Methods for the Examination of Waters and Associated Materials

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The Determination of Glyphosate, Aminomethylphosphonic acid and Glufosinate in Raw and Potable Waters

2026

The Determination of Glyphosate, Aminomethylphosphonic acid and Glufosinate in Raw and Potable Waters.

Methods for the Examination of Waters and Associated Materials

This booklet contains two methods for the determination of glyphosate, aminomethylphosphonic acid (AMPA) and glufosinate in raw and potable waters.

Method A using On-Line Solid Phase Extraction and Liquid Chromatography-Mass Spectrometry (LCMS)

Method B using Automated Sample Preparation and Liquid Chromatography-Mass Spectrometry (Automated LCMS)

Each method has been validated in only one laboratory and consequently details are included for information purposes only as an example of the type of procedures that are available to analysts. Information on routine multi-laboratory use of these methods would be welcomed to assess their full capabilities.

Whilst this booklet may report details of the materials used, this does not constitute an endorsement of these products but serves only as an illustrative example. Equivalent products are available, and the performance characteristics of the method may differ when other materials are used. It is left to users to evaluate methods in their laboratory.

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Blue Book No. 292
Methods for Examination of Water and Associated Materials Series
292: The Determination of Glyphosate, Aminomethylphosphonic acid and Glufosinate in Raw and Potable and Raw Waters (2026).

Foreword

Whilst specific commercial products may be referred to in this document, this does not constitute an endorsement of those products and serves only to illustrate the types of products available. Equivalent products may be available, and it should be understood that method performance may differ when alternative materials are used. The suitability of any such materials should therefore be confirmed during method validation.

This book does not endorse any particular instrument manufacturer or supplier. Any instruments referenced are provided solely as examples of the configurations used in the analytical procedures described and are included to enhance understanding of the method.

Contents

About This Series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, wastewater and effluents as well as sewage sludges and biota.

In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of Methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection - often, full evaluation is not possible, and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series "Methods for the Examination of Waters and Associated Materials" and their continuing revision is the responsibility of the Standing Committee of Analysts (SCA) - Established 1972 by the Department of the Environment.

At present, there are several working groups, each responsible for one section or aspect of water quality analysis:

1. General principles of sampling and accuracy of results
2. Microbiological methods
3. Inorganic and physical methods, metals and metalloids
4. Organic methods
5. Biological, biodegradability and inhibition methods
6. Radiochemistry methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with these methods are listed at the back of this booklet.

Publication of new or revised methods will appear on our website – the library for which serves as a record of the bona fide methods developed and produced by the Standing Committee of Analysts.

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Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary.

secretary@standingcommitteeofanalysts.co.uk

Users should ensure they are aware of the most recent version they seek.

Warning to Users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed, and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc. Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety. Amongst such resources are:

HSE: Information about health and safety at work

RSC: Laboratory best practices

The Determination of Glyphosate and Aminomethylphosphonic acid in Raw and Potable Waters.

1 Introduction

N-(phosphonomethyl)glycine is the IUPAC name for glyphosate, a widely used broad-spectrum systemic herbicide and crop desiccant. It is an organophosphorus compound, specifically a phosphonate, which acts by inhibiting the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) preventing synthesis of aromatic amino acids, which eventually kills susceptible plants - especially annual broadleaf weeds and grasses that compete with crops. Its herbicidal effectiveness was discovered by Monsanto chemist John E. Franz in 1970. Monsanto brought it to market for agricultural use in 1974 under the trade name Roundup. Glyphosate has become one of the most important pesticides in the world.

Glyphosate has four ionisable sites, with pKa values of <2.0, 2.6, 5.6 and 10.6. Therefore, it is a zwitterion in aqueous solutions and is expected to exist almost entirely in zwitterionic forms in the environment. When reaching the soil, Glyphosate sorbs strongly to its components such as clay minerals, iron oxides or humic acids. Glyphosate is readily degraded by soil microorganisms to aminomethylphosphonic acid (AMPA), which like glyphosate strongly adsorbs to soil solids and is therefore generally considered to have a low leaching potential, although both glyphosate and AMPA are commonly detected in water bodies. A portion of the AMPA detected in environmental samples may originate not only from glyphosate degradation but also from the degradation of other aminophosphonates used in detergents, other products and industrial applications

Glufosinate (also known as phosphinothricin and often formulated as the ammonium salt) is a naturally occurring broad-spectrum herbicide produced by several species of the soil bacterium *Streptomyces*. Glufosinate-ammonium is a non-selective contact herbicide with limited systemic action, used for the control of a wide range of weeds. It is highly soluble in water and volatile but is generally considered to have a low potential for leaching to groundwater because it is rapidly degraded by soil microorganisms. It may persist for longer in aquatic systems.

