Determination of Metribuzin, Metribuzin Desamino, Metribuzin Diketo, and Metribuzin Desaminodiketo in Groundwater by Liquid Chromatography Electro Spray Ionization Mass Spectrometry

1. Scope:

This Section Method (SM) provides a stepwise procedure for the analysis of metribuzin, metribuzin desamino (DA), metribuzin diketo (DK) and metribuzin desaminodiketo (DADK) in groundwater using Liquid Chromatography Electro Spray Ionization Mass Spectrometry (LC/ESI/MS/MS). The reporting limit for all analytes is 0.05 ppb.

2. Principle:

A Waters Oasis MCX Cartridge is used to retain the analytes from groundwater samples. The cartridge is placed under vacuum to eliminate any remaining water. The analytes are eluted with 7% ammonium hydroxide in methanol. The eluant is then concentrated, reconstituted in 1:1 acetonitrile/water and analyzed by LC/ESI/MS/MS.

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 shall 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. Interferences:

There were no matrix interferences at the time of method development and validation that caused quantitative problems for any of the analytes.

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 reservoirs)

5.6 Sample filtration apparatus

5.7 Liquid Chromatograph equipped with an Electro Spray Ionization Mass Spectrometer

6. Reagents and Supplies:

6.1	Metribuzin	CAS#3397-62-4
6.2	Metribuzin Desamino (DA)	CAS# 1007-28-9
6.3	Metribuzin Diketo (DK)	CAS#6190-65-4
6.4	Metribuzin Desaminodiketo (DADK)	CAS#21087-64-9
6.5	Propazine (surrogate)	CAS#139-40-29

6.6 Methanol, MS grade, Burdick & Jackson or equivalent

6.7 Acetonitrile, MS grade, Burdick & Jackson or equivalent

6.8 Water, MS grade, Burdick & Jackson or equivalent

6.9 Glacial acetic acid, HPLC grade

6.10 Ammonium hydroxide, reagent grade or equivalent

6.11 Elution reagent: 7% ammonium hydroxide in methanol

6.12 Hydrochloric acid 6 N

6.13 Reconstitution reagent: 1:1 Acetonitrile/water

6.14 Mobile phase A: Add 0.4 mL glacial acetic acid to 1 liter of acetonitrile

6.15 Mobile phase B: Add 0.4 mL glacial acetic acid to 1 liter of MS grade water

6.16 Solid phase extraction cartridges: Waters Oasis® MCX 6 cc (150 mg), 60-micron particle size cartridge

6.17 Test tubes (15 mL)

6.18 LCMS Column: Mac-Mod Ace Excel 2 C18-AR 100mm X 2.1 mm or equivalent

7. Standards Preparation:

7.1 Standards are purchased through an ISO 17034 accredited supplier.

7.1.1 A combination stock standard of 2.0 µg/mL for all five analytes is prepared in acetonitrile.

7.1.2 Propazine (surrogate spike standard) is prepared at 2.0 µg/mL in acetonitrile for spiking samples.

7.2 All standards are kept in the designated refrigerator for storage.

7.3 The expiration date of each standard is twelve months from the preparation date or the expiration date of the stock standards whichever comes first.

8. Sample Preservation and Storage:

Store all samples waiting for extraction in a separate refrigerator (1-4 °C).

9. Test Sample Preparation:

9.1 Background Preparation

9.1.1 The Department of Pesticide Regulation (DPR) provides the background groundwater.

9.1.2 Record the background water identification number on the extraction sheet.

9.2 Preparation of Blank and Spike

9.2.1 Matrix Blank

9.2.1.1 Weigh out 100 ± 0.1 g of background water into a 250 mL beaker and record the weight on the extraction sheet.

9.2.1.2 Follow the test sample extraction procedure (see Section 9.3).

9.2.2 Matrix Spike

9.2.2.1 Weigh out 100 ± 0.1 g of background water into a 250 mL beaker and record the weight on the extraction sheet.

9.2.2.2 Spike a client requested amount of analytes into the background water and let it stand for 1 minute.

9.2.2.3 Follow the test sample extraction procedure (see Section 9.3).

9.3 Test Sample Extraction

9.3.1 Remove sample from refrigerator and allow to come to ambient temperature.

9.3.2 Weigh 100 ± 0.1 g of water sample into a 250 mL beaker and record the weight on the extraction sheet.

9.3.3 Add 0.1 µg propazine (50 µL of 2.0µg/mL spiking solution) as a surrogate to each sample except blank and spike.

Note: the volume of acetonitrile in spiking solution added to the sample should be ≤ 0.1% of the sample volume.

