Progress Report

Glufosinate-ammonium – validation and ILV of soil and water methods

1. Introduction

This report summarizes the status of the development, validation and independent laboratory validation (ILV) of the analytical method used for the analysis of Glufosinate Ammonium (GA), N-acetylglufosinate (NAG), Glufosinate-MPP(MPP) and Glufosinate-MPA(MPA) in Soil, Sediment and Water Using LC/MS/MS.

2. Original Method – GC/FPD

The original method for the analysis of Glufosinate Ammonium and its metabolites in soil and water[1] included a sample clean up using either an anion or cation exchange column, derivitization with acetic acid and trimethylorthoacetate, SPE clean-up followed by analysis using gas chromatography using a flame photometric detector (GC/FPD). An ILV of this method was performed in 2009 to 2010 at EN-CAS Laboratories, Winston Salem, NC, and was unsuccessful.

3. Successor Method – LC/MS/MS

A successor method to the GC/FPD method was developed at Bayer Research Park, Stilwell, KS in 2011. In this method, residues of Glufosinate Ammonium and its metabolites are extracted from soil and sediment by shaking with water. An isotopic internal standard containing GA-d_3, NAG-d_3, and MPP-d_3 (MPP-d_3 is also used as a surrogate internal standard for Glufosinate-MPA) is added to the sample and an aliquot cleaned by passing through MAX and MCX SPE cartridges. Water samples are amended with internal standard and concentrated using MAX SPE cartridges. The samples were analyzed for GA, NAG, MPP and MPA by LC/MS/MS with quantification based on a comparison of peak areas with those of known standards. The method was assigned a method number of GL-003-S11-01[2] and is attached in Appendix 1.

The method limit of quantitation (LOQ) in soil and sediment for GA, NAG, MPP, and MPA is 10 ng/g. The LOQ for GA, NAG, MPP and MPA in water is 0.05 ng/mL.

4. Validation of LC/MS/MS Method

This method was successfully validated at BRP in Study Number MEGLP006[3] in both soil and water. The results obtained are quantitation MRM transition are summarized in Table 1 and Table 2.

5. ILV of LC/MS/MS Method

An ILV (Study Number RAGLP073[4]) of the revised method was initiated in 2011 and is ongoing. The first trials on both soil and water proved unsuccessful.

After discussion between the method developers and ILV laboratory, changes were made to the MS/MS parameters which resulted in improved instrument sensitivity. Using these conditions the soil samples were reinjected and acceptable recoveries were obtained for Glufosinate Ammonium, N-acetylglufosinate, Glufosinate-MPP the three targets with an isotopic internal standard in soil. The results for the final analyte, Glufosinate-MPA, which employed a surrogate internal standard, were low. It is suspected that the loss of Glufosinate-MPA is occurring during the SPE clean-up, and work is currently on-going at the development laboratory to modify the clean-up step before beginning the second trial. The results obtained to date are summarized in Table 3.

The data from the initial trial on water are summarized in Table 4. Acceptable recoveries were obtained for N-acetylglufosinate, Glufosinate-MPP and Glufosinate-MPA at 10xLOQ(0.5ng/mL) while low recoveries were obtained for Glufosinate Ammonium. Erratic recoveries were obtained for all analytes in the LOQ samples. These samples were not reinjected using the modified MS/MS parameters and it is suspected that the modified MS/MS parameters will result in improved sensitivity for the LOQ samples. The second trial will be performed upon successful completion of the soil ILV.

6. Conclusion

The LC/MS/MS method for the analysis of Glufosinate Ammonium, N-acetylglufosinate, Glufosinate-MPP and Glufosinate-MPA in Soil and Water Using LC/MS/MS was successfully performed at BRP. The ILV is currently on-going with low recoveries currently being obtained for one of the metabolites: Glufosinate-MPA. Work is currently on-going at the development laboratory to duplicate the results obtained by the laboratory performing the ILV and to determine a solution before beginning the second soil trial. On successful completion of the soil trial, the second water trial will be performed.

