ABSTRACT

The purpose of this study was to demonstrate that the analytical method (R0085/01) [1] could be performed successfully to determine the D- (Reg. No. 6113988) and L- (Reg. No.6113987) enantiomers of BAS 1000 H (Glufosinate) individually in soil and water by chiral LC-MS/MS at an outside facility with no prior experience with the method.

Principle of the Method

Residues of the D- and L- enantiomers of glufosinate are extracted from soil samples by shaking in water. A glufosinate internal standard (IS) is added to the sample and an aliquot of the extract is then cleaned with a Waters® Oasis MAX SPE column. The eluate is then evaporated and reconstituted in water. Residues are determined using a chiral analytical column by LC-(DMS)-MS/MS.

Residues of the D- and L- enantiomers of glufosinate are analyzed directly from an aliquot of the water samples. The sample is spiked with internal standard (IS) prior to determination using a chiral analytical column by LC-(DMS)-MS/MS.

Test conditions

For validation, untreated soil and water samples were fortified and analyzed according to the established method validation guidelines. Analytical sets for soil matrix typically consisted of a method blank, two unfortified control samples, five replicates fortified with BAS 1000 H (D- and L-glufosinate enantiomers mixed solution) at 2 µg/kg (ppb), the method limit of quantitation (LOQ) and five replicates fortified at 20 µg/kg, corresponding to 10xLOQ. Analytical sets for water matrix typically consisted of a method blank, two unfortified control samples, five replicates fortified with a mixed solution of the D- and L- enantiomers of glufosinate at 2.5 µg/L (ppb), the method limit of quantitation (LOQ) and five replicates fortified at 25 µg/L, corresponding to 10xLOQ.

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The LOQ was defined as the lowest fortification level tested for the analyte. The LOQ for each glufosinate enantiomer was 2 µg/kg in soil and was 2.5 µg/L in water. For the course of this ILV study, the LOD for soil and water were set at 0.4 µg/kg and 0.5 µg/L, respectively, which represented 20% of the defined method LOQ.

Selectivity

The selectivity of the method was confirmed with the collection of mass spectra (product ion scans) to justify the selection of ion transitions used for LC-MS/MS determination. In addition, neither of the test systems evaluated were found to contain interferences which met or exceeded the LOD (0.4 µg/kg for soil and 0.5 µg/L for water) at the retention times of BAS 1000 H (Glufosinate).

Linearity

Acceptable linearity was observed using solvent-based calibration standards for all standard ranges and mass transitions tested. The method-detector response for glufosinate was linear over the range of 0.4 – 50 ng/mL in soil and 0.5 – 50 ng/mL in water ($r \geq 0.995$).

1. INTRODUCTION

1.1 Scope of the Method

The analytical method R0085/01 was developed to determine residues of D- and L-glufosinate enantiomers individually in soil and water matrices by chiral LC-MS/MS at BASF Corporation. This method was independently validated for both D- and L-enantiomers of glufosinate at ADPEN Laboratories, Inc. during this ILV study.

2. MATERIALS AND METHODS

2.1 Test Systems

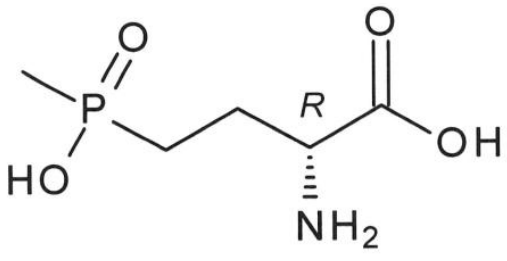
The test systems used in this study were soil and water as shown below.

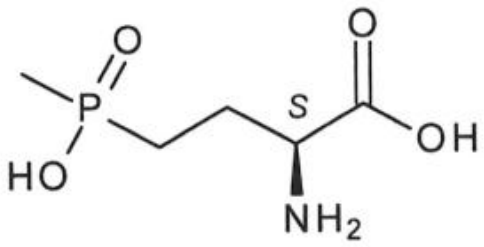
Sample Description	Customer Sample ID
Soil (Washington) – Loamy sand	R21G0560001
Soil (MSL-PF) – Sandy Loam	R21G0550001
Surface Water	R21G0350001R05
Well Water	R21G0350002R05

The control samples used in this study were provided by BASF Corporation and were received at ADPEN Laboratories, Inc. on July 29, 2021. Upon arrival at the laboratory, the samples were inspected and were assigned unique numbers through ADPEN's Laboratory Information Management System (LIMS). The samples were received in good condition, then stored in frozen (average temperature -20°C) until needed for analyses.

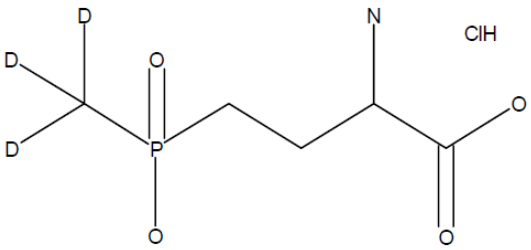
2.2 Reference Substance

The following reference standards were provided by the Sponsor and were stored frozen (-20°C) upon receipt at the testing facility. Characterization and stability data for the substances is maintained by the Sponsor, and a reserve sample of these standards is retained at BASF Corporation. Detailed information regarding the test substances, including the certificates of analysis are presented in Appendix A. A brief description of the analyte follows:

Common Name	Glufosinate (enantiomer)	
IUPAC Name	(2R)-2-Amino-4-[hydroxy(methyl)phosphoryl] butanoic acid	
BASF Reg. No.	6113988	
CAS-No.	Not Assigned	
Molecular Formula	$C_5H_{12}NO_4P$	
Molecular Weight	181.1 g/mol	
Purity	98.6%	
Batch Number	M20/27	
Expiration Date	August 1, 2026	
Storage	Keep in freezer	

Common Names	Glufosinate-P	
IUPAC Name	(2S)-2-Amino-4-[hydroxy(methyl)phosphoryl] butanoic acid	
BASF Reg. No.	6113987	
CAS-No.	35597-44-5	
Molecular Formula	$C_5H_{12}NO_4P$	
Molecular Weight	181.1 g/mol	
Purity	98.8%	
Batch Number	KR6451	
Expiration Date	12/1/22	
Storage	Keep in freezer	

Internal Standard:

Code/Common Name	Glufosinate Hydrochloride-methyl-d3	
IUPAC Name	1-carboxy-3-{hydroxy[(2H3)methyl]phosphoryl} propan-1-aminium chloride	
Molecular Formula	$C_5H_9D_3NO_4P$ – HCl	
Molecular Weight	217.6 g/mol	
Purity	95.4%	
Batch Number	0811201701	
Expiration Date	08/31/27	
Storage	Freezer	

2.3 Route of Administration

In this method validation study, the test substances were applied to the test systems as analytical standard solutions (in water) by a pipette to ensure precise delivery of a small amount of the test substances.

