1. INTRODUCTION

1.1 Background and Purpose of Study

The objective of this validation study was to demonstrate the applicability of BASF analytical method No. R0085/01, used for the determination of the D- (Reg. No. 6113988) and L- (Reg. No. 6113987) enantiomers of glufosinate (BAS 1000 H) residues in soil and water by chiral LC-MS/MS. 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.

2. MATERIALS AND METHODS

2.1 Test Systems

The following test systems were considered in this validation study:

Test System 1: Ground Water

Test System 2: Surface Water

Test System 3: Washington Soil (Loamy Sand)

Test System 4: MSL-PF Soil (Sandy Loam)

For characterization data see Appendix B.

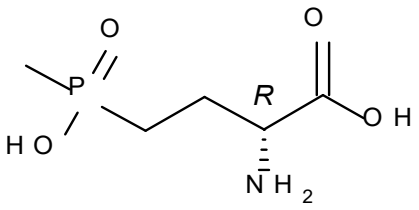
Each analysis set was uniquely identified with a Worklist Number, which consisted of the study number plus a unique analysis package (AP) number (e.g., 919195AP00101). The test system samples were assigned unique numbers and these were recorded for each analytical set (e.g., a ground water sample is identified by a combination of Lab Sample Number R21L0060001R01 and Measurement Sample number F0001Ma, in Worklist No. 919195AP00101). The actual sample numbers used for the analysis were identified in the raw data found in PISAR and in this final report.

2.2 Test and Reference Substances

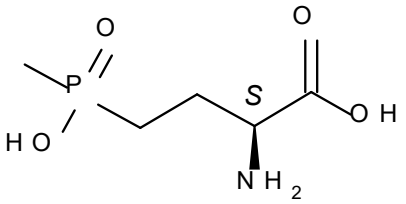
The certificate of analyses of the test and reference item as well as the internal standard are shown in Appendix A.

2.2.1 D- glufosinate and L- glufosinate

Internal-Code	N/A
Common Name	N/A
IUPAC Name	(2R)-2-Amino-4-[hydroxy(methyl)phosphoryl] butanoic acid
BASF Reg. No.	6113988
CAS-No.	N/A
Molecular Formula	$C_5H_{12}NO_4P$
Molecular Weight	181.1 g/mol
Lot/Batch No.	M20/27
Purity	98.6 %
Expiration Date	August 01, 2026

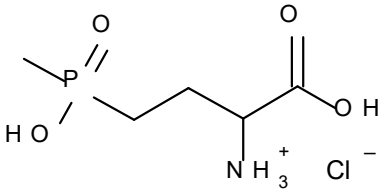


Internal-Code	N/A
Common Name	Glufosinate-P
IUPAC Name	(2S)-2-Amino-4-[hydroxy(methyl)phosphoryl] butanoic acid
BASF Reg. No.	6113987
CAS-No.	35597-44-5
Molecular Formula	$C_5H_{12}NO_4P$
Molecular Weight	181.1 g/mol
Lot/Batch No.	KR645I
Purity	98.8 %
Expiration Date	December 01, 2022



2.2.2 Internal Standard

Internal-Code	N/A
Common Name	Glufosinate Hydrochloride-methyl-d3
IUPAC Name	1-carboxy-3-{hydroxy[(2H3)methyl]phosphoryl}propan-1-aminium chloride
BASF Reg. No.	160561
CAS-No.	N/A
Molecular Formula	$C_5H_9D_3NO_4P$ - HCl
Molecular Weight	217.6 g/mol
Lot/Batch No.	K-1681
Purity	95.4 %
Expiration Date	August 31, 2027



The reference substances were used in the study to generate data for both the instrument and the method performance. Quantitation of all samples was achieved using calibration curves calculated by linear regression of the instrument responses for the reference substance at multiple concentrations, with 1/x weighting. Quantitation was derived by using

the ratio of the test substances to the isotopically-labeled internal standard. The performance of the instrument was evaluated during each injection set. The performance of the analytical method was evaluated during each sample set by fortifying the control matrix with standards of D- and L- glufosinate.