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Bayer Method GL-003-S11-01

An Analytical Method for the Determination of Residues of Glufosinate Ammonium, N-acetylglufosinate, Glufosinate-MPP and Glufosinate-MPA in Soil, Sediment and Water Using LC/MS/MS

1.0 SUMMARY

An analytical method was developed to determine the residues of Glufosinate Ammonium (GA), N-acetylglufosinate (NAG), Glufosinate-MPP (MPP), and Glufosinate-MPA (MPA) in soil, sediment and water.

Residues of glufosinate ammonium and its metabolites are extracted from soil and sediment by shaking with water. An isotopic internal standard is added to the sample and an aliquot cleaned through MAX and MCX SPE cartridges. Water samples are amended with internal standard and concentrated using MAX SPE cartridges. The samples were analyzed for GA, NAG, MPP and MPA by LC/MS/MS with quantification based on a comparison of peak areas with those of known standards.

The method limit of quantitation (LOQ) in soil and sediment for GA, NAG, MPP, and MPA is 10 ng/g. The LOQ for GA, NAG, MPP and MPA in water is 0.05 ng/mL.

2.0 BACKGROUND

Glufosinate (2-Amino-4-(hydroxymethylphosphinyl)butanoic acid) is a Bayer CropScience post-emergence herbicide with some systemic action (glutaminsynthestase-inhibitor) for the control of grasses and broadleaves in orchards, grapes, ornamentals, non-crops, rape, and soy beans. The analytical method presented in this report is designed to measure residues of GA, NAG, MPP, and MPA in soil, sediment, and water using isotopically labeled internal standards and LC/MS/MS detection.

3.0 APPARATUS

(Functional equivalents may be substituted)

- Various general laboratory glassware and utensils.
- MicroMan pipettors and tips (M100, M250, and M1000).
- Shaker
- Sonicator
- Vortex
- Manifold
- Turbovap
- Centrifuge
- Reservoir (60 mL, part no. 12131018)
- SPE adapters (Agilent part no 12131001)
- SeQuant ZIC-HILIC 150 mm X 4.6 mm, 5 µm particle size (Part#: 1504550001).

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- TSQ Quantum Ultra liquid chromatograph/mass spectrometer (LC-MS/MS) equipped with an electrospray interface, Surveyor HPLC pumps, CTC Analytics autosampler, and LCQuan data collection software (Thermo Electron Corporation)

4.0 REAGENTS AND CONSUMABLES

(Functional equivalents may be substituted)

- Acetonitrile (HPLC Grade)
- Water (HPLC grade)
- Methanol (HPLC grade)
- Ammonium formate (Fisher No. AC40115-2500)
- Formic acid, 99% (Acros Part No. 147930010)
- 100 mM ammonium formate; Dissolve 6.31 g of ammonium formate in 1 L of water. Mix well.
- 2% formic acid in methanol/water (1:1). Add 10 mL formic acid to 500 mL of 1:1 methanol:water. Mix well.
- Fisherbrand 125 mL 4oz glass jars (Cat. No. 02-911-455)
- Fisherbrand 500 mL 16 oz glass jars (Cat. No. 02-912-312)
- Culture tube, 20 x 150 mm (Fisherbrand No. 14-961-33)
- Waters Oasis MAX, 6 mL, 500 mg extraction cartridge (Waters Part No. 186000865)
- Waters Oasis MCX, 6 mL, 500 mg extraction cartridge (Waters Part No. 186000776)
- HPLC vials and caps (2-mL, National Scientific, Part Nos. C4011-5W and C4011-55)

5.0 PREPARATION OF STANDARD SOLUTIONS

Analytical standards of GA, NAG, MPP, and MPA as well as the isotopic internal standards of GA, NAG, and MPP are needed. These standards may be obtained from Bayer CropScience, 17745 South Metcalf, Stilwell, KS 66085. Additional details about these chemicals are given in Appendix 1.

The toxicities of these chemicals have not been precisely determined. Thus, each chemical must be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest reasonable level.

NOTE: The following procedure is an example description of how these standard solutions may be prepared. Alternate or additional standards of appropriate weight and volume may be prepared as needed.

Class "A" volumetric glassware or calibrated pipets should be used in the preparation of all analytical standards. All standard solutions should be stored in a refrigerator in glass bottles when not in use. Solutions should be allowed to warm to room temperature prior to use. Corrections for standard purities should be applied when expressing standard concentrations.