Detection of glyphosate at trace levels in environmental samples is difficult due to its zwitterionic structure and its complexation with metal ions. Analytical methods based on gas chromatography coupled with mass spectrometry are sensitive, but the sample preparation is tedious, as all ionic groups must be derivatised.

Glyphosate and AMPA have high polarity, low volatility, and lack of a chromophore. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is currently the method of choice for these polar analytes due to its high selectivity and sensitivity. However, when using LC-MS/MS, derivatisation of glyphosate, AMPA and

glufosinate is required to enable analysis and enrichment by reversed phase (RP) sorbent phases. Fluorenylmethylchloroformate (FMOC-Cl) is the most common pre-column derivatisation reagent used in combination with LC-MS/MS.

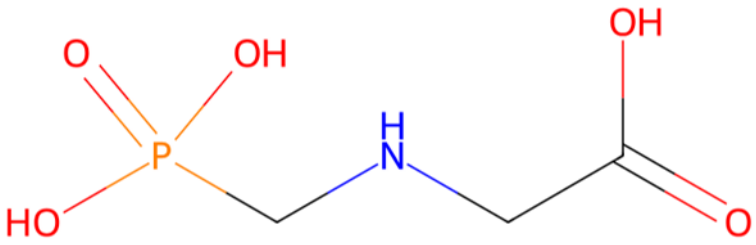
Due to the ionic nature of these polar pesticides and metabolites, an ion chromatography with mass spectrometry (IC-MS) direct injection method can also be used. An IC-MS method is not included in this book.

The methods described herein relate to the analysis of water samples. The methods have example target compound calibrations that are used to provide estimations of amounts of compounds present – the range of these calibrations may be adjusted accordingly to suit requirements.

2 Structures and description

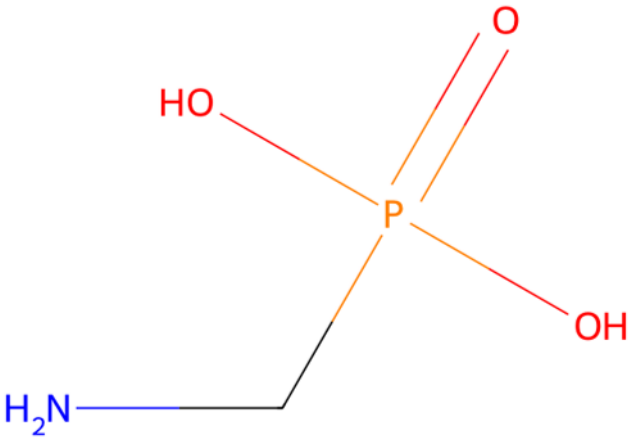
The following is a list of substances contained in this document, their structures, CAS numbers and other relevant information sources to aid in the use of this document.

Glyphosate
IUPAC name: N-(phosphonomethyl)glycine



Molecular formula: $C_3H_8NO_5P$
CAS number: 1071-83-6 (free acid)
Molecular weight: 169.073 Da
Monoisotopic mass: 169.014009 Da

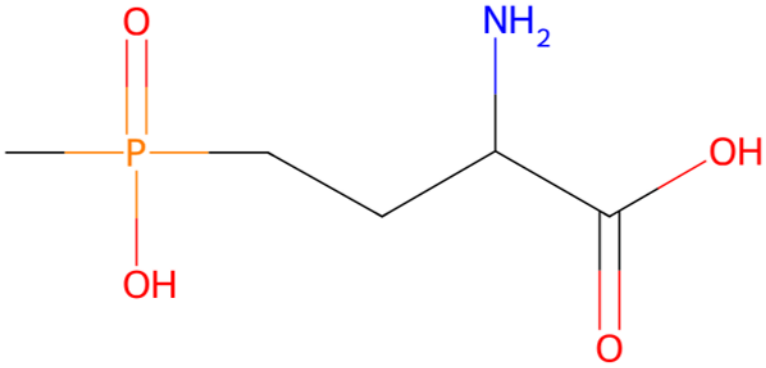
AMPA
IUPAC name: (Aminomethyl)phosphonic acid



Molecular formula: CH_6NO_3P
CAS number: 1066-51-9
Molecular weight: 111.037 Da
Monoisotopic mass: 111.008530 Da

Glufosinate

IUPAC name: 2-Amino-4-[hydroxy(methyl)phosphonoyl]butanoic acid



Molecular formula: $C_5H_{12}NO_4P$
CAS number: 51276-47-2
Molecular weight: 181.128 Da
Monoisotopic mass: 181.050394 Da

