacement, various volumes and types, Eppendorf or equivalent
6.21 Disposable Pasteur pipettes and other laboratory ware as needed
6.22 Solid phase extraction cartridges: Waters Oasis® HLB 6 cc (200 mg),
6.23 Graduated test tube, 15 mL (calibrated at 0.5mL with methanol)
6.24 LCMS Columns:
Analytical column: ACE Excel C18 2.0 µm, 2.1 x 100mm column or equivalent
6.25 Formic acid, HPLC grade
6.26 Aqueous Solution: Water with 0.1% Formic acid
6.27 Organic Solution: Methanol/Acetonitrile (50/50) with 0.1% Formic acid

7. Standards Preparation:

7.1 Individual stock standards of 1.0mg/mL were obtained from the CDFA/CAC Standards Repository. Standards are ordered from a 17034 accredited supplier when possible.

The standards were diluted to 10 µg/mL with acetonitrile. A combination standard of 10 µg/mL was prepared from the individual mg/mL standards in acetonitrile. The combination standard was also used to dilute to the following concentrations: 0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 µg/mL in acetonitrile which were later diluted 1:10 with clean background matrix for instrument calibration.

7.2 Keep all standards in the designated freezer for storage.

7.3 The expiration date of each standard is six months from the preparation date.

8. Sample Preservation and Storage:

Store all samples waiting for extraction in a designated refrigerator (4 ± 3 °C). Sample storage location and movement shall be recorded.

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the groundwater for background to be used in method validation and QC.

9.2 Preparation of blank and spike

Matrix blank: Weigh out 500 g of background water and follow the test sample extraction procedure.

Matrix spike: Weigh out 500 g of background water. Spike a client requested amount of pesticide (0.1 – 0.2 ppb) into the background water, mix well and let it stand for one minute. Follow the test sample extraction procedure.

9.3 Test Sample Extraction

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

9.3.2 Weigh 500 ± 0.5 g of water sample into a 600 mL beaker.

9.3.3 Add 0.1 µg 2,4,5 T / Simazine-d10 (100 µL of 1 ng/µL spiking solution) as a surrogate to each sample except blank.

9.3.4 Adjust pH to 2.0 – 2.5 with 3 N HCL.

9.3.5 Connect an HLB cartridge (200mg) to the vacuum manifold.

9.3.6 Condition the cartridge with ~5 mL of methanol at a flow rate ~ 8 mL/minutes followed by ~ 5 mL of acidified D.I. water (~ pH 2) by applying vacuum.

9.3.7 Turn off the vacuum when the D.I. water has just passed through the cartridges. Refill HLB cartridges with acidified D.I. water. Attach the sample delivery tubes to the cartridge and place weighted tube ends into water sample.

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

9.3.9 After all the water sample has passed through the cartridges, increase the vacuum to ~ 20 psi for 10 minutes. Detach the sample delivery tube from HLB cartridge. Shake out any excess water in the cartridge reservoir and dry inside of cartridge with Kimwipe.

9.3.10 Place the graduated test tubes into the vacuum manifold.

9.3.11 Elute and collect all analytes with 5 mL of acetonitrile/methanol (50/50) solution and at a flow rate of ~8 mL/minutes. Concentrate the eluant to ~1.8 mL in a water bath at 38 ± 2 °C under a gentle stream of nitrogen. Record the temperature into extraction sheet. Bring to a final volume of 2.0 mL with acetonitrile/methanol (50/50) solution.

9.3.13 Transfer the extract into an autosampler vial to be analyzed by ESI/LC/MS/MS.

10. Instrument Calibration:

10.1 A minimum of 3 levels of standards is required for linear curve.

10.2 The quadratic calibration standard curve consists of a minimum of five levels.

The recommended concentration levels of standards are 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 µg/mL.

10.3 The calibration may be achieved using linear or quadratic regression with a correlation coefficient (r) ≥ 0.995 or (r^2) ≥ 0.990.

11. Analysis:

11.1 Injection Scheme

The instrument may need to be conditioned with a few standards before running the following sequence of Standard Curve, Solvent, Matrix Blank, Matrix Spike, Test Samples and Standard Curve.