5.1 Primary Stock Standard Solution

Prepare individual ~100 µg/mL stock solutions of GA, NAG, MPP, and MPA. Standards used to prepare initial stock solutions should be weighed on an analytical balance capable of accurately weighing samples to ± 0.01 mg. Standards are typically provided in 9.0 to 12.0 mg aliquots. The standards are quantitatively transferred to a 100mL volumetric flask using water, and diluted to volume with water.

Prepare a mixed stock solution containing a 10 µg/mL of GA, NAG, MPP and MPA by taking an appropriate volume (~10 mL) of each of the primary stock solutions and diluting to 100 mL with water.

NOTE: Corrections for standard purities should be applied when expressing standard concentrations.

5.2 Fortification Standard Solutions

1 µg/mL mixed solution of GA, NAG, MPP, and MPA

Transfer 10 mL of the 10 µg/mL mixed stock standard solution into a 100 mL volumetric flask. Dilute to volume with water. Mix well.

100 ng/mL mixed solution of GA, NAG, MPP, and MPA

Transfer 5 mL of the 1 µg/mL mixed stock standard solution into a 50 mL volumetric flask. Dilute to volume with water. Mix well.

10 ng/mL mixed solution of GA, NAG, MPP, and MPA

Transfer 5 mL of the 100 ng/mL mixed stock standard solution into a 50 mL volumetric flask. Dilute to volume with water. Mix well.

5.3 Isotopic Internal Standard Solutions

Prepare individual ~100 µg/mL stock solutions of GA-d_3, NAG-d_3, and MPP-d_3. For quantification, the MPP-d_3 internal standard will be used as the internal standard for MPA since there is not a MPA internal standard available. Standards are typically provided in 2.0 to 5.0 mg aliquots. The standards are quantitatively transferred to a 50 mL volumetric flask using water, and diluted to volume with water.

Prepare a mixed 1 µg/mL internal standard solution containing a mixture of GA-d_3, NAG-d_3, and MPP-d_3 by taking an appropriate volume (~1.0 mL) of each of the stock internal standard solutions and diluting to 100 mL with water.

5.4 Calibration Standard Solutions

5.4.1 Soil and Sediment Calibration Standard Solutions

Prepare working calibration solutions consisting of 5, 10, 25, 50, 100 and 150 ppb of GA, NAG, MPP, and MPA by diluting to 10 mL with acetonitrile and water to bring the final concentration to 80/20 acetonitrile/water. Before bringing the calibration solutions to volume, add by pipet 0.2 mL of the 1 µg/mL internal standard solution to each of the calibration solutions. (see Section 5.3 Isotopic Internal Standard Solutions)

Concentration of Standard Solution used for dilution (µg/mL)	Concentration of Internal Standard Solution used for dilution (µg/mL)	Aliquot Native mix Taken (mL)	Aliquot Internal Standard Taken (mL)	ACN volume (mL)	Dilution Volume (mL)	Concentration of Calibration Solution (ppb)	Concentration of Internal Standard (ppb)
10	1	0.150	0.2	8	10	150	20
10	1	0.100	0.2	8	10	100	20
1	1	0.500	0.2	8	10	50	20
1	1	0.250	0.2	8	10	25	20
1	1	0.100	0.2	8	10	10	20
0.1	1	0.500	0.2	8	10	5	20

5.4.2 Water Calibration Standard Solutions

Prepare working calibration solutions consisting of 5, 10, 25, 50, 100 and 150 ppb of GA, NAG, MPP, and MPA by diluting to 10 mL with acetonitrile and water to bring the final concentration to 80/20 acetonitrile/water. Before bringing the calibration solutions to volume, add by pipet 0.25 mL of the 1 µg/mL internal standard solution to each of the calibration solutions. (see Section 5.3 Isotopic Internal Standard Solutions)

Concentration of Standard Solution used for dilution (µg/mL)	Concentration of Internal Standard Solution used for dilution (µg/mL)	Aliquot Native mix Taken (mL)	Aliquot Internal Standard Taken (mL)	ACN volume (mL)	Total Volume (mL)	Concentration of Calibration Solution (ng/mL)	Concentration of Internal Standard (ng/mL)
1	1	1.5	0.25	8	10	150	25
1	1	1	0.25	8	10	100	25
1	1	0.5	0.25	8	10	50	25
1	1	0.25	0.25	8	10	25	25
0.1	1	1	0.25	8	10	10	25
0.1	1	0.5	0.25	8	10	5	25

Further calibration solutions may be prepared but at least six calibration standards are needed.