3. ANALYTICAL METHOD

BASF Method R0085/01, “Method for the Separate Determination of the D- (Reg. No. 6113988) and L- (Reg. No. 6113987) Enantiomers of Glufosinate (BAS 1000 H) in Soil and Water by Chiral LC-MS/MS” (Reference 1) was used for the analysis of the samples. The method flow diagrams for residue analysis of Glufosinate in soil and water are presented in Appendix D.

3.1 Method Procedure

Procedure for Extraction of Glufosinate from Soil:
An aliquot (5 g) of control sample was weighed into 50-mL centrifuge tube, fortified with mixed solution containing the D- and L-enantiomers of glufosinate and the residues were extracted in water (25 mL) by shaking for 30 minutes. An aliquot (50 µL, concentration 1 µg/mL) of glufosinate internal standard was added to each sample and mixed well. Sample was

centrifuged and the supernatant was decanted into a new 50-mL centrifuge tube and centrifuged once again at high speed and subjected to SPE clean-up.

An Oasis MAX SPE cartridge (150 mg. 6 cc) was conditioned with methanol, followed by 5% ammonium hydroxide in water, allowing the solvents to flow down to the top of the frit without letting the column go dry. An aliquot (5 mL) of the sample extract was loaded to the column and the diluent was drained to waste. The cartridge was washed using 5% ammonium hydroxide in water, methanol, and 2% formic acid in methanol, in that order, discarding all to waste. A glass culture tube was placed under each column to collect sample eluent. The analyte of interest was eluted from the SPE column using 6 mL of 2% formic acid in water. The eluent was evaporated to dryness in a TurboVap with water bath set to ~60 ºC. The dried residue were reconstituted with 1 mL of water, sonicated, and vortex mixed well. Reconstituted final volume was transferred into a Thomson filter vial (0.2 µm PTFE) for LC-MS/MS injection. For analysis, a Sciex 6500 LC-MS/MS instrument fitted with SelexION Differential Mobility Separation device (DMS) was used.

Procedure for Extraction of Glufosinate from Water:
An aliquot (10 mL) of control sample was weighed into 15-mL centrifuge tube, fortified with mixed solution containing the D- and L-enantiomers of glufosinate. An aliquot (100 µL, concentration 1 µg/mL) of glufosinate internal standard was added to each sample and mixed well. An aliquot of the sample was transferred into a Thomson filter vial (0.2 µm PTFE) for LC-MS/MS injection. For analysis, a Sciex 6500 LC-MS/MS instrument fitted with SelexION DMS was used.

3.2 Method Selectivity

The method selectivity was evaluated with the collection of mass spectra (product ion scans) to justify the selection of ion transitions used for LC-MS/MS determination. Single-analyte stock solution was prepared according to the analytical method, diluted as necessary and infused directly into the MS-MS detector to confirm the quantification and confirmation ion transitions for the D- and L- enantiomers of glufosinate to confirm method selectivity. In addition, method selectivity was also evaluated by screening unfortified test system samples for the quantification and confirmation ion transitions listed in the method.

3.3 Validation of Method

For validation of the method to the glufosinate enantiomers individually, untreated samples of soil and water were fortified with mixed solution containing D- and L-glufosinate enantiomers then analyzed according to the method instructions. To test the repeatability of the method, the analytical sets for each test system consisted of a method blank, two unfortified control samples, five replicates fortified at the method LOQ (2 µg/kg for soil and 2.5 µg/L for water) and five replicates fortified at a higher level, corresponding to 10x the method LOQ (20 µg/kg for soil and 25 µg/L for water).

5. SUMMARY OF METHOD

Type of Method	Calibration standards and samples were analyzed using an LC-MS/MS system, consisting of an Agilent 1290 UHPLC system with a tandem Sciex 6500 mass spectrometer. The Sciex 6500 utilized a SelexION DMS device.
Test Systems	Soil and Water
Mass transitions (*m/z*)	182→136 *m/z* (quantification) and 182→119 *m/z* (confirmatory)
Analytical Procedure	Technical Procedure for the D- (Reg. No. 6113988) and L- (Reg. No. 6113987) enantiomers of glufosinate (BAS 1000 H) in soil and water by chiral LC-MS/MS (BASF Method R0085/01).
Confirmation Technique	A secondary MRM transition was used for confirmation.
Method of Quantitation	Quantitation is based on the monitoring of two mass transitions for glufosinate. Recovery data were reported for each mass transition considered.
LOD	0.4 µg/kg for soil (20% of method LOQ) 0.5 µg/L for water (20% of method LOQ)
LOQ	2 µg/kg for soil (lowest fortification level) 2.5 µg/L for water (lowest fortification level)
Levels of Fortification	2 µg/kg and 20 µg/kg for soil 2.5 µg/Land 25 µg/L for water
Time Required	A set of 13 samples requires approximately 4 hours of work for water and 8 hours of work for soil (analysis time and calculation of the results excluded). For matrix matched standards an additional number of controls samples need to be prepared.

11.9 Additional Table Summaries

Table 33 Example of Standard Solutions Preparation and Dilution

Primary Stock Solutions (Concentrated Stock Standards)

Standard ID #	Compound	Amount of Substance Weighed (mg)	Solvent	Final Volume (mL)	Final Concentration (ng/µL)	Prep Date
C9773‡	Glufosinate Hydrochloride-methyl-d3	3.83	Water	25	122	7/23/2021
C9774†	D-Glufosinate	10.14		50	200	
C9775^	D-Glufosinate	10.14		50	200	
C9776†	L-Glufosinate	10.12		50	200	
C9777^	L-Glufosinate	10.12		50	200	

‡ Internal Standard (IS)
† Stock solution used for preparation of fortification solutions.
^Stock solution used for preparation of calibration standard solutions.

Secondary Stock Solutions

Standard ID #	Primary Stock ID#	Parent Conc. (ng/µL)	Aliquot Volume (mL)	Final Volume (mL)*	Final Concentration (ng/µL)	Prep Date
I10474‡	C9773	122	0.082	10	1.0	7/23/2021
I10492†	C9774	200	0.250	10	5.0	8/9/2021
	C9776	200	0.250		5.0	
I10493^	C9775	200	0.250	10	5.0	8/9/2021
	C9777	200	0.250		5.0	

* Standards are prepared in water.
‡ Internal Standard (IS)
† Secondary stock solution used for preparation of fortification solutions.
^Secondary stock solution used for preparation of calibration standard solutions.

Mixed Fortification Solutions

Standard ID #	Secondary Stock ID#	Parent Conc. (ng/µL)	Aliquot Volume (mL)	Final Volume (mL)*	Final Concentration (ng/µL)	Prep Date
W16927-1	I10492	5.0	5	50	0.5	8/9/2021
W16927-2			0.5	50	0.05	

* Standards are prepared in water.