3 Sample stability

Compound	Matrix			Sample Container	Preservative	Comments	Number of replicates
	Drinking Water	Surface Water	Ground Water				
	Stability time (Days)						
Glyphosate	28	28	31	No specific requirement	No specific requirement	9-Fluorenylmethoxycarbonyl chloride (FMOC) method only	10 (ALS)
	(ALS Data)	(ALS Data)	(ALS Data)	(ALS, PE/HD bottles)	(ALS, sodium thiosulfate)		
AMPA	28	28	31	No specific requirement	No specific requirement	9-Fluorenylmethoxycarbonyl chloride (FMOC) method only	10 (ALS)
	(ALS Data)	(ALS Data)	(ALS Data)	(ALS, PE/HD bottles)	(ALS, sodium thiosulfate)		
Glufosinate	No data available	No data available	No data available	n/a	n/a	9-Fluorenylmethoxycarbonyl chloride (FMOC) method only	n/a

Data taken from - The Stability and Preservation of Drinking, Ground and Surface Water Samples November 2018' – unless other wise stated.

4 References

Water quality — Sampling Part 3: Preservation and handling of water samples (ISO 5667-3).

Standing Committee of Analysts. The Stability and Preservation of Drinking, Ground and Surface Water Samples 2018, November 2018.

Standing Committee of Analysts Blue Book 'Estimation of Uncertainty of Measurement for Chemical and Physico-chemical Determinands in Drinking Water 2018' and Table A3 of The Water Supply (Water Quality) (Amendment) Regulations 2018.

ISO 16308:2014, Water quality — Determination of glyphosate and AMPA — Method using high performance liquid chromatography (HPLC) with tandem mass spectrometric detection

Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry: performance tuning of derivatization, enrichment and detection. Irene Hanke, Heinz Singer, Juliane Hollender, Analytical and Bioanalytical Chemistry, 2008, Volume 1175, Issue 2, page 2265-2276.

Anatune / Element - 'Initial Development Work For the Automated Derivitisation of Glyphosate and Aminomethylphosphonic Acid in Water Followed by LC-QQQ Analysis'. AS220

InfinityLab Deactivator Additive User Guide – 5991-9516EN

A Turn-Key Guaranteed Analyser for the Routine Measurement of Glyphosate and AMPA in Drinking Water (European Drinking Water Directive 98/83/EC) and Other Environmental Water Samples.

A The Determination of Glyphosate and AMPA in Raw and Potable Water by On-Line Solid Phase Extraction and Liquid Chromatography with Mass Spectrometry Detection

A1 Performance characteristics of the method

A1.1	**Substances determined**	Glyphosate (N-(phosphonomethyl)glycine), AMPA ((Aminomethyl)phosphonic acid)
A1.2	**Type of sample**	Raw waters (ground and surface), potable waters.
A1.3	**Basis of method**	Samples are derivatised with 9-fluorenylmethyl chloroformate. Determinands are trapped on-line using a solid-phase extraction cartridge. Analysis is by liquid chromatography with mass spectrometric detection.
A1.4	**Range of application**	Typically up to 0.250 µg L^{-1}
A1.5	**Standard deviation**	See Table A1**.**
A1.6	**Limit of Quantification**	Glyphosate 0.005 µg L^{-1} and AMPA 0.006 µg L^{-1}, based on a low-level standard (0.010 µg L^{-1}). See Table A1.
A1.7	**Bias**	See Table A1.

A2 Principle

The method is a large volume direct aqueous injection on-line solid-phase extraction (SPE) procedure. Samples are derivatised with 9-fluorenylmethyl chloroformate (FMOC-Cl) prior to analysis. The FMOC derivatised sample is analysed by high performance liquid chromatography using a triple quadrupole mass spectrometer as a detector. The analytes are separated by an LC column and are identified and quantified with mass spectrometric detection (MSD) in selected reaction monitoring (SRM) mode. Quantitation is based on an internal standardisation procedure. In order to decrease the load to the LC column and mass spectrometer from derivatisation by-products (such as 9-fluorenylmethanol FMOC-OH) a liquid/liquid extraction clean up step is performed with dichloromethane. Addition of disodium ethylenediaminetetraacetate (EDTA-Na_2) is used to minimise complexation of the target compounds with metal ions.

A3 Interferences

HPLC-MS/MS is an extremely selective technique, and interferences are expected to occur only rarely. Any interfering compound would have to display the identical SRM transition at the same retention time as the analyte; this is extremely unlikely in potable water samples. However, any compound, which passes through the extraction procedure, and has a similar liquid chromatographic retention time and mass spectrometric properties to the compound of interest, will cause interference. Samples containing high humic or fulvic content have been shown not to cause significant ion suppression.

A4 Hazards/COSHH

Analysts using this method should familiarise themselves with the COSHH and risk assessments for the analysis. See Safety Data Sheet (SDS) provided by the chemical manufacturer or supplier.