Stock solutions of D- glufosinate and L- glufosinate are prepared in water and intermediate (fortification) solutions are prepared by serially diluting an aliquot of the stock solution with water. Mixed calibration standards were prepared from combining the intermediate solutions and further diluting serially with water. During the course of this study, the test/reference substance solutions were stored under refrigeration. Preparation and dilution data pertaining to the stock and working solutions are located in PISAR. Example standard dilution and use information, as performed in the subject study, are provided in Appendix H.

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.

2.4 Analytical Method

2.4.1 Principle of the Method

Using BASF Analytical Method No. R0085/01, D- glufosinate and L- glufosinate residues in soil are extracted by shaking with water. An aliquot of the extract has internal standard (IS) added (50 µL of 1 µg internal standard/mL, for final concentration 10 ng/mL in all samples). 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 (100 µL of 1 µg internal standard/mL, for final concentration 10 ng/mL in all samples). The residues are determined by chiral LC-MS/MS.
The method procedures validated in this study are provided in Appendix C.

2.4.2 Specificity/Selectivity

The residues of D- glufosinate and L- glufosinate are determined by LC-MS/MS monitoring in the positive mode ion transitions *m/z* 182→136 (primary quantitation ion) and 182→119 (confirmatory ion). Quantitation is obtained using an isotopically-labeled internal standard, Glufosinate -d3. Two mass transitions are available for the test substances, and due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary for parent quantitation. The multiple reaction monitoring (MRM) transitions used to identify D- glufosinate and L- glufosinate residues were determined by product ion scan (see Appendix G).

2.5 Validation of Method

For validation, untreated water and soil samples were fortified with D- glufosinate and L- glufosinate and analyzed according to the established method validation guidelines. To test the repeatability of the method, the analytical sets consisted of a reagent blank, two controls, five replicates fortified with the test item at the method limit of quantitation, and five replicates fortified at a higher level corresponding to 10X the limit of quantitation, for each matrix. Two mass transitions were evaluated. Refer to Appendix C for the full description of the method.

2.6 Standard Solution Stability

The stability of stock, fortification, and calibration standard solutions of glufosinate has been determined in previous studies and was not further investigated in this study. The stock, fortification and calibration solutions have been proven to be stable in water for 30 days when maintained refrigerated (Reference 2).

Table 3. Summary Parameters for the Analytical Method Used for the Quantitation of D- glufosinate and L- glufosinate Residues in Water and Soil

Method ID	BASF Analytical Method No. R0085/01
Analyte(s)	D- glufosinate and L- glufosinate residues in water and soil
Matrices tested	The crop commodities successfully tested in the subject method validation study included ground and surface water, and two types of soil: WA soil and MSL-PF soil.
Extraction solvent/technique	Briefly, residues of D- glufosinate and L- glufosinate in soil are extracted by mechanical 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.
Cleanup strategies	decantation; centrifugation; column purification
Instrument/Detector	Analyses are performed on a Waters Acquity with FTN -LC/MS/MS system (AB Sciex Triple Quad 6500+ Mass Spectrometer with Turbo Ion Drive detection) equipped with a Daicel Crownpak CR(+) column (150 X 4.0 mm, 5 µm particle size) using a mobile phase gradient of water (acidified with 4 mM ammonium formate and 2% formic acid):methanol (acidified with 0.5% formic acid), starting at 100:0, then transitioning to 99:1 before going to 95:5, then back to 100:0 v/v, over 10.0 minutes, flow rate 300 uL/minute), and monitoring in the positive mode ion transitions *m/z* 182→136 and 182→119 for native D- glufosinate and L- glufosinate, and 182 → 139 and 185→ 122 for the internal standard, D3- glufosinate. A divert valve was used to minimize sample introduction to the source, except for the portion of the run immediately surrounding the retention time of the analytes.
Standardization method	Linear regression (1/x weighting). Quantitation is obtained using an isotopically-labeled internal standard,glufosinate-d3, added at the time of extraction.
Stability of std solutions	The stability of stock, fortification, and calibration standard solutions of glufosinate has been determined in previous studies and was not further investigated in this study. The stock, fortification and calibration solutions have been proven to be stable in water for 30 days when maintained refrigerated (Reference 2). During the course of this study, the test/reference substance solutions were stored in a refrigerator and all solutions were used within the reported time period of stability.
Retention time (approx. minutes)	3.5 for D-glufosinate 4.0 for L-glufosinate