Mixed Solutions for Preparation of Calibration Standards

Standard ID #	Secondary Stock ID#	Parent Conc. (ng/µL)	Aliquot Volume (mL)	Final Volume (mL)*	Final Concentration (ng/µL)	Prep Date
W16926-1	I10493	5.0	5	50	0.5	8/9/2021
W16926-2			0.5	50	0.05	

* Standards are prepared in water.

Table 33 Example Standard Solutions Preparation and Dilution Data (continued)

Mixed Calibration Solutions

Standard ID #	Secondary Stock / Mixed Solution ID #	Parent Conc. (ng/µL)	Aliquot Volume (mL)	Final Volume (mL)*	Final Concentration (ng/mL)†	Prep Date
W16928-1	I10493	5.0	0.1	10	50	8/9/2021
	I10474 ***(IS)***	*1.0*	*0.1*			
W16928-2	W16926-1	0.5	0.5	10	25	
	I10474 ***(IS)***	*1.0*	*0.1*			
W16928-3	W16926-1	0.5	0.1	10	5	
	I10474 ***(IS)***	*1.0*	*0.1*			
W16928-4	W16926-2	0.05	0.5	10	2.5	
	I10474 ***(IS)***	*1.0*	*0.1*			
W16928-5	W16926-2	0.05	0.1	10	0.5[A]	
	I10474 ***(IS)***	*1.0*	*0.1*			
W16928-6	W16926-2	0.05	0.08	10	0.4[B]	
	I10474 ***(IS)***	*1.0*	*0.1*			

***** Standards are prepared in water.
† Each calibration solution has an Internal Standard concentration of 10 ng/mL (5 ng/mL per enantiomer).
[A] The 0.5 ng/mL standard is the lowest calibration point for water analysis, corresponding to the LOD concentration.
[B] The 0.4 ng/mL standard is the lowest calibration point for soil analysis, corresponding to the LOD concentration.

Matrix-Matched Calibration Solutions for Water Analysis

Standard ID #	Secondary Stock / Mixed Solution ID #	Parent Conc. (ng/µL)	Aliquot Volume (mL)	Final Volume (mL)*	Final Concentration (ng/mL)	Prep Date
W16969-1	I10493	5.0	0.02	2	50	8/26/2021
W16969-2	I10493	5.0	0.01	2	25	
W16969-3	W16926-1	0.5	0.02	2	5	
W16969-4	W16926-1	0.5	0.01	2	2.5	
W16969-5	W16926-2	0.05	0.02	2	0.5	

***** Standards are prepared in Control Matrix Final Volume: An adequate number of Water control samples (enough to generate the required final volume needed for the calibration standards) is taken through the sample preparation procedure, including the addition of internal standard. Ensure a 99% matrix load.

Note: The table above for preparation of matrix-matched standards shows the preparation scheme for calibration solutions for analysis of Well Water, using Sample Number R21G0350002R05. Calibration points with the Standard ID # *W16970* were prepared in the same manner shown here, using the final volume generated from Sample Number R21G0350001R05, for analysis of Surface Water.

Accuracy of standard calibration and fortification solutions was confirmed in order to show correct preparation of the solutions. This was achieved through independent preparation of the concentrated stock standards. One set of stock solutions was used for preparation of fortification solutions and a second set for calibration standards. Standard solutions were stored in refrigerator E-109 at an average temperature of 4 ºC for the duration of this study. All standards were used within their expiration date. Preparation and dilution data forms pertaining to the standard solutions can be found in the raw data.

Table 34 Instrument Conditions and Parameters for BAS 1000 H (Glufosinate) Analysis Used in Soil

HPLC Conditions				
Chromatographic System:	Agilent 1290 Infinity			
Column:	Daicel Crownpak CR(+), 5 µm particle size, 4 x 150 mm			
Column Temperature:	6 °C			
Flow rate:	300 µL/min			
Gradient:	Time (min)	Flow (µL/min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	300	100	0
	3.75	300	100	0
	4.00	300	99	1
	8.00	300	95	5
	8.01	500	100	0
	10.00	500	100	0
Mobile Phase A:	4 mM Ammonium formate in Water with 2% Formic Acid			
Mobile Phase B:	Methanol with 0.5% Formic Acid			
Injection Volume:	40 µL			
MS/MS Conditions				
Detection System:	Sciex 6500 Triple Quad Mass Spectrometer with SelexION DMS			
Ionization:	Electrospray (ESI)			
Polarity:	Positive			
Ion Spray Voltage:	5500 V			
Curtain gas (CUR):	20			
Source Temperature (TEM):	700 °C			
Collision gas setting (CAD):	8.00			
GS1:	45.00			
GS2:	55.00			
Entrance potential (EP):	10.00			
Scan type:	MRM			
DMS Parameters				
DMS Temperature (DT):	Low			
Modifier (MD):	None			
Separation Voltage (SV):	2750 V			
Compensation Voltage (COV):	0.2 V			
DMS Offset (DMO):	-3.0 V			
DMS Resolution Enhancement (DR):	Open			

Table 34 Instrument Conditions and Parameters for BAS 1000 H (Glufosinate) Analysis Used in Soil (continued)

MRM Conditions	Transition (*m/z*)	Dwell Time (msec)	DP	CE	CXP	Retention Time (min)
D-Glufosinate	182.0 → 136.0*	200	91.00	19.00	14.00	approx. 3.6
	182.0 → 119.0			25.00	10.00	
D-Glufosinate-d_3 IS	185.0 → 139.0	200	106.00	19.00	6.00	approx. 3.6
	185.0 → 122.0			27.00	18.00	
L-Glufosinate	182.0 → 136.0*	200	91.00	19.00	14.00	approx. 4.0
	182.0 → 119.0			25.00	10.00	
L-Glufosinate-d_3 IS	185.0 → 139.0	200	106.00	19.00	6.00	approx. 4.0
	185.0 → 122.0			27.00	18.00	
Divert Sequence:	0 to 2 minutes: Waste					
	2 to 7.5 minutes: MS					
	7.5 to 10 minutes: Waste					

* Designated quantitation ion transition

Table 35 Instrument Conditions and Parameters for BAS 1000 H (Glufosinate) Analysis Used in Water

HPLC Conditions				
Chromatographic System:	Agilent 1290 Infinity			
Column:	Daicel Crownpak CR(+), 5 µm particle size, 4 x 150 mm			
Column Temperature:	6 °C			
Sample Temperature:	Ambient			
Flow rate:	300 µL/min			
Gradient:	Time (min)	Flow (µL/min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	300	100	0
	3.75	300	100	0
	4.00	300	99	1
	8.00	300	95	5
	8.01	500	100	0
	10.00	500	100	0
Mobile Phase A:	4 mM Ammonium Formate in Water with 2% Formic Acid			
Mobile Phase B:	Methanol with 0.5% Formic Acid			
Injection Volume:	100 µL			
MS/MS Conditions				
Detection System:	Sciex 6500 Triple Quad Mass Spectrometer with SelexION DMS			
Ionization:	Electrospray (ESI)			
Polarity:	Positive			
Ion Spray Voltage:	5500 V			
Curtain gas (CUR):	20			
Source Temperature (TEM):	700 °C			
Collision gas setting (CAD):	8.00			
GS1:	45.00			
GS2:	55.00			
Entrance potential (EP):	10.00			
Scan type:	MRM			
DMS Parameters				
DMS Temperature (DT):	Low			
Modifier (MD):	None			
Separation Voltage (SV):	2750 V			
Compensation Voltage (COV):	0.2 V			
DMS Offset (DMO):	-3.0 V			
DMS Resolution Enhancement (DR):	Open			