6.0 PROCEDURE

6.1 Soil and Sediment Extraction

Appendix 2 shows the analytical scheme for the extraction of GA, NAG, MPP, and MPA in soil and sediment. The detailed stepwise procedure is as follows:

1. Weigh 5 ± 0.05 grams of soil/sediment into a 125 mL glass jar.
2. Fortify the recovery samples at the desired fortification level with the appropriate mixed standard solution (see Section 5.2 Fortification Stock Solutions). Let the fortified samples sit for a minimum of 5 minutes.
3. Add 25 mL of water to each sample. Place samples on shaker for ~ 30 minutes.
4. Add 0.1 mL of the 1 ug/mL internal standard solution to each sample. Mix well.
5. Centrifuge for 5 minutes at 2000 rpm.
6. Remove a 10-mL aliquot and place into a culture tube.
7. Apply the aliquot to a preconditioned MCX cartridge that is placed above a MAX cartridge using an SPE adapter. Cartridges are preconditioned with 10 mL of water. Vacuum can be used to pull the sample through the cartridges.
8. Add 5 mL of water to the culture tube, rinse and apply to the MCX cartridge.
9. Remove the MCX cartridge and rinse the MAX cartridge with one column volume of methanol. Discard the effluent.
10. Apply 15 mL of 2% formic acid in methanol:water (1:1) to the MAX cartridge and collect in a clean culture tube. Do not use vacuum to elute the cartridge.
11. Place the culture tube in a Turbovap set at 60°C and evaporate the sample to dryness.
12. Add 0.5 mL of 0.1 M ammonium formate to each sample tube, vortex and sonicate to dissolve all residues.
13. Add 1.5 mL of acetonitrile to each sample tube and vortex.
14. Place an aliquot of each sample into an hplc vial for LC/MS analysis.
15. Analyze 20 uL aliquots for GA, NAG, MPP and MPA analyses.

6.2 Water Extraction

Appendix 3 shows the analytical scheme for the extraction of GA, NAG, MPP and MPA in water. The detailed stepwise procedure is as follows:

1. Place 400 mL of water into a 500 mL glass jar.
2. Fortify the recovery samples at the desired fortification level with the appropriate mixed standard solution (see Section 5.2 Fortification Stock Solutions).
3. Add 0.05 mL of the 1 ug/mL internal standard solution to each sample. Mix well.
4. Apply the sample to a preconditioned MAX cartridge (a SPE reservoir can be added above the cartridge using a SPE adapter). Cartridges are preconditioned with 10 mL of water. Vacuum can be used to pull the sample through the cartridge.
5. Add 5 mL of water to the jar, rinse and apply to the MAX cartridge.
6. Rinse the MAX cartridge with one column volume of methanol. Discard the effluent.
7. Apply 15 mL of 2% formic acid in methanol:water (1:1) to the MAX cartridge and collect in a clean culture tube. Do not use vacuum to elute the cartridge.
8. Place the culture tube in a Turbovap set at 60°C and evaporate the sample to dryness.
9. Add 0.5 mL of 0.1 M ammonium formate to each sample tube, vortex and sonicate to dissolve all residues.
10. Add 1.5 mL of acetonitrile to each sample tube and vortex.
11. Place an aliquot of each sample into an hplc vial for LC/MS analysis.
12. Analyze 10 µL aliquots for NAG, MPP and MPA analyses and 20 uL aliquots for GA analyses.

7.0 ANALYSIS BY LC/MS/MS

7.1 Analytical Procedure

Step 1. Using the recommended procedures listed below, analyze an aliquot of each of the calibration standard solutions (if necessary, additional standard solutions).