Appendix C. Procedure used for Method Validation

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.

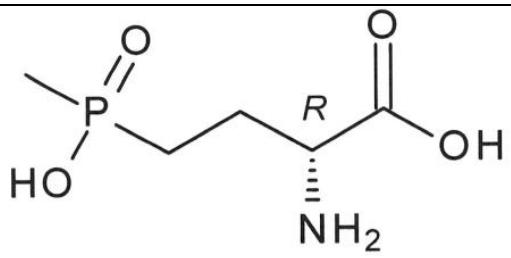
Water samples are aliquoted, then spiked with internal standard (IS). The residues are determined by chiral LC-MS/MS.

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.

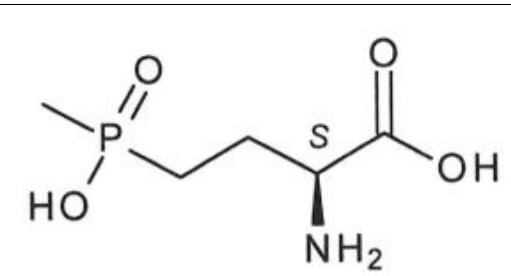
2.2 Test and Reference Items

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

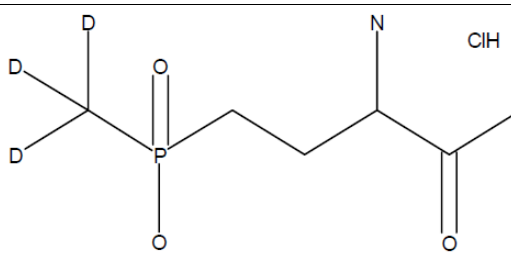


Common Names	Glufosinate-P
IUPAC Name	(2S)-2-Amino-4-[hydroxy(methyl)phosphoryl] butanoic acid
BASF Reg. No.	6113987
CAS-No.	35597-44-5
Molecular Formula	$C_5H_{12}NO_4P$
Molecular Weight	181.1 g/mol



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 30 days when stored refrigerated (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
**Per enantiomer

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.

Soil:

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
**Per enantiomer

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]