Table 35 Instrument Conditions and Parameters for BAS 1000 H (Glufosinate) Analysis Used in Water (continued)

MRM Conditions	Transition (*m/z*)	Dwell Time (msec)	DP	CE	CXP	Retention Time (min)
D-Glufosinate	182.0 → 136.0*	200	91.00	19.00	14.00	approx. 4.2
	182.0 → 119.0			25.00	10.00	
D-Glufosinate-d_3 IS	185.0 → 139.0	200	106.00	19.00	6.00	approx. 4.2
	185.0 → 122.0			27.00	18.00	
L-Glufosinate	182.0 → 136.0*	200	91.00	19.00	14.00	approx. 4.6
	182.0 → 119.0			25.00	10.00	
L-Glufosinate-d_3 IS	185.0 → 139.0	200	106.00	19.00	6.00	approx. 4.6
	185.0 → 122.0			27.00	18.00	
Divert Sequence:	0 to 2 minutes: Waste					
	2 to 7.5 minutes: MS					
	7.5 to 10 minutes: Waste					

* Designated quantitation ion transition

Table 36 Example Residue Calculations

Quantitation of residues in all samples was achieved using an external calibration curve calculated by linear regression of instrument responses for the reference substances at multiple concentrations. The performance of the instrument was evaluated during each injection set. The correlation coefficient for each calibration curve was found to be $r \geq 0.99$. A standard curve was prepared by injecting standard solutions at appropriate concentrations for the analytes. Calibration standard concentrations for glufosinate enantiomers ranged from 0.4 – 50 ng/mL in soil and 0.5 – 50 ng/mL in water matrix. A calibration standard was typically injected every five to six sample injections. Data processing software, Analyst® 1.6.3, created the standard curve based on linear regression, using 1/x weighting. The regression functions were used to calculate the amount found (native/IS concentration ratio) on the x-axis versus the detector's peak response (peak area ratio) on the y-axis.

Recovery results and concentration found (µg/kg) were calculated for each analytical set within LIMS and reported in Microsoft® Office Excel spreadsheet data reports, which are presented in Appendix B.

Statistical treatment of the data included calculation of means, standard deviations (SD), and percent relative standard deviations (%RSD). These calculations were performed using Microsoft® Excel®. Results were rounded only for reporting purposes. No calculations were made with rounded numbers.

The following equations are used for residue and recovery calculations for glufosinate in soil:

a) Calibration curve $y = mx + b$ Solving for x: $x = \frac{y - b}{m}$

Where, m = Slope
b = y-intercept
x = Amount found (ng)/ng of IS
y = Ratio of analyte peak area/internal standard peak area
µg/kg = ng/g = ppb

b) amount of sample injected (g) = (sample weight × injection size)/final sample volume

c) ppb found = ng found / g of sample injected

d) Percent recovery = (µg/kg found / µg/kg added) × 100%

Appendix D. Method Flow Diagrams

Method R0085/01 Flow Diagram for the Residue Analysis of D- and L- enantiomers of Glufosinate in Soil

Sample (5 g)

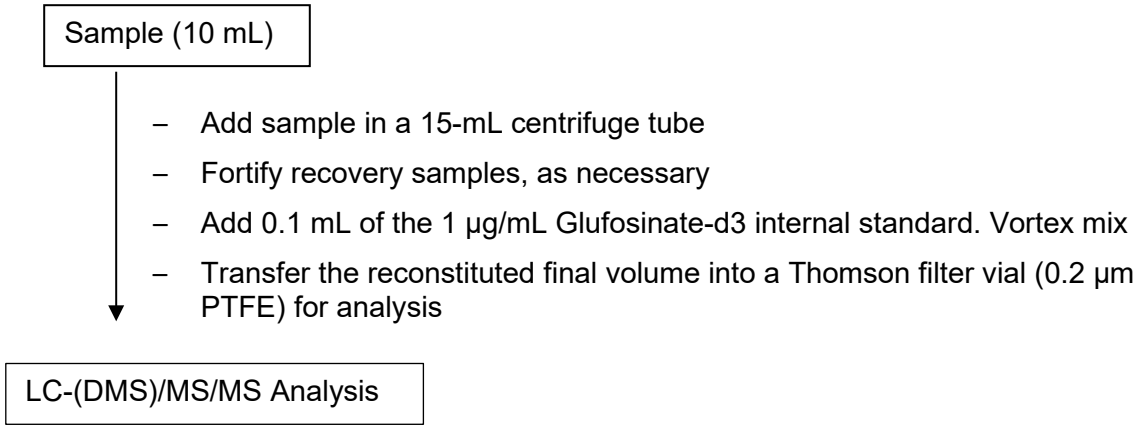
- Add sample in a 50-mL centrifuge tube
- Fortify recovery samples, as necessary
- Add 25 mL of water and shake on a mechanical shaker for 30 minutes
- Remove from shaker and add 50 µL of the 1 µg/mL Glufosinate-d3 internal standard. Vortex mix
- Centrifuge samples at ~3400 rpm for 5 minutes
- Decant supernatant into a new 50-mL plastic centrifuge tube
- Centrifuge again at maximum speed for 10 minutes

SPE Clean-up

- Condition Oasis MAX SPE (150 mg,6 cc) cartridge by adding one column volume of methanol followed by one column volume of 5% Ammonium Hydroxide in water. Allow solvents to flow down to the top of the frit, do not let column go dry. Discard eluate
- Load 5 mL of sample extract onto the column. Allow sample load to flow through column to waste, then wash the column with: one column volume of 5% Ammonium Hydroxide in water, followed by methanol, and then 2% formic acid in methanol. Discard eluate
- Place a glass culture tube under each column to collect sample eluent
- Elute analytes of interest from the SPE column using 6 mL of 2% formic acidin water
- Evaporate the eluent to dryness in a TurboVap with water bath set to 60 ºC
- Reconstitute the dried residue with 1 mL water, sonicate and vortex mix well
- Transfer the reconstituted final volume into a Thomson filter vial (0.2 µm PTFE) for analysis

LC-(DMS)/MS/MS Analysis

Method R0085/01 Flow Diagram for the Residue Analysis of D- and L- enantiomers of Glufosinate in Water



Technical Procedure:

Method for the separate determination of the D- (Reg. No. 6113988) and L- (Reg. No. 6113987) enantiomers of glufosinate (BAS 1000 H) in soil and water by chiral LC-MS/MS

BASF Method Number R0085/01

Authors
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Date
July 13, 2021

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Number of Pages
20

ABSTRACT

Glufosinate (BAS 1000 H) is a BASF post-emergence herbicide with some systemic action (glutaminsynthestase-inhibitor) for the control of grasses and broadleaves in orchards, grapes, ornamentals, non-crops, rape, and soybeans.