9.3.4 Adjust pH to ≤ 2 with 6N HCL. Check using pH test strips.

9.3.5 Connect the MCX cartridges to the vacuum manifold.

9.3.6 Condition the cartridges with approximately 10 mL of methanol at a flow rate of approximately 1 drop/second followed by approximately 10 mL of pH 2.0 adjusted D.I. water at a flow rate of approximately 1 drop/second by applying vacuum if necessary.

9.3.7 Turn off the vacuum when the D.I. water has just passed through the top frit of the cartridges. Refill MCX cartridges with D.I. water. Attach the sample reservoirs to the top of the cartridges.

9.3.8 Add 60 to 70 mL's of the QC samples **and** the samples to the reservoirs.

9.3.9 Allow the sample to pass through the conditioned cartridges by applying vacuum if necessary. Adjust the flow rate to approximately 1 drop/second.

9.3.10 Add remaining QC samples **and** samples to the reservoirs before the water in the cartridge is below the top frit.

9.3.11 After all the water sample has passed through the cartridges, increase the vacuum to approximately 10 psi for about 20 minutes to dry the cartridges.

9.3.12 Place 15 mL test tubes into the vacuum manifold.

9.3.13 Elute and collect all analytes with 10 ± 0.5 mL of 7% ammonium hydroxide (prepare fresh with each batch) in methanol at a flow rate of approximately 1 drop/second.

9.3.14 Evaporate the eluant to just dryness in a water bath at 38 ± 2 °C under a gentle stream of nitrogen.

9.3.15 Reconstitute the QC samples **and** samples to 1.0 mL with 1:1 acetonitrile/water. Vortex for 30 seconds. Transfer the extract into an autosampler vial. Analyze by LC/ESI/MS/MS.

10. Instrument 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 recommended working standard levels are 0.00625, 0.0125, 0.025, 0.050, 0.100, 0.25, 0.50 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.

10.1.3 Standards do not need to be prepared in matrix.

10.2 Injection Scheme

10.2.1 Calibration Standard Curve

10.2.2 Matrix Blank

10.2.3 Matrix Spike

10.2.4 Test Samples (maximum of 20)

10.2.5 Calibration Standard Curve

10.3 LC System

10.3.1 LC Controller: Shimadzu CBM20A

10.3.2 LC Pumps: Shimadzu LC30AD

10.3.3 Autosampler: Shimadzu SIL30AC

10.3.4 Column Oven: Shimadzu CTO30A

10.3.5 Column: Mac-Mod Ace Excel 2 C18-AR 100mm X 2.1 mm

10.3.6 Column Temperature: 40 °C

10.3.7 Suggested Mobile Phase: Gradient

Table 1 – LC Mobile Phase Gradient Parameters

Time (min)	Flow Rate (mL/min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	0.4	95	5
2.0	0.4	80	20
10.5	0.4	5	95
12.5	0.4	5	95
13.0	0.4	95	5
15.0	0.4	95	5

10.3.8 Injection Volume: 6.0 µL

10.4 Mass Spectrometer and Operating Parameters:

10.4.1 Model: AB Sciex 6500

10.4.2 Ion Source Type: Electrospray ionization (ESI)

10.4.3 Source Polarity: Positive

10.4.4 ESI Vaporizer Temperature: 450 °C

10.4.5 Capillary Temperature: 220 °C

10.4.6 Curtain Gas Flow Rate: 35

10.4.7 GS1 Gas Flow: 50

10.4.8 GS2 Gas Flow: 50

Table 2 - LC/ESI/MS/MS Instrument Conditions

Analyte	Transition [1]	Dwell (msec)	Declustering Potential	Collision Energy	Exit Potential
Metribuzin 1	214.9 - 187.1	50	76	23	26
Metribuzin 2	214.9 - 48.9	50	76	47	12
Metribuzin DA 1	199.9 - 172.0	50	65	23	13
Metribuzin DA 2	199.9 - 115.8	50	65	29	13
Metribuzin DK 1	185.0 - 157.0	50	70	20.5	13
Metribuzin DK 2	185.0 - 68.9	50	70	22	10
Metribuzin DADK 1	170.0 - 142.0	50	70	22	13
Metribuzin DADK 2	170.0 - 99.9	50	70	21.5	12
Propazine 1 (Surr.)	229.9 - 188.0	50	66	23	24
Propazine 2 (Surr.)	229.9 - 145.9	50	66	31	18

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 are spiked at 0.05 ng/ml 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 for each analyte 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.

The results for the standard deviations and MDL are in Appendix I.

[1] Quantitation transitions are in bold font

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. The reporting limit for this method is 0.05 ppb.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included five levels of fortification and a method blank. All spikes and method blanks were processed through the entire analytical method. Spike levels and recoveries for the analytes are shown in Appendix II. All these results are in agreement with client's need and request.