11.2 Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer

11.1.1 LC Instrument: Shimadzu LC30
Column: ACE Excel C18 2.0 µm, 2.1 x 100 mm column
Column Temperature: 40 °C
Mobile Phase: Gradient
Solvent 1: Aqueous Solution
Solvent 2: Organic Solution

Time (min)	Flow rate (mL/min)	Solvent 1	Solvent 2
0.01	0.3	65	35
2.0	0.3	65	35
12.0	0.3	15	85
15.0	0.3	15	85
16.0	0.3	65	35
17.0	0.3	65	35

Injection Volume: 3.0 µL

11.2.2 Mass Spectrometer and Operating Parameters

Model:	ABSciex QTRAP 6500
Experiment 1	
Ion ProbeType:	Electrospray Ionization (ESI)
Ion Mode:	Positive

Curtain Gas:	30
Ion Spray Voltage:	4500
Temp:	350
Ion Source Gas 1	40
Ion Source Gas 2	40
Collision Gas:	High

Compound	RT	Precursor Ion	Product Ion	Declustering Potential	Collision Energy	Entrance Potential	Exit Potential
Bensulfuron-methyl	8.14	410.9	**149**	66	25	10	18
		410.9	182	66	25	10	24
Bispyribac sodium	8.86	430.9	**275**	51	17	10	14
		430.9	413	51	23	10	20
Clomazone	7.95	240	**125**	26	23	10	14
		240	89.1	26	65	10	10
Halosulfuron methyl	9.52	434.9	**182**	61	25	10	22
		434.9	138.9	61	63	10	22
Molinate	9.14	187.9	**126**	41	17	10	16
		187.9	55	41	35	10	14
Orthosulfamuron	7.38	424.9	**199**	51	17	10	26
		424.9	226.9	51	19	10	30
Penoxsulam	7.28	483.9	**195**	80	37	10	26
		483.9	443.9	80	33	10	20
Propanil	8.22	217.8	**161.9**	56	21	10	18
		217.8	126.9	56	33	10	16
Propiconazole	11.06	341.9	158.9	56	31	10	20
		341.9	69	56	23	10	12
Thiobencarb	11.51	257.9	**124.9**	41	23	10	16
		257.9	89	41	65	10	14
simazine-d10 (surrogate)	4.52	212.1	**136.8**	86	27	10	16
		212.1	104.9	86	35	10	16
Diclofenac-d4 (internal std)	10.25	299.8	**218.9**	31	27	10	28
		299.8	253.9	31	19	10	32

Experiment 2

Ion ProbeType:	Electrospray Ionization (ESI)
Ion Mode:	Negative
Curtain Gas:	30
Ion Spray Voltage:	-4500
Temp:	350
Ion Source Gas 1	40
Ion Source Gas 2	40
Collision Gas:	High

Compound	RT	Precursor Ion	Product Ion	Declustering Potential	Collision Energy	Entrance Potential	Exit Potential
MCPA	7.54	199	**141**	-25	-18	-10	-9
		199	155	-25	-12	-10	-9
Triclopyr	8.13	254	**195.9**	-10	-14	-10	-11
		254	217.8	-10	-8	-10	-13
2,4,5 T(surrogate)	8.93	253	**194.8**	-10	-16	-10	-11
		253	158.8	-10	-38	-10	-11
Diclofenac-d4 (internal std)	10.25	298	**254**	-20	-16	-10	-15
		298	217.1	-20	-28	-10	-13

12. Quality Control:

12.1 Method Detection Limits (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 groundwater samples were spiked at 0.1ppb for the selected rice herbicides and processed through the entire method along with a blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL using the following equation:

MDL = tS

Where t is the Student single tailed 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. The results for the standard deviations and MDL are in Appendix 1.

12.2 Reporting Limit (RL)

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 requirement. The reporting limits for the selected rice herbicides are 0.05ppb.

12.3 Method Validation

The method validation consisted of 5 sample sets in background matrix. Each set included five levels of fortification for the selected rice herbicides (0.10, 0.25, 0.5, 1.25 and 2.5 ppb) and a method blank. All spikes and method blanks are processed through the entire analytical method. Recoveries for the selected rice herbicides are tabulated in Appendix 2.

12.4 Control Charts and Limits

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

12.5 Acceptance Criteria

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

12.5.2 For positive results the retention time shall not vary from standards more than ± 0.1 minute.

12.5.3 Presence of both Qual and Quan ion.

12.5.4 The recoveries of the matrix spikes shall be within the control limits. See Appendix2.

12.5.5 The sample shall be diluted if result exceed 10% of the highest calibration standard on the curve.

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

13. Calculations:

Quantitation is based on an external standard (ESTD) calculation using either the peak area or height. The software uses linear or quadratic curve fit. 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.

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

14. Reporting Procedure:

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

15. Discussion:

Diclofenac-d4 was used as an internal standard added at the end of extraction to calculate results. Both external standard (ESTD) and internal standard (ISTD) were used to calculate results during this method validation. We found that the quantitation based on an external standard (ESTD) with matrix matching standards worked better.