A4.1 **Acetonitrile (**CH_3CN)
CAS No. 75-05-8

A4.2 **Dichloromethane (**CH_2Cl_2)
CAS No. 75-09-2

A4.3 **Methanol** (CH_3OH)
CAS No. 67-56-1

A4.4 **Ammonia solution** (NH_3 (aq), 25%)
CAS No. 1336-21-6

A4.5 **9-Fluorenylmethylchloroformate** ($C_{15}H_{11}ClO_2$)
CAS No. 28920-43-6

A4.6 **Formic acid** (CH_2O_2)
CAS No. 64-18-6

A4.7 **di-Sodium tetraborate, decahydrate** ($Na_2B_4O_7.10H_2O$)
CAS No. 1303-96-4

A5 Reagents

All reagents should be of sufficient purity that they do not give rise to interferences during analysis and distilled, deionised or similar grade water should be used throughout. A procedural blank should be run with each batch of samples to check for interferences. All solutions should be mixed thoroughly prior to use.

A5.1 **Acetonitrile**, LC-MS grade

A5.2 **Dichloromethane**, alcohol free

A5.3 **Methanol**, LC-MS grade

A5.4 **Formic acid**, LC-MS grade

A5.5 **Ammonia solution, 25%**, LC-MS grade

A5.6 **Sodium thiosulfate pentahydrate**, AR grade

A5.7 **di-Sodium tetraborate, decahydrate**, AR grade

A5.8 **9-Fluorenylmethyl chloroformate**, HPLC grade

A5.9 **Ethylenediaminetetraacetic acid, disodium salt, dihydrate**, AR grade

A5.10 **Ammonium acetate,** LC-MS grade

A5.11 **Water**, LC-MS grade

Ultrapure grade water should contain no measurable amounts of the compounds of interest. Millipore 'MilliQ SP' water has been found to be satisfactory. This water is used to prepare aqueous stock, intermediate and working solutions and aqueous mobile phases.

A5.12 **Matrix Water**, a treated drinking water

Matrix water should contain no measurable amounts of the compounds of interest. This 'real' water is used for the preparation of calibration standards and analytical quality control (AQC) samples.

A5.13 **Sodium thiosulfate pentahydrate solution**, 1.8% $^{w}/_{v}$ or 18 g L^{-1}

Prepared by dissolving 9.00 ± 0.01 g of sodium thiosulfate pentahydrate in 500 mL of ultrapure water in a volumetric flask. This is used as matrix modifier for all samples and to produce the calibration and AQC matrix water. Store under refrigeration at 3 ± 2 °C for no longer than 1 month.

A5.14 **Calibration / AQC matrix water**, 18 mg L^{-1} sodium thiosulfate pentahydrate

Prepared by measuring 1000 mL of an appropriate 'analyte free' treated drinking water into a 1 L coloured glass bottle and using a pipette add 1 mL 18 g L^{-1} sodium thiosulfate solution. This is used for the preparation of the calibration and AQC working standards, instrument sensitivity and calibration drift standards and to dilute samples that are over the calibration range. Store under refrigeration at 3 ± 2 °C for no longer than 1 month.

A5.15 **di-Sodium tetraborate, decahydrate solution**, 40 mM (aq)

Prepared by dissolving 15.25 ± 0.01 g di-sodium tetraborate, decahydrate in ultrapure water in a 1000 mL volumetric flask. Use an ultrasonic bath at 30 °C to aid preparation. Store at ambient room temperature for no longer than 1 week.

A5.16 **9-Fluorenylmethyl chloroformate, (FMOC-Cl) solution,** 6.5 mM in acetonitrile

Prepared by dissolving 1.68 ± 0.01 g FMOC-Cl in acetonitrile in a 1000 mL volumetric flask. Store under refrigeration at 3 ± 2 °C for no longer than 1 week.

A5.17 **Ethylenediaminetetraacetic acid, disodium salt, dihydrate solution**, 0.1 M (aq)

Prepared by dissolving 37.22 ± 0.01g ethylenediaminetetraacetic acid, disodium salt, dihydrate, in ultrapure water in a 1000 mL volumetric flask. Use an ultrasonic bath at 30 °C to aid preparation. Store at ambient room temperature for no longer than 1 month.

A5.18 **Ammonium acetate solution**, 1 M (aq)

Prepared by dissolving 7.71 ± 0.01 g ammonium acetate in ultrapure water in a 100 mL volumetric flask. Store at ambient room temperature for no longer than 1 month.