Step 2. Analyze an aliquot of each of the analytical samples.
Note: Up to 20 sample analyses can be made after the analysis of the standard solutions. In the case of over 20 samples, extra standard solutions could be added between sample analyses.

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Step 3. Again, analyze an aliquot of each of the calibration standard solutions (and, if necessary, additional standard solutions).

Step 4. When necessary, analyze additional samples and standard solutions. Always finish the procedure with the analysis of a set of standard solutions.

7.2 HPLC Conditions

Note: The analyst should optimize chromatographic conditions to obtain satisfactory chromatography. The following recommended conditions were used on a Finnigan Ultra instrument.

Mobile Phase A: 100 mM aqueous ammonium formate
Mobile Phase B: Acetonitrile

HPLC column: SeQuant ZIC-HILIC, 150 mm X 4.6 mm, 5 µm particle size
Injection volume: 10-20 µL
(Adjust injection volume as necessary for LC/MS/MS system being used)

Time (min)	Mobile Phase A	Mobile Phase B	Flow rate (µL/min)
0.0	40	60	800
1.0	40	60	800
3.0	70	30	800
4.0	70	30	800
4.5	40	60	800
8.0	40	60	800

7.3 Mass Spectrometer Conditions

Note: The analyst should optimize the mass spectrometer conditions to obtain satisfactory system response. The following conditions were used on a Finnigan Ultra instrument.

Positive ion mode for GA

Spray Voltage (V)	3000
Vaporizer Temperature (°C)	375
Sheath Gas Pressure (psi)	25
Ion Sweep Gas Pressure (psi)	2
Auxiliary Gas Pressure (psi)	10
Capillary Temperature (°C)	375

Negative ion mode for NAG, MPP and MPA

Spray Voltage (V)	3000
Vaporizer Temperature (°C)	375

Sheath Gas Pressure (psi)	25
Ion Sweep Gas Pressure (psi)	2
Auxiliary Gas Pressure (psi)	10
Capillary Temperature (°C)	375

7.4 Mass Spectrometer Data Collection

Note: The analyst should optimize the mass spectrometer data collection to obtain satisfactory system response. As the HPLC column ages, the retention times of the analytes will change. The time between the ion mode transition from negative mode to positive mode may change with the retention time change. A standard solution should be analyzed before each set of samples to confirm the data collection parameters.

The daughter ions used in this method were chosen due to their optimum sensitivity on the instrument used for this study. The following recommended ion transitions and conditions were example conditions used on a Thermo Finnigan Ultra instrument:

Primary ion

Analyte Name	Q1 Mass (amu)	Q3 Mass (amu)	Scan Width	Time	CE	Resolution Q1	Resolution Q3	RT (min)
GA	182.10	136.1	0.2	0.1	15	0.5	0.7	~4.0
GA IS	185.10	139.1	0.2	0.1	15	0.5	0.7	~4.0
NAG	222.03	136.0	0.1	0.1	20	0.5	0.7	~3.8
NAG IS	225.03	139.0	0.1	0.1	20	0.5	0.7	~3.8
MPP	151.00	133.0	0.1	0.1	13	0.5	0.7	~4.2
MPP IS	154.00	136.0	0.1	0.1	13	0.5	0.7	~4.2
MPA	136.98	78.1	0.1	0.1	17	0.5	0.7	~4.1
Confirmatory ion								
Analyte Name	Q1 Mass (amu)	Q3 Mass (amu)	Scan Width	Time	CE	Resolution Q1	Resolution Q3	RT (min)
GA	182.10	119.10	0.2	0.1	19	0.5	0.7	~4.0
GA IS	185.10	122.10	0.2	0.1	19	0.5	0.7	~4.0
NAG	222.03	180.00	0.1	0.1	16	0.5	0.7	~3.8
NAG IS	225.03	183.00	0.1	0.1	16	0.5	0.7	~3.8
MPP	151.00	107.00	0.1	0.1	15	0.5	0.7	~4.2
MPP IS	154.00	110.00	0.1	0.1	15	0.5	0.7	~4.2
MPA	136.98	63.10	0.1	0.1	20	0.5	0.7	~4.1

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8.0 CALCULATION OF RESULTS

The example calculation displayed below was used by the laboratory developing this method. Alternate calculation procedures appropriate to the reporting requirements may be substituted.