16. References:

16.1 Tsai, Cindy, *Determination of Selected Rice Herbicides in Water Samples by Solid Phase Extraction (SPE) and LC/ MSMS,* Department of Fish and Wildlife, SOP: WPCL-LC-009

16.2 Fitch, P., Tran, D., updated by Lee, P., Hsu, J., White, J. *Determination of Atrazine, Bromacil, Cyanazine, Diuron, Hexazinone, Metribuzin, Norflurazon, Prometon, Prometryn, Simazine, Deethyl Atrazine (DEA), Deisopropyl Atrazine (ACET), Diamino Chlorotraizine (DACT), Tebuthiuron and the metabolites Tebuthiuron-104, Tebuthiuron-106, Tebuthiuron-107 and Tebuthiuron-108 in Well and River Water By Liquid Chromatography- triple quadrupole mass spectrometry (LC/MS/MS).* 2014*,* Environmental Monitoring Method, Center for Analytical Chemistry, CDFA

Appendix 1

The Determination of Method Detection Limit (MDL) and Reporting Limit (RL) in Groundwater

calculated without internal std spike 0.1ppb

Compound Name	Spk 1	Spk 2	Spk 3	Spk 4	Spk 5	Spk 6	Spk 7	SD	MDL
Bensulfuron-methyl	0.117	0.103	0.104	0.0957	0.0975	0.100	0.0997	0.007048	0.02215
Bispyribac-sodium	0.115	0.0988	0.101	0.0925	0.0960	0.0972	0.0950	0.007412	0.02330
Clomazone	0.115	0.101	0.102	0.0936	0.0981	0.0969	0.0983	0.006876	0.02161
Halosulfuron-methyl	0.115	0.0976	0.0999	0.0903	0.0948	0.0941	0.0959	0.007974	0.02506
Molinate	0.114	0.0974	0.102	0.0873	0.0966	0.0956	0.0976	0.008081	0.02540
Orthosulfamuron	0.112	0.102	0.102	0.0934	0.0985	0.101	0.0977	0.005743	0.01805
Penoxsulam	0.117	0.102	0.103	0.0966	0.0994	0.101	0.100	0.006622	0.02081
Propanil	0.118	0.102	0.102	0.0932	0.101	0.102	0.104	0.007403	0.02327
Propiconazole	0.117	0.0984	0.102	0.0934	0.0961	0.0994	0.0993	0.007645	0.02403
Thiobencarb	0.114	0.0926	0.0951	0.0799	0.0898	0.0870	0.0943	0.010525	0.03308
Simazine-d10	0.121	0.104	0.104	0.0972	0.102	0.104	0.102	0.007503	0.02358
MCPA	0.116	0.102	0.101	0.0911	0.100	0.0994	0.100	0.007397	0.02325
Triclopyr	0.117	0.102	0.0996	0.0890	0.0975	0.0980	0.100	0.008397	0.02639
2,4,5 T	0.116	0.0952	0.0982	0.0850	0.0965	0.0940	0.0982	0.009291	0.02920

Reporting Limit =0.05ppb for all compounds

Appendix 2

		Spike Level					Control Limits	
Compound	Set#	0.1 ppb	0.25ppb	0.5 ppb	1.25ppb	5ppb		%
Bensulfuron-methyl	1	95.5	97.2	91.4	92.8	89.6	Mean:	95.5
	2	89.5	97.2	86.4	98.4	78.8	SD:	7.95
	3	106	109	103	104	99.2	UCL:	119
	4	92.9	89.6	76.2	96.8	96.0	uwl	111
	5	98.8	91.2	105	103	100	lwl	79.6
							LCL:	71.6
Bispyribac sodium	1	93.6	91.6	90.6	89.6	88.0	Mean:	92.0
	2	85.7	92.0	83.2	95.2	74.0	SD:	6.96
	3	101	100	98.0	100	95.2	UCL:	113
	4	89.6	87.2	78.2	95.2	92.8	uwl	106
	5	95.1	86.4	99.4	99.2	99.2	lwl	78.1
							LCL:	68.8
Clomazone	1	94.5	93.6	90.4	87.2	88.4	Mean:	92.5
	2	88.2	94.8	83.2	95.2	77.2	SD:	7.74
	3	103	99.6	97.6	101	98.4	UCL:	116
	4	89.0	84.0	74.6	95.2	93.6	uwl	108
	5	96.9	83.2	101	102	100	lwl	77.0
							LCL:	69.3
Halosulfuron methyl	1	90.0	89.2	88.6	89.6	86.8	Mean:	92.0
	2	85.6	95.2	84.8	95.2	74.8	SD:	7.56
	3	101	101	100	102	96.8	UCL:	115
	4	89.3	85.2	77.6	95.2	92.4	uwl	107
	5	93.3	84.4	100	102	100	lwl	76.9
							LCL:	69.3
Molinate	1	92.1	93.2	90.0	85.6	89.2	Mean:	92.0
	2	86.3	96.4	86.6	95.2	76.8	SD:	7.26
	3	99.9	96.4	97.2	100	95.2	UCL:	114
	4	91.2	88.0	78.6	99.2	95.2	uwl	106
		94.3	75.2	101	98.4	98.0	lwl	77.5
	5						LCL:	70.2