An analytical method was previously developed to measure residues of both enantiomers of glufosinate (BAS 1000 H) together in soil and water (Reference 1). This method was developed from that method but includes the ability to determine residues of the D- (Reg. No. 6113988) and L- (Reg. No. 6113987) enantiomers of glufosinate individually.

Soil and Water

Soil samples are extracted by shaking with water. An aliquot of the extract has internal standard (IS) added. An aliquot of the extract is then cleaned with a MAX column, evaporated and reconstituted in water. The residues are determined by chiral LC-MS/MS.

Water samples are aliquoted, then spiked with internal standard (IS). The residues are determined by chiral LC-MS/MS.

The limit of quantitation of BAS 1000 H (Glufosinate) is 2 ppb per enantiomer (4 ppb combined) for soil. The limit of quantitation of BAS 1000 H (Glufosinate) is 2.5 ppb per enantiomer (5 ppb combined) for water. The limit of detection is 20% of LOQ, equivalent to 0.4 ppb per enantiomer (0.8 ppb combined) in soil and 0.5 ppb per enantiomer (1 ppb combined) in water.

TABLE OF CONTENTS

DEFINITIONS AND ACRONYMS

Sample Set:	A group of samples that are extracted and cleaned up at the same time using the same method represented.
Untreated Sample:	A sample that has not been treated with the test substance.
Control Sample:	Usually an untreated sample used for fortification experiments (can be acquired from same study or from a different source).
Unknown Sample:	The samples with unknown residues.
Treated Sample:	A sample that has been treated with the test substance.
Blank:	Solvent, solution or mobile phase injected together with a sample set.
Reagent Blank:	A complete analysis conducted using solvents and reagents only in absence of any sample. Also known as blank of reagents or procedural blank. This sample is analyzed within the sample set to evaluate possible contamination of chemicals or reagents.
Procedural Recovery:	A control sample to which a known amount of analyte has been added before sample work up. This sample is then carried through the method and analyzed with the unknown samples to determine the reliability of the method.
Instrument Recovery:	A control sample which is carried through the method and to which a known amount of analyte has been added before injection. This sample is analyzed within the sample set to evaluate the matrix effect in the instrument.
Analytical Run:	A group of samples that undergo a determinative measurement on an analytical instrument (such as GC, HPLC, CE, GC/MS, or LC/MS/MS) in a defined and continuous sequence under identical instrumental conditions.
Limit of Quantitation (LOQ):	Lowest tested concentration of the analyte in a sample that can be determined with acceptable accuracy and precision according to the method.
Limit of Detection (LOD):	Concentration of analyte equivalent to a defined percentage of the limit of quantitation of the method (e.g. 20% of LOQ). At this concentration, the analyte must be qualitatively detectable in sample matrix (analyte peak height at least 3-5 x baseline noise).
Standards:	Non-isotopically labeled analyte standards are referred to as ‘native’ standards. Isotopically labeled standards are referred to as ‘internal’ standards (IS).

1 INTRODUCTION

Analytical Method R0085 determines the residues of the D- (Reg. No. 6113988) and L- (Reg. No. 6113987) enantiomers of glufosinate (BAS 1000 H) in soil and water using chiral LC-MS/MS.

Version	TP Date	Change
01	June 24, 2021	Creation of TP

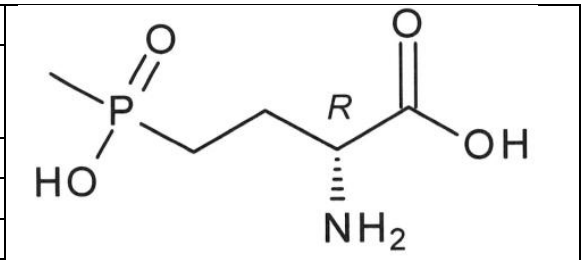
2. MATERIALS

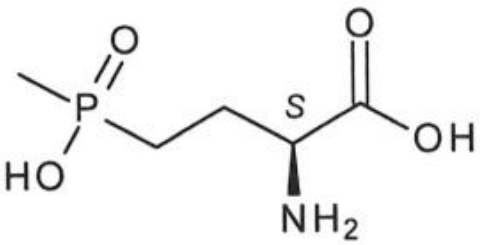
2.1 Safety

The test and reference items, as well as the chemicals required for this analysis, should be handled in accordance with good industrial hygiene and safety practice. Avoid contact with the skin, eyes and clothing. Wearing of closed work clothing is recommended. Remove contaminated clothing. Ensure work clothing is stored separately. Keep away from food, drink and animal feed stuffs. No eating, drinking, smoking or tobacco use at the place of work. Hands and/or face should be washed before breaks and at the end of the shift. Details are given in the Materials Safety Data Sheets (MSDS) of the individual substances. All procedures involving organic solvents should be performed in a well-ventilated hood.
Disposal of samples and chemicals must be done in compliance with on-site safety policies and procedures.

2.2 Test and Reference Items

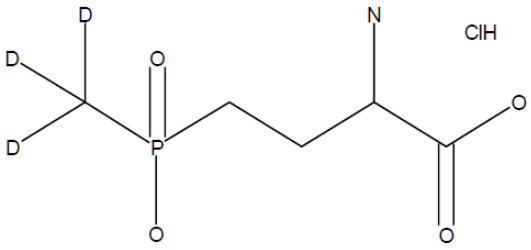
Test and reference items should be stored as stated on the substances certificate of analysis.