12.4 Control Charts and Limits

Control charts were generated using the data from the method validation for each analyte. The upper and lower control limits are set at $\pm$ 3 standard deviations of the percent recovery.

12.5 Acceptance Criteria

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

12.5.2 The retention time should be within $\pm$ 0.1minute of that of the standards.

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

12.5.4 The recoveries of the matrix spike and surrogate shall be within the control limits.

12.5.5 When QC spike results are out of the control limits appropriate corrective action will be taken, which may include initiating a formal corrective action with root cause investigation.

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

12.6 Measurement Uncertainty:

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

13. Calculations:

Quantitation is based on an external standard (ESTD) calculation using either the peak area or height. The LC/ESI/MS/MS software uses a linear curve fit, with all levels weighted equally. Alternatively, at the analyst's discretion, concentrations may be calculated using the response factor for the standard whose value is less than 30% to the level in the sample.

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

14. Reporting Procedure:

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

15. Discussion:

15.1 A storage stability study was done with this project. The storage stability study consisted of a 1.0 ppb spike level and 3 replicates over a 28-day period. 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 (0.1 ppb) were also extracted along with the storage spikes. This storage stability study showed all analytes have storage stability through day 28. The results are shown in Appendix III.

15.2 Propazine is used as a surrogate. 0.1 µg of propazine is added to each sample and processed through the entire analytical method. This allows the extraction steps to be monitored.

16. References:

16.1 Center of Analytical Chemistry, California Department of Food and Agriculture, "Determination of Atrazine, Bromacil, Diuron, Hexazinone, Metribuzin, Norflurazon, Prometon, Prometryn, Simazine, Deethyl Atrazine (DEA), Deisopropyl Atrazine (ACET), Diamino Chlorotraizine (DACT),

Desmethyl Norflurazone, 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" EMON-SM-69.2 . A revision of 5/14/2020

Appendix I

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

Analyte	Set 1 (ppb)	Set 2 (ppb)	Set 3 (ppb)	Set 4 (ppb)	Set 5 (ppb)	Set 6 (ppb)	Set 7 (ppb)	SD	MDL (ppb)	RL (ppb)
Metribuzin	0.0339	0.0337	0.0400	0.0297	0.0332	0.0304	0.0326	0.0033	0.011	0.05
Metribuzin DA	0.0160	0.0155	0.0364	0.0331	0.0320	0.0271	0.0310	0.0084	0.026	0.05
Metribuzin DK	0.0421	0.0349	0.0303	0.0146	0.0287	0.0183	0.0379	0.0101	0.032	0.05
Metribuzin DADK	0.0386	0.0389	0.0228	0.0189	0.0250	0.0223	0.0296	0.0080	0.025	0.05
Propazine (Surr.)	0.0385	0.0390	0.0577	0.0536	0.0513	0.0468	0.0544	0.0076	0.024	0.05

Definitions
MDL = Method Detection Limit
ppb = Part Per Billion
RL = Reporting Limit
SD = Standard Deviation

Appendix II

Determination of Method Control Limits in Groundwater

Analyte	Spike Level (ppb)	Day 1 Spike Recovery (%)	Day 2 Spike Recovery (%)	Day 3 Spike Recovery (%)	Day 4 Spike Recovery (%)	Day 5 Spike Recovery (%)
Metribuzin	0.1	75.4	85.3	79.6	77.2	79.2
Metribuzin	0.25	72.8	84.0	83.6	73.2	77.6
Metribuzin	0.5	80.8	75.2	89.6	90.8	85.8
Metribuzin	1.25	79.8	77.4	84.8	78.4	89.6
Metribuzin	2.5	74.5	75.2	82.0	74.8	82.4
Metribuzin DA	0.1	65.6	86.7	80.6	80.6	82.4
Metribuzin DA	0.25	76.0	97.6	96.2	77.6	84.4
Metribuzin DA	0.5	88.6	96.6	105	98.4	97.4
Metribuzin DA	1.25	87.2	92.0	90.3	88.0	98.4
Metribuzin DA	2.5	84.0	90.4	90.2	87.6	90.0
Metribuzin DK	0.1	79.0	64.8	56.6	71.0	69.3
Metribuzin DK	0.25	74.8	74.8	81.6	69.2	71.2
Metribuzin DK	0.5	77.8	71.6	80.6	87.6	80.0
Metribuzin DK	1.25	80.8	68.0	75.3	77.8	83.2
Metribuzin DK	2.5	71.6	70.8	74.0	72.4	75.2
Metribuzin DADK	0.1	86.9	73.6	75.0	91.0	75.9
Metribuzin DADK	0.25	85.6	96.4	103	69.6	86.4
Metribuzin DADK	0.5	96.2	88.8	106	105	101
Metribuzin DADK	1.25	92.0	94.4	96.8	83.2	92.8
Metribuzin DADK	2.5	88.0	84.8	90.8	81.6	84.0