A5.19 **Certified reference materials**

Certified reference materials are stored at room temperature in the dark or under refrigeration at 3 ± 2 °C or in a freezer at less than -17 °C according to the manufacturer's instruction. The expiry date is recorded on the certificate when supplied. Calibration and AQC certified reference materials are sourced from separate independent suppliers where possible (only if

this is not possible – then as a minimum – different lot numbers from the same supplier are used).

A5.20 **Certified reference stock solution, internal standard, Glyphosate 1,2-$^{13}C_2$ ^{15}N**, 100 mg L^{-1} in ultrapure water

Certified reference stock solutions are purchased from approved analytical suppliers, e.g., LGC, part number DRE-XA14050100WA. Shelf life: as stated by supplier. Store in the dark under refrigeration at 3 ± 2 °C.

A5.21 **Certified reference stock solution, internal standard, Aminomethyl phosphonic acid (AMPA) ^{13}C ^{15}N**, 100 mg L^{-1} in ultrapure water

Certified reference stock solutions are purchased from approved analytical suppliers, e.g., LGC, part number DRE-XA10205100WA. Shelf life: as stated by supplier. Store in the dark under refrigeration at 3 ± 2 °C.

A5.22 **Calibration stock solutions**, 400 mg L^{-1} in 9:1, ultrapure water: acetonitrile

Accurately weigh out 20 mg of each of the target compounds to four decimal places. Transfer to separate 50 mL volumetric flasks, dissolve and dilute to the 50 mL mark with 9:1, ultrapure water : acetonitrile, stopper and mix. Transfer contents to a 125 mL PE/HD bottle and store in the dark under refrigeration at 3 ± 2 °C for no longer than 6 months or the expiry date of the certified reference material, if before.

Note: The concentration of the stock solution (mg L^{-1}) is determined by applying the following calculation: $C_{STOCK} = M_{CERTIFIED}/V_{FLASK}$

Where; C_{STOCK} is the concentration of the stock solution, $M_{CERTIFIED}$ is the mass of certified reference material in mg and V_{FLASK} is the volume of solution in litres.

A5.23 **Calibration intermediate solution**, 4.00 mg L^{-1} in 9:1, ultrapure water : acetonitrile

Using a micropipette transfer 200 µL of each of the target compound calibration stock solutions to a 50 mL volumetric flask and make up to volume with 9:1, ultrapure water : acetonitrile, stopper and mix. Transfer contents to a 125 mL PE/HD bottle and store in the dark under refrigeration at 3 ± 2 °C for no longer than 6 months or the first expiry date of the calibration stock solutions, if before.

A5.24 **Calibration spiking solution**, 0.020 mg L^{-1} in 9:1, ultrapure water : acetonitrile

Using a micropipette transfer 250 µL of the calibration intermediate solution to a 50 mL volumetric flask and make up to volume with 9:1, ultrapure water : acetonitrile, stopper and mix. Transfer contents to a 125 mL PE/HD bottle and store in the dark under refrigeration at 3 ± 2 °C for no longer than 2 months, or the first expiry date of the calibration stock solutions, if before.

A5.25 **Calibration working solutions**, 0 (Blank), 0.010, 0.050, 0.125, 0.200 and 0.250 µg L^{-1} in calibration / AQC matrix water

Remove the calibration spiking solution from refrigeration and allow to equilibrate to room temperature. On each occasion a batch of samples is analysed prepare calibration working solutions by adding the volume of calibration spiking solution in the table below into 20 mL of calibration / AQC matrix water in a 50 mL PE/HD centrifuge tube. Then follow the analytical procedure from **A8.4**.

Calibration Working Solution	Calibration Spiking Solution Added (µL)	Concentration (µg L^{-1})
Cal Blank	0	0.000
Cal Level 1	10	0.010
Cal Level 2	50	0.050
Cal Level 3	125	0.125
Cal Level 4	200	0.200
Cal Level 5	250	0.250

A5.26 **Internal standard spiking solution**, 0.040 mg L^{-1} in 9:1, ultrapure water : acetonitrile

Using a micropipette transfer 40 µL of each certified internal standard stock solution to a 100 mL volumetric flask and make up to volume with 9:1, ultrapure water : acetonitrile, stopper and mix. Transfer contents to a 125 mL PE/HD bottle and store in the dark under refrigeration at 3 ± 2 °C for no longer than 2 months or the first expiry date of the certified internal standard stock solutions, if before.

A5.27 **AQC stock solutions**, 400 mg L^{-1} in 9:1, ultrapure water : acetonitrile

Accurately weigh out 20 mg of each of the target compounds to four decimal places. Transfer to separate 50 mL volumetric flasks, dissolve and dilute to the 50 mL mark with 9:1, ultrapure water : acetonitrile, stopper and mix. Transfer contents to a 125 mL PE/HD bottle and store in the dark under refrigeration at 3 ± 2 °C for no longer than 6 months or the expiry date of the certified reference material, if before.