Common Names		
IUPAC Name	(2R)-2-Amino-4-[hydroxy(methyl)phosphoryl] butanoic acid	
BASF Reg. No.	6113988	
CAS-No.		
Molecular Formula	$C_5H_{12}NO_4P$	
Molecular Weight	181.1 g/mol	

Common Names	Glufosinate-P	
IUPAC Name	(2S)-2-Amino-4-[hydroxy(methyl)phosphoryl] butanoic acid	
BASF Reg. No.	6113987	
CAS-No.	35597-44-5	
Molecular Formula	$C_5H_{12}NO_4P$	
Molecular Weight	181.1 g/mol	

Internal Standards

Code/Common Name	Glufosinate Hydrochloride-methyl-d3
IUPAC Name	1-carboxy-3-{hydroxy[(2H3)methyl]phosphoryl}propan-1-aminium chloride
Molecular Formula	$C_5H_9D_3NO_4P$ - HCl
Molecular Weight	217.6 g/mol



2.3 Equipment

Equipment	Size, Description	Manufacturer
Balance, Analytical	Model AT200	Mettler
Balance, Top Loader	LB 820	Sartorius
Beakers	Various Sizes	Various
Centrifuge	Multifuge X4R Pro	Thermo Scientific
Centrifuge Adapter	for 50- and 15-mL tubes	Thermo
Centrifuge Tubes, disposable	15 mL	Globe Scientific Inc.
Centrifuge Tubes, disposable	50 mL	VWR
Culture Tube	16X100mm	VWR
Cylinder, Graduated	Various sizes	Various
LC column	Crownpak CR (+), 4 x 150 mm, 5um	Daicel
LC Vials	2 mL injection vials	Agilent Technologies
LC Vials (filtered)	PTFE 0.45 um	Thomson
LC-MS/MS	API 6500+ w/ SelexION DMS	AB Sciex
SPE column	Oasis MAX 150 mg, 6 mL	Waters
Turbovap	----	Biotage
Xplorer Electronic Pipettes	100-10,000 µL	Eppendorf
Various Flask, Volumetric	100, 50, 25 ,10 and 5 mL	Various

Note: The equipment and instrumentation listed above may be substituted by that of similar specifications. The applicability is confirmed if the recoveries of the fortification experiments are in the expected concentration range.

2.4 Reagents

2.4.1 Chemicals

Chemical	Grade	Manufacturer/Supplier
Formic acid	LC-MS	Sigma Aldrich (F0507-100ML)
Methanol	LC-MS	Supelco
Water	LC-MS	VWR
Ammonium hydroxide (28-30%)	ACS	Acros Organics

Note: Equivalent reagents and chemicals from other suppliers may be substituted.

2.4.2 Solutions and Solvent Mixtures

Description	Code	Composition
SPE Solution	**SPE1**	5% Ammonium Hydroxide in Water Add 179 mL of ammonium hydroxide (28%) to in a, e.g., 1L Erlenmeyer flask, complete volume to 1 L with water and mix well to ensure complete homogenous solution.
SPE Solution	**SPE2**	2% Formic Acid in Methanol Add 20 mL formic acid to 980 mL methanol in a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogenous solution.
SPE Solution	**SPE3**	2% Formic Acid in Water Add 20 mL formic acid to 980 mL water in a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogenous solution.
HPLC mobile phase A	**LC1**	4 mM ammonium formate in water with 2% formic acid Add 0.25 g of ammonium formate and 20 mL of formic acid to 980 mL of water into a, e.g., 1L volumetric flask and mix well to ensure complete homogeneous solution.
HPLC mobile phase B	**LC2**	Methanol with 0.5% formic acid Add 5 mL formic acid to 995 mL methanol into a, e.g., 1L volumetric flask and mix well to ensure complete homogeneous solution.

Note: If necessary, the solutions may also be prepared in different volumes as long as the proportions are not modified.

Standard Solutions

This analytical method uses isotopically labeled internal standards to improve the reliability and precision of the calibration process, to correct for potential LC/MS matrix suppression, and to provide chromatographic retention time markers. Non-isotopically labeled analyte standards are referred to as 'native' standards. Isotopically labeled standards are referred to as 'internal' standards (IS).

Stock Solutions (Native)

Prepare a 0.2 mg/mL stock solution by weighing an appropriate amount of the analytes separately into a flask and add the required volume of water.

For example, to prepare 50 mL of 0.2 mg/mL stock solution of R-glufosinate in water, weigh 10 mg R-glufosinate into a 50 mL volumetric flask. Dissolve and dilute to mark with water. Ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Accuracy of standard calibration and fortification solutions should initially be confirmed to show correct preparation of the solutions. This can be achieved for example using one of the following approaches:

- Two stock solutions are independently prepared. One is used for preparation of fortification solutions, the other for calibration standard solutions.
- Fortification and calibration standard solutions should be prepared from one stock solution in separate dilution series.
- Testing one series of solutions from one stock solution against the second series from the other stock solution

For subsequent preparations of solutions, freshly prepared solutions may be compared directly to previous standard solutions to verify the accuracy of the preparation.

A correction for purity is done if the purity is ≤ 95%. If the purity is > 95 % correction is optional.

Stock Solutions (Internal Standard Stock Solutions)

Prepare a 0.1 mg/mL stock solution by weighing an appropriate amount of the analytes into a flask and add the required volume of water.

For example, to prepare 50 mL of 0.1 mg/mL stock solution of GA-d_3 in water, weigh 5 mg GA-d_3 into a 50 mL volumetric flask. Dissolve and dilute to mark with water. Ensure a complete homogeneous solution (e.g. by sonication or vortexing).

A correction for purity is done if the purity is ≤ 95%. If the purity is > 95 % correction is optional.

Native Fortification Solutions

Dilute with appropriate solvents as shown in the table below. Mix well to ensure a homogeneous solution (e.g. by sonication or vortexing).

Preparation of mixed Fortification solutions

Take solution	Volume (mL)	Dilute with water to a final volume of (mL)	Final Concentration (µg/mL)
0.2 mg/mL (each stock)	0.25	10	5
5 µg/mL	5	50	0.5
5 µg/mL	0.5	50	0.05

Note: A different concentration scheme may be used and the volume of solution prepared may be changed.

Internal Standard Solutions

Dilute with appropriate solvents as shown in the table below. Mix well to ensure a homogeneous solution (e.g. by sonication or vortexing).

Preparation of mixed Internal Standard solutions

Take solution	Volume (mL)	Dilute with water to a final volume of (mL)	Final Concentration (µg/mL)
0.1 mg/mL	0.1	10	1

Note: A different concentration scheme may be used and the volume of solution prepared may be changed.

Calibration Standard Solutions

Prepare standard calibration solutions for LC-MS/MS analysis by using the native fortification and internal standard solutions (notated by **IS** in the table) that have been prepared. Dilute with the appropriate solvents as shown in the table below. Mix well to ensure a homogeneous solution (e.g. by sonication or vortexing).

Preparation of mixed standard solutions for calibration

Take solution (µg/mL)	Volume (mL)	Dilute with water to a final volume of (mL)	Concentration (ng/mL)*
5	0.1	10	50
1 (**IS**)	0.1		
0.5	0.5	10	25
1 (**IS**)	0.1		
0.5	0.1	10	5
1 (**IS**)	0.1		
0.05	0.5	10	2.5
1 (**IS**)	0.1		
0.05	0.1	10	0.5**
1 (**IS**)	0.1		
0.05	0.08	10	0.4**
1 (**IS**)	0.1		

* The concentration for all solutions for the internal standard is 10 ng/mL
**The 0.5 ng/mL standard should be the low standard for water analysis and the 0.4 ng/mL should be used instead of the 0.5ng/mL level for soil analysis. These levels correspond to the LODs for each matrix.

Note: A different concentration scheme may be used and additional standards may be prepared as needed. If necessary, the volume of solution prepared may be changed.