Residue concentrations were determined using calibration curves which were generated after each analysis using LCQuan software (Version 2.5) using linear regression with 1/x weighting.

The standards were fit to the linear equation:

$$Y = MX + B \text{ with } 1/x \text{ weighting.}$$

where: X is the concentration of the reference standard in ng/mL
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area: isotopic peak area ratio

After regression coefficients were calculated, the residue in ng/g for soil samples or ng/mL for water samples was determined using the following equation:

$$\text{Residue found} = \frac{(Y-B)}{M} \times D$$

For soil samples:

$$\text{Dilution Factor (D)} = \frac{\text{Initial volume (25 mL)}}{\text{Initial sample wt. (5 g)}} \times \frac{\text{Final dilution volume (2 mL)}}{\text{Aliquot taken (10 mL)}} = 1$$

For water samples:

$$\text{Dilution factor (D)} = \frac{\text{Final Dilution volume (2 mL)}}{\text{Initial Volume (400 mL)}} = 0.005$$

LCQuan software was used to calculate the residue for each sample and the percent recovery for the spiked samples.

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8.1 Fortification Experiments

Note: Fortification experiments may be performed as needed to monitor method efficiency and reproducibility, but are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing and validating method efficiency.

With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries using the following equation:

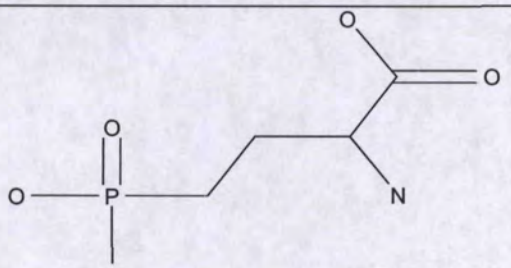
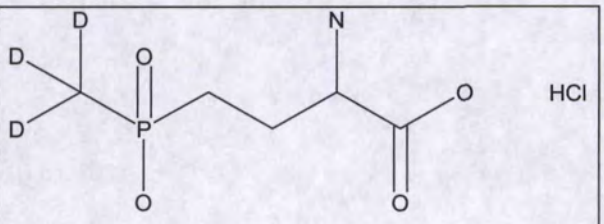
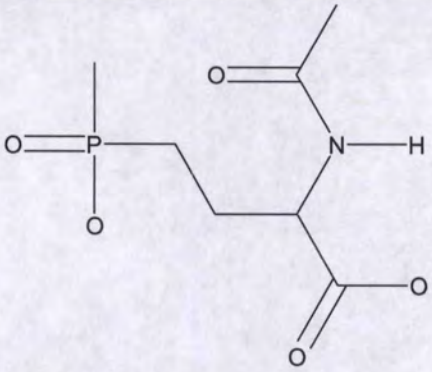
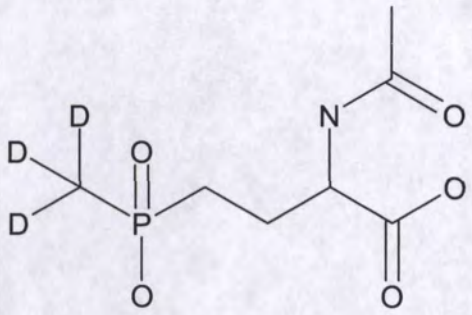
$$\text{Recovery (\%)} = \frac{(R - S)}{T} \times 100$$

Where: R = ppb of target analyte found in fortified sample
S = ppb of target analyte found in control sample, real or apparent
T = theoretical ppb in fortified sample