Note: The concentration of the stock solution (mg L^{-1}) is determined by applying the following calculation: $C_{STOCK} = M_{CERTIFIED}/V_{FLASK}$

Where; C_{STOCK} is the concentration of the stock solution, $M_{CERTIFIED}$ is the mass of certified reference material in mg and V_{FLASK} is the volume of solution in litres.

A5.28 **AQC intermediate solution**, 4.00 mg L^{-1} in 9:1, ultrapure water : acetonitrile

Using a micropipette transfer 200 µL of each of the target compound AQC stock solutions to a 50 mL volumetric flask and make up to volume with 9:1, ultrapure water : acetonitrile, stopper and mix. Transfer contents to a 125 mL PE/HD bottle and store in the dark under refrigeration at 3 ± 2 °C for no longer than 6 months or the first expiry date of the AQC stock solutions, if before.

A5.29 **AQC spiking solution**, 0.020 mg L^{-1} in 9:1, ultrapure water : acetonitrile

Using a micropipette transfer 250 µL of the AQC intermediate solution to a 50 mL volumetric flask and make up to volume with 9:1, ultrapure water : acetonitrile, stopper and mix. Transfer contents to a 125 mL PE/HD bottle and store in the dark under refrigeration at 3 ± 2 °C for no longer than 2 months or the first expiry date of the AQC stock solutions, if before.

A5.30 **AQC working solution**, 0.100 µg L^{-1} in calibration / AQC matrix water

Remove the AQC spiking solution from refrigeration and allow to equilibrate to room temperature. On each occasion a batch of samples is analysed prepare an AQC Blank and an AQC Spike by adding the volume of AQC spiking solution in the table below into 20 mL of calibration / AQC matrix water in a 50 mL PE/HD centrifuge tube. Then follow the analytical procedure from **A8.4**.

AQC Working Solution	AQC Spiking Solution Added (µL)	Concentration (µg L^{-1})
AQC Blank	0	0.000
AQC Spike	100	0.100

A5.31 **Quaternary Pump, Mobile Phase A, used for On-line Solid Phase Extraction, Water, 0.1% formic acid.**

Dispense 1000 mL of ultrapure water into a 1000 mL measuring cylinder and add 1 mL of formic acid. Stopper and invert to mix.

A5.32 Quaternary Pump, Mobile Phase B, used for On-line Solid Phase Extraction, Methanol, 0.1% formic acid.

Dispense 1000 mL of LCMS grade Methanol into a 1000 mL measuring cylinder and add 1 mL of formic acid. Stopper and invert to mix.

A5.33 Binary Pump, Mobile Phase A used for the analytical column, Water, 5mM ammonium acetate, 0.01% ammonia, pH 9.25 ± 0.25

Prepared in a 1000 mL stoppered measuring cylinder by adding 5 mL of 1M ammonium acetate and 0.4 mL of ammonia solution (25%) into the measuring cylinder then making up to the 1000 mL mark with ultrapure water. Stopper and invert to mix. Check pH using a manual pH meter.

A5.34 Binary Pump, Analytical, Mobile Phase B used for the analytical column, Acetonitrile, LC-MS grade

A6 Apparatus

In addition to normal laboratory glassware and apparatus the following are required.

A6.1 Refrigerator and freezer, set at 3 ± 2 °C and less than -17 °C, respectively with valid calibration status.

A6.2 Analytical Balance, four decimal place of a gram, for the preparation of stock solutions

A6.3 Top Pan Balance, two decimal places of a gram.

A6.4 Ultrasonic Bath

A6.5 Centrifuge

A6.6 Micropipettes, positive displacement, with valid calibration status, various sizes

A6.7 Electronic digital autopipette (EDP) with valid calibration status, 10 mL and 20 mL capacity

A6.8 Dispenser, bottle-top

A6.9 Dispenser, bottle-top, acid resistant, used for formic acid

A6.10 Flasks, volumetric, glass, various sizes

A6.11 Measuring Cylinder, stoppered, 1000 mL capacity, used for mobile phase preparation

A6.12 Sample bottles – polyethylene high-density, PE/HD, bottles, for sample collection add sodium thiosulfate 1.8% $^{w}/_{v}$ solution for sample preservation.

A6.13 Centrifuge tubes – 50 mL, polypropylene, used for analytical procedure

A6.14 Auto-sampler vials, glass, 2 mL capacity

A6.15 Pasteur pipettes, glass, disposable

A6.16 Liquid Chromatograph Mass Spectrometer

A high-performance liquid chromatograph triple quadrupole mass spectrometer system fitted with binary and quaternary pumps, auto-sampler and column oven connected to a triple quadrupole mass spectrometer capable of unit mass resolution and operating in dynamic multiple reaction monitoring (dMRM) mode, with an appropriate data station, e.g., Agilent, 1260 series liquid chromatograph and 6470 triple quadrupole mass spectrometer

The following instrument conditions have been used and found to be satisfactory.

