Analysis of Glyphosate, Glufosinate and Aminomethylphosphonic Acid (AMPA) in Ground Water

1. Scope

This method provides determination of Glyphosate, Glufosinate and Aminomethylphosphonic Acid (AMPA) as a glyphosate metabolite in ground water using IC-MS/MS. The reporting limit is 0.05 ppb for all compounds.

2. Principle

Polar ionic pesticides in ground water have been determined by using IC-MS/MS. In this direct analysis, we demonstrate fast determinations of three ionic polar compounds. This Ion Chromatography-Mass Spectroscopy (IC-MS/MS) provides a separation and quantitation technique for Glyphosate, Glufosinate and AMPA without derivatization and preparation of complex mobile phases.

3. Safety

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

3.2 Polar pesticides are extremely toxic hazardous substances. The analyst should avoid all contact with, and inhalation of the materials containing pesticides and wear appropriate protective clothing 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 should be used and handled with care in a ventilated area.

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

4. Interference

There were no matrix interferences for Glyphosate, Glufosinate and Aminomethylphosphonic Acid (AMPA) compounds by using background ground water at the time of method development.

5. Apparatus and Equipment

5.1 A Dionex IC system comprising of autosampler, AXP-MS auxiliary pumps, EGC 500 KOH eluent generator cartridge, anion dynamically regenerated suppressor and TSQ Altis Thermo scientific triple quadrupole mass spectrometer

5.2 Dionex Ion Pack AS24, 2 x 250 mm column with Dionex Ion Pack AG24, 2 x 50 mm Guard column

5.3 Thermo Scientific Dionex AS-AP autosampler with sample syringe, 250 µL

Thermo Scientific Dionex AXP-MS auxiliary pumps for make-up flow and ADRS 600 regeneration

5.4 Thermo Scientific Dionex ADRS 600 anion dynamically regenerated suppressor

5.5 Thermo Scientific Dionex CR-ATC 600 continuously regenerated anion trap column

5.6 Thermo Scientific Dionex EGC 500 KOH eluent generator cartridge

5.7 Thermo Scientific TSQ Altis triple quadrupole mass spectrometer

5.8 Thermo Scientific Barnstead Gen Pure water purification system

5.9 Vortex vibrating mixer

5.10 Micropipettes, adjustable, recommended sizes as follows: 200 µL and 1000 µL

6. Reagents and Supplies

6.1 Deionized (DI) Water, type I reagent grade, with 18 MΩ.cm resistivity

6.2 Methanol, Optima LC/MS grade or higher purity

6.3 Glyphosate, 1000 µg/ml in water, Chem Services, CAS# 1071-83-6

6.4 Glufosinate ammonium, 1000 µg/ml in water, Chem Services, CAS# 77182-82-2

6.5 Aminomethylphosphonic acid, 1000 µg/ml in water, Chem Services, CAS# 1066-51-9

6.6 Thermo Scientific Autosampler Vial Kit, polystyrene, 10ml, with caps and septa, P/N 074228

6.7 KOH solution

7. Standards Preparation

7.1 All Glyphosate, Glufosinate and AMPA standards were purchased from a ISO 17043 reference material producer. A concentration of 1.0 mg/ml was prepared for each Glyphosate, Glufosinate and AMPA in CDFA/CAC standard repository.

7.2 All these three compounds were received at a concentration of 1.0 mg/ml and were diluted to 1.0 µg/ml (intermediate standard solution 1). Then 0.1 µg/ml was prepared by dilution of 1.0 µg/ml for each Glyphosate, Glufosinate and AMPA standards (intermediate standard solution 2). See standard preparation sheet.

The combination calibration standards (working standard solutions) were prepared to the following concentrations: 10.0, 5.0, 2.5, 0.5, 0.1, 0.05 and 0.04 ng/ml in type I reagent grade DI water. The calibration standards should be prepared freshly every time before use. See standard preparation sheet.

7.3 Use plastic flasks, stopper for preparation of standard solutions of Glyphosate, Glufosinate and AMPA because they tend to interact with glass surfaces. Use the plastic vial for storing samples, spikes and calibration standards.

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

7.5 Keep all stock standard solutions in the freezer at -20°C. The frozen solution was allowed to thaw at room temperature before further dilutions were made.

8. Sample Preservation and Storage

Store all samples waiting for analysis in a refrigerator at 0-4°C. Based on 28 days storage study, holding time for the ground water samples is 10 days from sampling.

9. Background Preparation

The Department of Pesticide Regulations (DPR) provides the background water for matrix blank and matrix spike.

10. Preparation of Blanks and Spike

10.1 Reagent Blank (Method Blank)

Prepare a reagent blank by delivering approximately 5 ml of the type I reagent grade DI water into a 10 ml plastic vial and analyze it with a sample batch.