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

II. Residue [mg/kg]

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

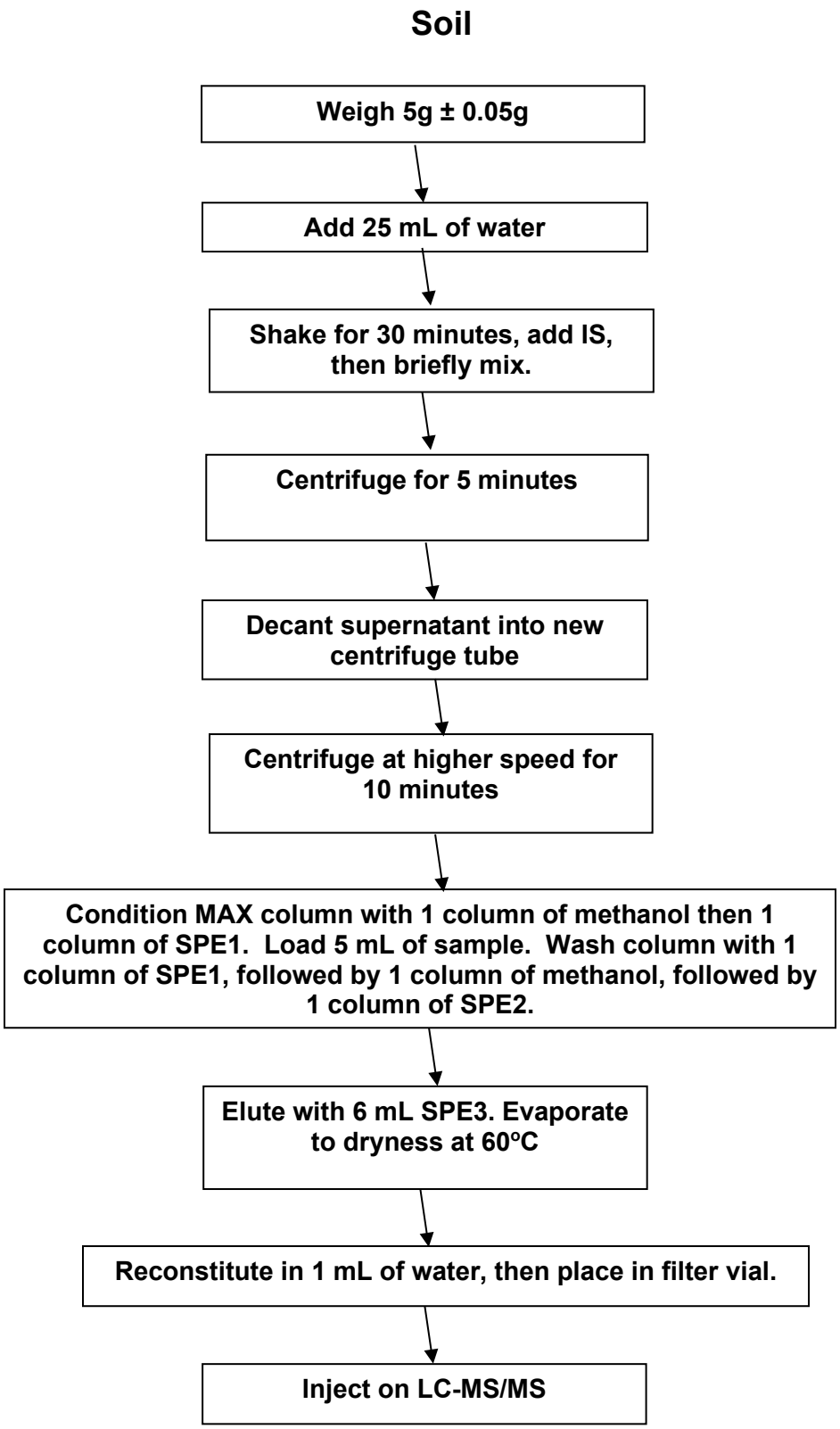
Vol	=	Final volume of the extract after all dilution steps [mL]
Conc	=	Concentration of analyte as read from the calibration curve [ng/mL]
Dil	=	Dilution Factor
Weight	=	Weight of the sample extracted [g]
AF	=	Aliquot Factor
1000	=	Factor remaining after all unit conversions

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

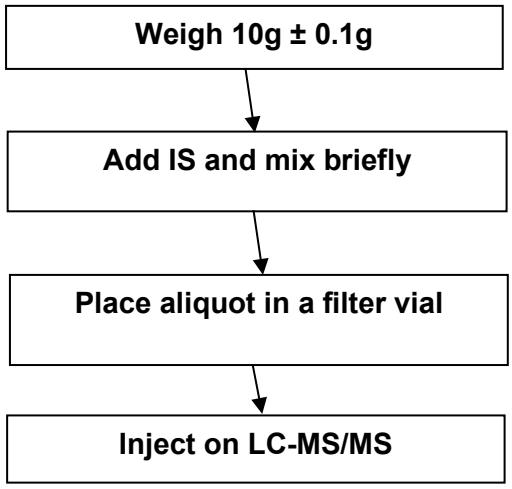
III. Recovery %

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

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 60°C
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