Typical Operating Conditions for the Liquid Chromatograph

Quaternary Pump, On-line Solid Phase Extraction Conditions	
Quaternary Pump	Agilent 1260 Infinity Series
On-line SPE Cartridge	Agilent PLRP-S cartridge, 12.5 mm x 2.1 mm, 15-20 µm particles, part number 5982-1271
Temperature	Ambient, room temperature
Injection Volume	900 µL
Mobile Phase A Mobile Phase B	Water, 0.1% formic acid Methanol, 0.1% formic acid
Flow Rate (Loading)	0.900 mL minute^{-1}

Quaternary Pump, On-line Solid Phase Extraction Gradient Program		
Time (minutes)	Flow Rate (mL minute^{-1})	Mobile Phase B (%)
0.00	0.900	2
0.10	0.900	2
0.25	0.900	98
0.75	0.900	98
0.90	0.900	2
3.90	0.900	2
4.00	0.450	2
9.50	0.450	2
9.60	0.900	2
10.00	0.900	2

Auto-Sampler	
Injector Program	**Command**
DRAW	Draw 900 µL from sample with 360 µL minute^{-1} using default offset
WASH	Wash needle as specified in the method
VALVE	Switch valve to "Mainpass"
WAIT	Wait 2.50 minutes
REMOTE	Set remote line "Start" for 125 ms
WAIT	Wait 4.00 minutes
EJECT	Eject maximum volume to seat with 200 µL minute^{-1}

Binary Pump, Analytical	
Binary Pump	Agilent 1260 Infinity Series
Analytical Column	Waters XBridge BEH C18, 50 mm x 2.1 mm, 3.5 µm particles, part number 186003021
Analytical Guard Column	Waters XBridge BEH C18, 5 mm x 2.1 mm, 3.5 µm particles, part number 186007766
Temperature	60 °C
Injection Volume	900 µL
Mobile Phase A Mobile Phase B	Water, 5 mM Ammonium acetate, 0.01% Ammonia, pH 9.25 ± 0.25 Acetonitrile
Flow Rate	0.300 mL minute^{-1}

Binary Pump, Analytical Gradient Program		
Time (minutes)	Flow Rate (mL minute^{-1})	Mobile Phase B (%)
0.00	0.300	2
0.50	0.200	2
1.00	0.200	2
2.00	0.200	20
7.00	0.200	45
7.25	0.300	90
9.50	0.300	90
9.75	0.300	2
10.00	0.300	2

Typical Operating Conditions for the Tandem Mass Spectrometer

Triple Quadrupole Mass Spectrometer	
Mass Spectrometer	Agilent 6470
Ionisation Source	Jet Stream Electrospray Ionisation
Polarity	Negative

Ionisation Source Parameters	
Drying Gas Temperature	300 °C
Drying Gas Flow	8 L minute^{-1}
Nebulizer	40 psi
Sheath Gas Temperature	300 °C
Sheath Gas Flow	11 L minute^{-1}
Nebulizer Voltage	2000 V
Capillary Voltage	4000 V

Time Segment 1 – 3:				
Time Segment #	Time	Div Valve	Delta EMV (V)	Store
1	0.00	To Waste	0	No
2	2.00	To MS	400	Yes
3	7.00	To Waste	0	No

Dynamic MRM							
Compound Name	Prec. Ion	MS1 Res	Prod. Ion	MS2 Res	Frag. (V)	CE (V)	Cell Acc (V)
Glyphosate 13C2 15N	393.1	Unit	171.0	Wide	105	8	5
Glyphosate 13C2 15N	393.1	Wide	153.0	Wide	105	28	5
Glyphosate	390.1	Unit	168.0	Wide	105	8	5
Glyphosate	390.1	Wide	150.0	Wide	105	28	5
AMPA 13C 15N	334.1	Wide	138.0	Wide	75	12	5
AMPA 13C 15N	334.1	Unit	112.0	Wide	75	2	5
AMPA	332.1	Wide	136.0	Wide	75	12	5
AMPA	332.1	Unit	110.0	Wide	75	2	5

Typical Retention Times and SRM Transition Information

Compound Name	Rt (min)	Quantifier SRM		Qualifier SRM		Qualifier SRM Ratio (%)
		Precursor	Product	Precursor	Product	
Glyphosate 13C2 15N	4.18	393.1	171.0	393.1	153.0	35
Glyphosate	4.19	390.1	168.0	390.1	150.0	35
AMPA 13C 15N	5.14	334.1	112.0	334.1	138.0	50
AMPA	5.15	332.1	110.0	332.1	136.0	50

All compound retention times will vary depending on the condition of the analytical column therefore they should only be used as a guide. Compound elution order will remain unchanged.