2.4.3 Stability of Standard Solutions

The stock, fortification and calibration solutions have been proven to be stable in water for 11 months when maintained at 4°C (Reference 2).

3. ANALYTICAL PROCEDURE

3.1 Sample Preparation

Samples must be sufficiently homogenized beforehand, to assure that the aliquot taken for residue analysis is representative for the whole sample. Cryomilling soil samples in dry ice is highly recommended.

3.2 Sample Storage

Samples are stored frozen until analysis.

3.3.1 Weighing and Fortification - Soil

Weigh 5.0 g +/- 0.05 g of soil into a 50 mL centrifuge tube

For fortified samples, use the following fortification scheme.

Soil:

Sample Type	Sample Weight	Concentration of Spiking Solution [µg/mL]	Volume of Spiking Solution [mL]	Level of Fortification in ppb [ug/kg]
Control	5.0 g	-	-	0
Fortification (LOQ)	5.0 g	0.05	0.2	2*
Fortification (10 × LOQ)	5.0 g	0.5	0.2	20
Treated	5.0 g	-	-	-

* Limit of quantification

Note: Volume of spiking solution added to generate the fortified sample should not exceed 10% of sample weight or volume.

3.3.2 Weighing and Fortification - Water

Place 10 mL of water into a 15 mL centrifuge tube.

For fortified samples, use the following fortification scheme.

Water:

Sample Type	Sample Weight	Concentration of Spiking Solution [µg/mL]	Volume of Spiking Solution [mL]	Level of Fortification in ppb [ug/L]
Control	10 g	-	-	0
Fortification (LOQ)	10 g	0.05	0.5	2.5*
Fortification (10 × LOQ)	10 g	0.5	0.5	25
Treated	10 g	-	-	-

* Limit of quantification

Note: Volume of spiking solution added to generate the fortified sample should not exceed 10% of sample weight or volume.

3.4 Extraction of Sample Material

3.4.1 Extraction of soil

a) Add 25 mL of water to each sample.

b) Place on shaker and shake on high for 30 minutes.

c) Add 0.05 mL of the 1 ug/mL internal standard solution to each sample. Mix well.

d) Centrifuge the samples at ~3700 rpm for 5 minutes.

e) Decant the supernatant into new 50 mL centrifuge tubes

f) Centrifuge at 12,000 xg for 10 minutes.

g) Proceed to Sample Clean up (Section 3.5)

3.4.2 Extraction of water

a) Add 0.1 mL of the 1 ug/mL internal standard solution to each sample. Mix well.
b) Proceed to Preparation for Measurement (Section 3.6)

3.5 Sample Cleanup (soil only)

a) Condition an Oasis MAX column with a column volume of methanol followed by a column volume of **SPE1** (5% ammonium hydroxide in water). Load 5 mL of sample into the column. Wash the column with a column volume of **SPE1**, followed by a column volume of methanol, followed by a column volume of **SPE2** (2% formic acid in methanol). Discard the eluents from load and wash steps.

b) Elute with 6 mL **SPE3** (2%formic acid in water). Collect the eluent in a culture tube and pull column to dryness under vacuum.

c) Evaporate the eluent to dryness in a Turbo-vap set to 60°C.

d) Reconstitute the dried residue with 1 mL of water and mix well (i.e. sonicate and vortex).

3.6 Preparation for Measurement

a) Aliquot sample into filter vial, and slowly plunge filter piston. Samples are ready for injection.

3.7 Influence of matrix effects on analysis

Matrix effects are compensated by the use of the internal standard.

The analyst should verify that no interference peaks are present in the retention window of the analyte of interest. This can be verified by injecting a control and a control with internal standard for comparison.

3.8 Stability of Extracts and Final Volumes

The use of internal standard will compensate for any losses due to stability of the analytes in extract and final volume.

4. QUANTIFICATION AND CALCULATION

4.1 Set-up of the analytical run

A sequence for measurement generally consists of:
- Calibration standards with internal standard
- Control samples and Procedural recovery samples
- Unknown samples

Reagent Blanks or blanks may also be injected if desired. Each injection set should begin and end with an injection of a calibration standard. Standards should be interspersed with samples. Each

calibration standard should be injected at least twice. At least 5 calibration levels need to be injected.

4.2 Instrumental analysis

4.2.1 Instrumentation and Conditions

Chromatography Method

	Parameter			
Chromatographic System	Waters Acquity with FTN			
Analytical-column	Daicel Crownpak CR(+) 150 x 4 mm, 5 um			
Injection Volume	25 µL			
Mobile Phase A	4 mM ammonium formate in water with 2% formic acid			
Mobile Phase B	Methanol with 0.5% formic acid			
Flow Rate	300 µL/min			
Gradient (including wash and equilibration)	Time (min)	Flow (µL/min)	Phase A	Phase B
	0.00	300	100	0
	3.75	300	100	0
	4.00	300	99	1
	8.00	300	95	5
	8.01	500	100	0
	10.00	500	100	0
Detection System	AB Sciex Triple Quad 6500+ Mass Spectrometer with Turbo Ion Drive			
Ionization	ESI			
API Temperature	650 °C			
DMS Temperature	High**			
Analyte	**Transitions**		**Polarity**	**Expected Retention Time**
D-Glufosinate	182 → 136* 182 → 119		positive	approx. 3.5 min
D-Glufosinate IS	185→ 139 185→ 122		positive	approx. 3.5 min
L-Glufosinate	182 → 136* 182 → 119		positive	approx. 4 min
L-Glufosinate IS	185→ 139 185→ 122		positive	approx. 4 min

* proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time.

** COV values should be optimized at the given SV and captured in the raw data

Note: Instruments with similar specifications may substitute the equipment listed above. The instruments used are applicable for analysis if the recoveries of the fortification experiments are in the acceptable range.
A divert valve may be used to reduce the matrix load on the detection system.
Instrument conditions, e.g. injection volumes, columns, gradient steps or mass transitions may be modified, but any changes must be recorded in the raw data. Changes are acceptable, when the recoveries of the fortification experiments are in the acceptable range.
Other parameters like gas flows and voltages are dependent of the equipment used and therefore not listed. Those parameters may need to be adapted for the used instrument.

Calibration procedures

Calculation of results is based on peak area measurements using a calibration curve of the native/IS response ratio. At least 5 calibration levels need to be injected (e.g., required for enforcement). The calibration curve is obtained by direct injection of glufosinate standards for LC-MS/MS in the range of 50 ng/mL to 0.5/0.4 ng/mL or a smaller range maybe used. The internal standard concentration is the same for all standards at 10 ng/mL (5 ng/mL per enantiomer). In a given injection run, the same injection volume is used for all samples and standards.

Linear calibration functions are preferred for evaluation. If other functions are used (e.g. quadratic), this should be fully justified.