Analyte	Spike Level (ppb)	Day 1 Spike Recovery (%)	Day 2 Spike Recovery (%)	Day 3 Spike Recovery (%)	Day 4 Spike Recovery (%)	Day 5 Spike Recovery (%)
Propazine (Surr.)	0.1	83.1	97.6	93.4	90.5	82.5
Propazine (Surr.)	0.25	76.0	88.8	92.0	72.8	78.0
Propazine (Surr.)	0.5	86.8	76.4	84.0	81.6	90.4
Propazine (Surr.)	1.25	83.2	75.0	78.1	71.2	88.9
Propazine (Surr.)	2.5	79.6	76.4	75.2	69.6	86.8

Analyte	Mean Spike Recovery (%)	Standard Deviation	Lower Control Limit	Upper Control Limit
Metribuzin	80.36	5.277	64.53	96.19
Metribuzin DA	88.47	8.622	62.61	114
Metribuzin DK	74.36	6.512	54.82	93.9
Metribuzin DADK	89.15	9.677	60.12	118
Propazine (Surr.)	82.32	8.200	57.72	106

Appendix III

Storage Stability Study

Analyte	Sample Type	Day 0 Spike Rec. (%)	Day 2 Spike Rec. (%)	Day 4 Spike Rec. (%)	Day 8 Spike Rec. (%)	Day 14 Spike Rec. (%)	Day 21 Spike Rec. (%)	Day 28 Spike Rec. (%)
Metribuzin	Blank	ND	ND	ND	ND	ND	ND	ND
Metribuzin	Spike	99.0	69.5	89.0	91.0	98.0	81.0	89.5
Metribuzin	Spike 1	89.9	73.8	79.5	81.3	94.0	68.8	76.8
Metribuzin	Spike 2	86.2	69.9	73.1	85.5	97.3	67.3	74.7
Metribuzin	Spike 3	84.7	77.2	78.7	81.0	92.6	79.4	76.0
Metribuzin DA	Blank	ND	ND	ND	ND	ND	ND	ND
Metribuzin DA	Spike	101	95.0	86.0	69.0	100	89.0	104
Metribuzin DA	Spike 1	85.7	88.8	74.8	68.4	93.8	76.5	89.5
Metribuzin DA	Spike 2	91.3	91.2	86.8	67.7	97.8	75.2	89.4
Metribuzin DA	Spike 3	88.0	89.7	76.6	62.0	90.0	85.6	89.8
Metribuzin DK	Blank	ND	ND	ND	ND	ND	ND	ND
Metribuzin DK	Spike	89.0	68.0	79.0	88.0	93.0	69.0	72.0
Metribuzin DK	Spike 1	83.0	72.7	76.5	80.8	91.4	63.2	68.1
Metribuzin DK	Spike 2	84.2	72.6	67.6	85.3	99.2	58.4	67.1
Metribuzin DK	Spike 3	77.4	73.6	71.6	80.8	90.5	69.6	71.0
Metribuzin DADK	Blank	ND	ND	ND	ND	ND	ND	ND
Metribuzin DADK	Spike	110	101	104	102	105	95.0	99.0
Metribuzin DADK	Spike 1	96.0	95.1	94.2	93.3	90.7	83.3	83.9
Metribuzin DADK	Spike 2	103	96.4	93.6	94.4	93.4	76.0	89.5
Metribuzin DADK	Spike 3	95.1	89.0	94.5	89.2	90.6	79.5	89.4

Analyte	Sample Type	Day 0 Spike Rec. (%)	Day 2 Spike Rec. (%)	Day 4 Spike Rec. (%)	Day 8 Spike Rec. (%)	Day 14 Spike Rec. (%)	Day 21 Spike Rec. (%)	Day 28 Spike Rec. (%)
Propazine (Surr.)	Blank	ND	ND	ND	ND	ND	ND	ND
Propazine (Surr.)	Spike	97.0	81.0	93.0	93.0	106	86.0	95.0
Propazine (Surr.)	Spike 1	77.9	71.3	85.0	85.5	102	70.8	80.1
Propazine (Surr.)	Spike 2	79.6	66.7	81.8	87.5	103	68.6	79.6
Propazine (Surr.)	Spike 3	76.6	68.4	82.7	82.2	97.7	78.1	80.8

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