10.2 Matrix Blank

Deliver approximately 5 ml of the background ground water into a 10 ml plastic vial and analyze it in a sample batch. If the background ground water is not clear, a filtration step is recommended.

10.3 Matrix Spike

Prepare a concentration of 0.5 ng/ml for all Glyphosate, Glufosinate and AMPA in background ground water as a matrix spike. If the background ground water is not clear, a filtration step after spiking is recommended. See standard preparation sheet.

11. Sample Preparation

This analysis requires no sample preparation. Remove samples from refrigerator and allow them to reach ambient temperature. Shake the sample well and deliver approximately 5 ml into a 10 ml plastic vial.

12. Sequence Arrangement

Use the following recommended sequence: two conditioning samples, reagent blank, seven calibration standards, matrix blank, reagent blank, matrix spike, samples, reagent blank, the continuing calibration verification, and reagent blank. All analytes of interest elute and are detected within 17 minutes.

13. Instrument Conditions

13.1 IC Separation Condition

Thermo Scientific Dionex Ion Chromatography system is equipped with AS-AP autosampler, AXP-MS auxiliary pumps for make-up and ADRS 600 regeneration, EGC 500KOH eluent generator cartridge, CR-ATC 600 continuously regenerated anion trap column, ADRS 600 anion dynamically regenerated suppressor and TSQ Altis triple quadrupole mass spectrometer. The chromatographic separation is carried out using a polymer-based Dionex IonPac AS24 column with guard in 2 mm format. Instrument parameters and settings are shown in Table 1. The hydroxide eluent was prepared in-situ

using an eluent generator, the Dionex EluGen KOH cartridge and a Dionex CR-ATC. See the IC concentration conditions in Table 2. After separation, the eluent passed the electrochemically regenerated AERS suppressor. In order to improve desolvation, a second pump added methanol as a make-up solvent.

Table 1. IC Conditions

Parameter	Setting
Mobile Phase	KOH
Eluent Source	Eluent Generator
Analytical Column	IonPac AS24 (2 x 50 mm) with guard column
Suppressor	Dionex AERS 600
Make-Up Pump Flow	0.1 ml/min
Make-Up Solvent	Methanol
Anion Regeneration Pump Flow	0.3 ml/min
Injection Volume	100 µL
Column Temperature	21°C
Pump 1 Flow	0.3 ml/min

Table 2. EGC Concentration Gradient

Step	Time (min)	Concentration [mM]
0	0.00	25.0
1	0.20	25.0
2	11.0	80.0
3	11.1	100.0
4	12.5	100.0
5	12.6	25.0
6	17.0	Stop Run

13.2 MS Conditions

To achieve a mass spectrum of the Glyphosate, Glufosinate and AMPA, a TSQ Altis triple quadrupole mass spectrometer is used. See the Table 3 for mass parameters. The

mass spectrometer operates in selected reaction monitoring mode (SRM) described in Table 4, by monitoring transitions for each compound.

Table 3. Mass Spectrometer Conditions

Ionization Mode	H-ESI
Scan Type	SRM
Polarity	Negative Ion Mode
Spray Voltage	3000 V
Sheath Gas Pressure	42 Arb
Aux Gas Pressure	12 Arb
Sweep Gas Pressure	1 Arb
Ion Transfer Tube Temperature	300 °C
Vaporizer Temperature	300 °C
Q1 Resolution	0.7 FWHM
Q3 Resolution	1.2 FWHM
Collision Gas Pressure (CID) Gas	1.5 mTorr
Source Fragmentation	0 V

Table 4. IC-MS/MS Parameters for Selected Reaction Monitoring Transitions

Compound	RT (min)	Transition Type	Precursor (m/z)	Product (m/z)	Collision Energy	RF Lens (V)
Glufosinate	4.8	Quantifier	180.1	94.94	17.55	75
		Qualifier 1	180.1	119.13	15.75	
		Qualifier 2	180.1	136.16	16.25	
AMPA	5.2	Quantifier	109.975	63.042	18.31	68
		Qualifier 1	109.975	79.042	28.34	
		Qualifier 2	109.975	81.113	12.71	
		Quantifier	167.962	63.06	21.18	
		Qualifier 1	167.962	78.899	38.91	

Glyphosate	8.89	Qualifier 2	167.962	81.042	15.15	69
		Qualifier 3	167.962	124	11.78	

14. Quality Control

14.1 Initial Calibration

Analyze at least five working calibration standard solutions from Section 7.2 starting with the lowest concentration and ending with the highest, using a quadratic regression of the calibration data with all levels weighted 1/x. If the correlation coefficient (r) is at 0.995 or greater, the system is calibrated, and the analysis of the samples may proceed.