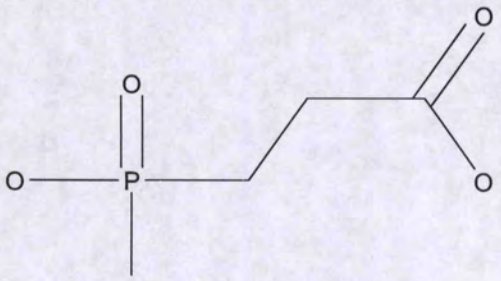
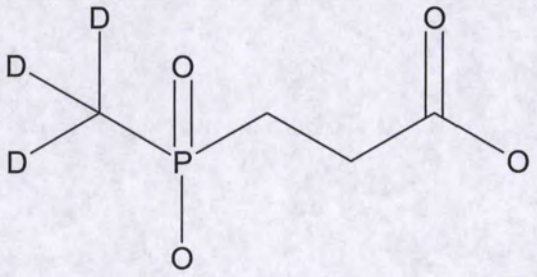
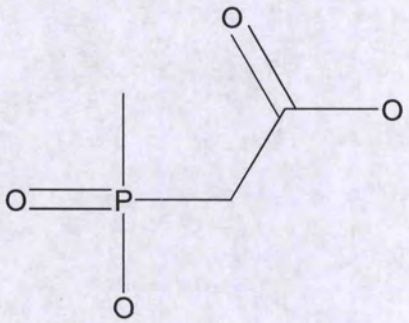
Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at the LOQ or other appropriate level with fortification solutions. Calculate the final residue for the control (S) and fortified control (R) samples.

Appendix 1 Test and Reference Substances

The toxicities of these chemicals have not been precisely determined. Thus, each chemical must be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest reasonable level.

 **Glufosinate, AE F035956** 2-Amino-4-(hydroxymethylphosphinyl)butyric acid Vial: K-1666 Purity: 98.5 Expiration Date: 1/19/2013 CAS Registry No.: 51276-47-2	 **Glufosinate Hydrochloride-methyl-d3** Vial K-1681 Purity: 81.3% Expiration Date: 8/30/2017
 **N-acetyl Glufosinate (NAG)** 2-(Acetylamino)-4-(hydroxymethylphosphinyl)butanoic acid Vial: K-1248 Purity: 99.1 Expiration Date: 6/17/2016 CAS Registry No.: 73634-73-8	 **N-acetylglufosinate-methyl-d3** Vial K-1679 Purity: 92.1% Expiration Date: 3/2/2017

Appendix 1 Test and Reference Substances (Cont'd)

 **Glufosinate propanoic acid** 3-(Hydroxymethylphosphinyl) propanoic acid Vial: K-1667 Purity: 98.9 Expiration Date: 9/29/2021 CAS Registry No.: 15090-23-0	 **Propanoic Acid-methyl-d3** Vial K-1658 Purity: 99.6% Expiration Date: 11/17/2016
 **Glufosinate acetic acid** (Hydroxymethylphosphinyl) acetic acid Vial: K-1940 Purity: 99.4 Expiration Date: 12/17/2014 CAS Registry No.: 72651-25-3	

Appendix 2 Extraction Scheme for Soil and Sediment Samples

Weigh an aliquot of soil/sediment into a 125 mL glass jar

↓

Add 25 mL of water

↓

Shake samples for 30 minutes

↓

Add 0.1 mL of the 1 µg/mL internal standard solution

↓

Centrifuge at 2000 rpm for 5 minutes

↓

Apply a 10-mL aliquot to a pre-conditioned MCX cartridge placed above a MAX cartridge and rinse with water and methanol

↓

Elute the sample extract with 15 mL of 2% formic acid in methanol/water (1:1, v/v)

↓

Evaporate sample to dryness

↓

Add 0.5 mL of 100 mM ammonium formate, vortex and sonicate

↓

Add 1.5 mL of acetonitrile and mix well

↓

Transfer to vial for LC/MS/MS analysis

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Appendix 3 Extraction Scheme for Water Samples

Place 400 mL of water into a 500 mL glass jar

↓

Add 0.05 mL of the 1 µg/mL internal standard solution

↓

Apply sample to a pre-conditioned MAX cartridge and rinse with water and methanol

↓

Elute the sample extract with 15 mL of 2% formic acid in methanol/water (1:1, v/v).

↓

Evaporate sample to dryness

↓

Add 0.5 mL of 100 mM ammonium formate, vortex and sonicate

↓

Add 1.5 mL of acetonitrile and mix well

↓

Transfer to vial for LC/MS/MS analysis