4.2.2 Calculation of Residues and Recoveries

Calculation of results is based on peak area measurements using a calibration curve of the native/IS response ratio. For the procedural recoveries, the sample weight will be considered 5 g for soil and 10 g for water in the final calculation of residues [mg/kg]. The method requires that the sample weight to be 5 ± 0.05 g for soil and 10 ± 0.1 g for water for fortification samples. The recovery is the percentage of the fortified amount (µg or ng), which is recovered through the method and the weights cancels out, as shown in the equation below, during the final calculation step.

Since all standards were weighed corrected for parent equivalents, all recoveries and residue values will be in parent equivalents.

The residues of glufosinate and the metabolites in mg/kg are calculated as shown in equations **I** and **II**. An example calculation is in Section 9.1

I. Concentration [ng/mL]

$$Concentration = \frac{\frac{Response}{IS\ Area} - Intercept}{Slope}$$

II. Residue [mg/kg]

$$Residue = \frac{Vol\ X\ Conc\ X\ Dil}{Weight\ X\ 1000}$$

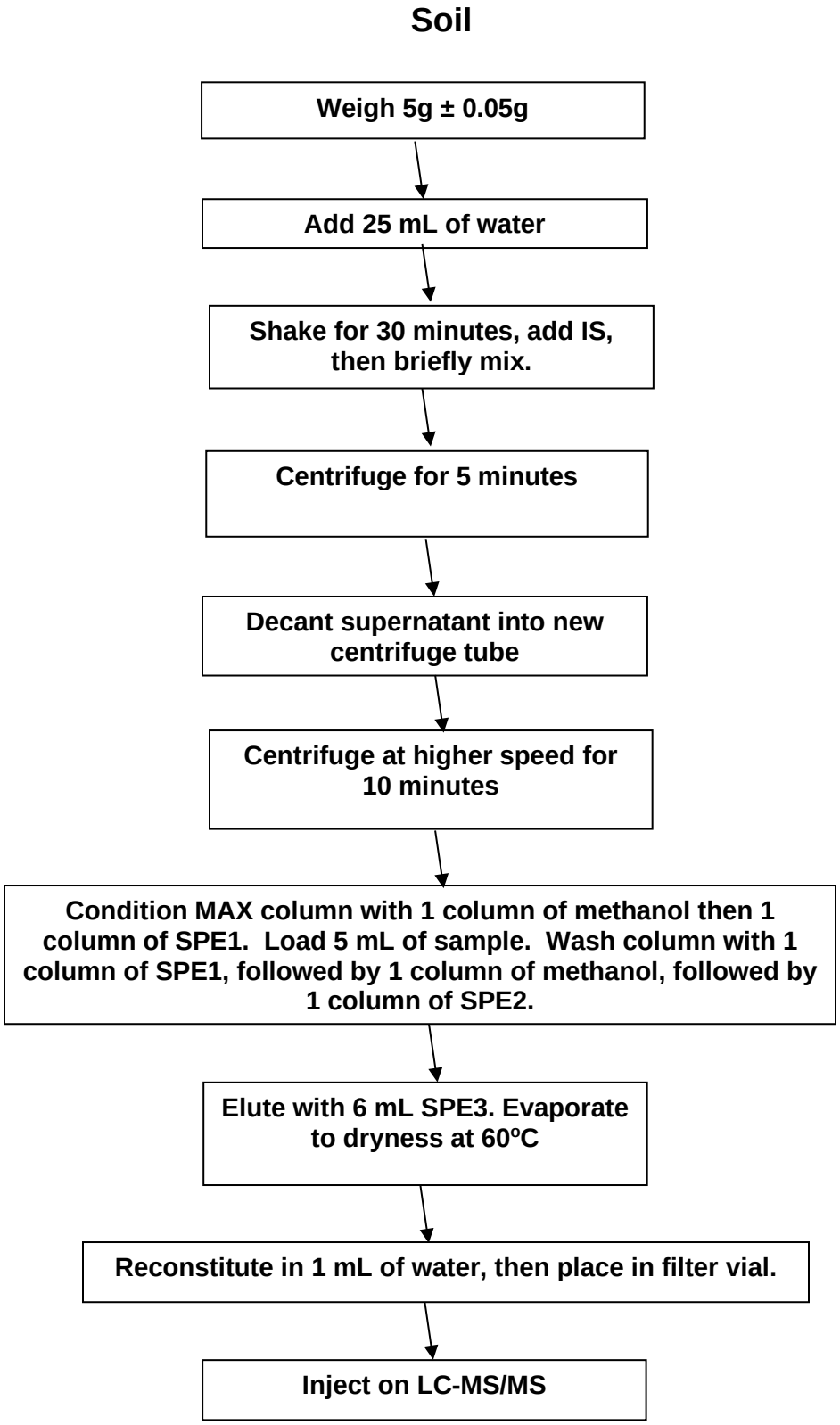
Vol	=	Final volume of the extract after all dilution steps [mL]
Conc	=	Concentration of analyte as read from the calibration curve [ng/mL]
Dil	=	Dilution Factor
Weight	=	Weight of the sample extracted [g]
1000	=	Factor remaining after all unit conversions

The recoveries of spiked compounds are calculated according to equation **III**:

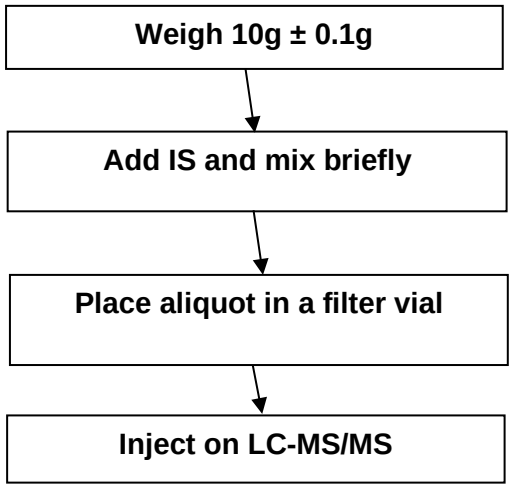
III. Recovery %

$$Recovery\ (\%) = \frac{(Residue\ in\ fortified\ sample - Residue\ in\ conrol)\ X\ 100}{Amount\ of\ analyte\ fortified}$$

5. FLOWCHART


Soil
Weigh 5g ± 0.05g
Add 25 mL of water
Shake for 30 minutes, add IS, then briefly mix.
Centrifuge for 5 minutes
Decant supernatant into new centrifuge tube
Centrifuge at higher speed for 10 minutes
Condition MAX column with 1 column of methanol then 1 column of SPE1. Load 5 mL of sample. Wash column with 1 column of SPE1, followed by 1 column of methanol, followed by 1 column of SPE2.
Elute with 6 mL SPE3. Evaporate to dryness at 60ºC
Reconstitute in 1 mL of water, then place in filter vial.
Inject on LC-MS/MS

Water


Weigh 10g ± 0.1g
Add IS and mix briefly
Place aliquot in a filter vial
Inject on LC-MS/MS