14.2 Carry Over

Analyze a reagent blank to demonstrate that the system is clean and free of interferences. A type I reagent grade DI water containing no small polar pesticides was injected to check for carryover.

14.3 Recovery

Recovery values for matrix spike should be within the control limits. When spike recoveries fall outside the limits, the analyst must investigate the cause. The 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-prepared and re-analyzed. If the spike recoveries fall within the limit, then the results from the re-analyzed samples can be reported.

14.4 Continuing Calibration Verification

Use the established calibration from Section 7.2, check the calibration of the instrument throughout and at the end of the analytical sequence. A mid-level standard of known concentration as an indicator of continuing calibration should result in a found value that is $\leq$20% difference of the true value. If not, then the instrument should be recalibrated (all standards reinjected) and all the samples analyzed since the last acceptable calibration should be re-analyzed.

$$\text{Difference \%} = (\text{found value} / \text{true value}) \times 100$$

14.5 Sample Dilution

Analytical solution concentrations must be less than the highest standard concentration. Dilute with type I reagent grade DI water if necessary.

14.6 Method Detection and Reporting Limits

Method detection limit (MDL) refer to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 background ground water samples are spiked at 0.10 ng/ml for each analyte along with blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL using this equation:

MDL=tS (n=7 replicates, t=3.143)

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 for this method is

0.05 ng/ml for all three compounds in this work as client requested. See the calculated MDL for all compounds in Appendix 1.

Trace values will be reported when results fall within the MDL and reporting limit.

14.7 Validation

The method validation consisted of five sample sets. Each set includes five levels of fortification. Spike levels and recoveries are shown in Appendix 2.

14.8 Control Charts

Control charts were generated using the data from the method verification for each analyte. The upper and lower control limits are set at ± 3 standard deviation of the percent of recovery, shown in Appendix 2.

14.9 Acceptance Criteria

14.9.1 Each set of samples will have a reagent blank, a matrix blank, and a matrix spike.

14.9.2 The recoveries of the matrix spikes shall be within the control limits.

14.9.3 The sample should be diluted if results fall outside of the calibration curve.

14.9.4 Relative abundance of confirmation ion within 30% of the standard.

14.9.5 The retention time of the analytes must match within 0.1 minute of the analyte in the standards within the same sequence.

15. Calculations

Quantitation is based on external standard calculation using either the peak area or height. The results are analyzed using a quadratic regression of concentration versus peak area with all levels weighted 1/x.

16. Reporting Procedure

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

17. References

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Appendix 1

Calculated MDL for Glufosinate, AMPA and Glyphosate in Ground Water (ng/ml)

Compound	Spike (ng/ml)	Spike 1	Spike 2	Spike 3	Spike 4	Spike 5	spike 6	Spike 7	SD	MDL
Glufosinate	**0.1**	0.104	0.097	0.102	0.097	0.101	0.099	0.096	0.00299	0.00940
AMPA	**0.1**	0.108	0.108	0.102	0.097	0.099	0.1	0.098	0.00457	0.01437
Glyphosate	**0.1**	0.096	0.098	0.094	0.094	0.092	0.09	0.095	0.00261	0.00820

Appendix 2

Method Validation Study for Glufosinate, AMPA and Glyphosate in Ground Water

		Spike Level					Control Limits	
Compound	Set#	0.1 ppb	0.25ppb	0.5 ppb	1.25 ppb	2.5 ppb		%
Glufosinate	1	95.0	90.4	92.8	88.1	86.2	Mean:	94.5
	2	91.0	92.0	88.6	94.7	84.0	SD:	8.33
	3	81.0	92.8	93.2	96.7	97.1	UCL:	119
	4	91.0	90.0	91.6	89.4	91.0	uwl	111
	5	105	111.0	112	111	106	lwl	77.8
							LCL:	69.5
AMPA	1	111	95.6	98.6	94.5	93.7	Mean:	100
	2	95.0	94.4	91.8	95.7	85.2	SD:	7.64
	3	99.0	98.0	91.0	95.0	97.8	UCL:	123
	4	103	99.6	112	108	110	uwl	116
	5	99.0	109	109	113	108	lwl	85.0
							LCL:	78.8
Glyphosate	1	98.0	87.2	89.8	85.4	84.5	Mean:	94.8
	2	88.0	90.8	89.4	93.3	80.7	SD:	7.17
	3	99.0	100	96.8	94.2	96.6	UCL:	116
	4	95.0	90.8	99.4	97.3	93.6	uwl	109
	5	110	108	104	103	95.2	lwl	80.5
							LCL:	73.3

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