Orthosulfamuron	1	83.2	87.2	87.0	88.0	86.0	Mean:	93.3
	2	93.6	108	92.8	104	86.4	SD:	7.64
	3	95.0	103	101	100	98.4	UCL:	116
	4	86.1	85.6	77.4	97.6	94.0	uwl	109
	5	89.4	90.4	99.0	99.2	101	lwl	78.1
							LCL:	70.4
Penoxsulam	1	92.2	90.0	88.6	88.6	87.6	Mean:	93.2
	2	92.7	93.6	84.2	95.2	80.0	SD:	6.99
	3	104	99.6	96.8	100	97.2	UCL:	114
	4	93.9	85.6	76.8	97.6	94.8	uwl	107
	5	98.4	88.8	101	102	101	lwl	79.2
							LCL:	72.2
Propanil	1	90.4	91.6	87.2	88..0	87.6	Mean:	91.6
	2	81.9	94.8	84.6	91.2	72.0	SD:	7.97
	3	101	101	93.8	100	95.6	UCL:	116
	4	88.6	86.0	76.6	95.2	92.4	uwl	108
	5	96.7	86.4	101	101	102	lwl	75.7
							LCL:	67.7
Propiconazole	1	92.3	90.4	89.0	90.4	88.8	Mean:	92.5
	2	88.3	94.4	83.8	97.6	76.8	SD:	7.91
	3	104	102	98.0	102	96.0	UCL:	116
	4	93.6	87.6	78.0	96.0	80.4	uwl	108
	5	95.9	82.4	101	101	102	lwl	76.6
							LCL:	68.7
Thiobencarb	1	89.1	88.0	85.8	84.8	88.0	Mean:	88.2
	2	73.7	92.4	81.2	81.6	70.0	SD:	8.95
	3	105	98.8	98.4	97.6	93.6	UCL:	115
	4	83.8	82.8	73.8	91.2	84.8	uwl	106
	5	89.1	78.4	94.4	98.4	99.2	lwl	70.3
							LCL:	61.3
Simazine-d10	1	91.5	83.6	80.8	76.7	78.4	Mean:	89.2
	2	87.1	94.0	88.0	99.2	77.2	SD:	8.64
	3	104	94.8	88.4	93.6	93.6	UCL:	115
	4	93.9	80.8	74.8	92.8	89.2	uwl	106
	5	98.3	76.4	92.8	94.4	105	lwl	71.9
							LCL:	63.2

MCPA	1	96.7	93.6	91.0	92.8	91.6	Mean:	93.5
	2	85.2	95.2	84.2	92.0	72.0	SD:	7.63
	3	105	102	97.6	100	98.4	UCL:	116
	4	93.8	88.0	79.2	96.8	93.6	uwl	109
	5	98.5	88.4	101	101	101	lwl	78.3
							LCL:	70.7
Triclopyr	1	95.5	95.6	91.8	95.2	94.0	Mean:	94.8
	2	83.4	93.2	83.6	92.0	71.2	SD:	8.24
	3	104	107	100	102	101	UCL:	120
	4	94.8	87.2	84.0	99.2	96.8	uwl	111
	5	97.6	90.4	104	102	104	lwl	78.3
							LCL:	70.1
2,4,5,T	1	96.7	94.4	91.4	94.4	92.4	Mean:	93.3
	2	77.6	92.8	84.0	86.4	69.2	SD:	8.81
	3	104	104	99.6	102	98.8	UCL:	120
	4	91.2	88.8	81.0	97.6	91.6	uwl	111
	5	97.9	88.4	102	102	104	lwl	75.7
							LCL:	66.9

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Revision Log:

Date	What was revised? Why?