A7 Sample collection and preservation

Sampling, samples are taken in 125 mL PE/HD bottles, with screw tops (lined with an appropriate inert material) containing 0.125 mL sodium thiosulfate pentahydrate solution, 1.8% w/v or 18 g L^{-1}.

A8 Analytical Procedure

A8.1 Remove samples from storage and allow samples to equilibrate to room temperature.

A8.2 Remove internal standard spiking solution from refrigeration. Allow contents to equilibrate to room temperature.

A8.3 Add 20 mL of sample to a 50 mL polypropylene centrifuge tube using a calibrated electronic digital autopipette.

A8.4 Add 50 µL of internal standard spiking solution using a micropipette. This will produce a nominal concentration of 0.100 µg L^{-1} for each internal standard compound.

A8.5 Add 2.50 mL of di-sodium tetraborate, decahydrate solution, 40 mM, using a fixed volume 2.5 mL dispenser. This will produce a sample of approximately pH 9.

Note: Pump borate buffer bottle top dispenser at least twice into a waste container before using the dispenser to add borate buffer.

A8.6 Add 2.50 mL of 9-fluorenylmethyl chloroformate solution (FMOC-Cl), 6.5mM, using a fixed volume 2.5 mL dispenser. This will produce an FMOC-Cl concentration of 650 µM and a sample composition of 10% acetonitrile. Cap and shake vigorously to mix.

Note: Pump FMOC-Cl bottle top dispenser at least twice into a waste container before using the dispenser to add FMOC-Cl.

A8.7 Leave sample for at least two hours at room temperature for the FMOC-Cl derivatization reaction to take place.

A8.8 Add 0.25 mL of formic acid, using a 0.25-2.50 mL bottle top dispenser in a fume cabinet. This will stop the FMOC-Cl derivatization reaction and acidify the sample to approximately pH 3.

Note: Pump formic acid bottle top dispenser at least twice into a waste container before using the dispenser to add formic acid. This action will ensure the formic acid added has not been in contact with the bottle top dispenser for any period of time and that the first 0.25 mL of formic acid is dispensed accurately.

A8.9 Add 1.00 mL of ethylenediaminetetraacetic acid, disodium salt, dihydrate solution, 0.1M, using a 1.0 - 10.0 mL electronic digital pipette. This will reduce complex reformation by the FMOC derivatives and cations.

A8.10 Add 5 mL of dichloromethane to each sample using a bottle top dispenser, replace sample cap securely and shake vigorously.

Note: Pump dichloromethane bottle top dispenser at least twice into a waste container before using the dispenser to add dichloromethane.

A8.11 Transfer centrifuge tube to a centrifuge and centrifuge for 2 minutes at a setting of 2800 rpm.

A8.12 Using a Pasteur pipette transfer standards and samples (aqueous layer) to 2 mL auto-sampler vials ready for instrumental analysis.

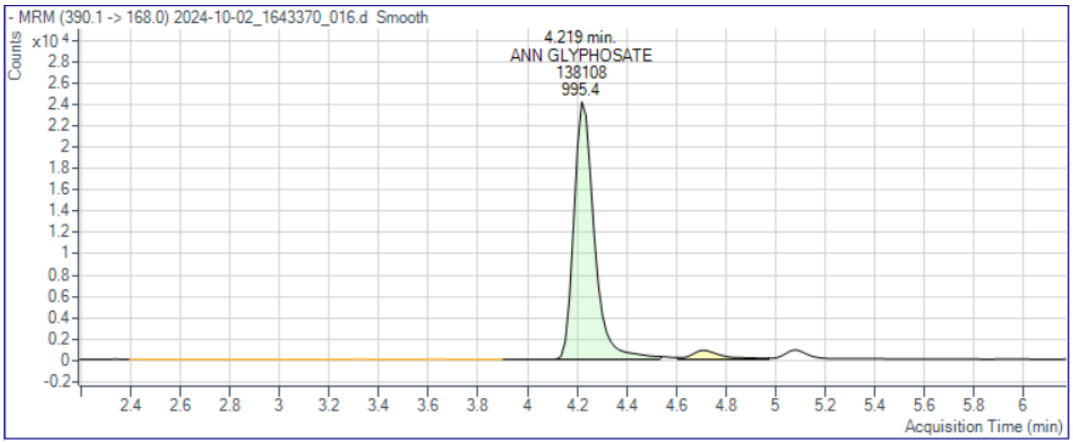
A9 Calculation

Results for target compounds are calculated using the following equation: -

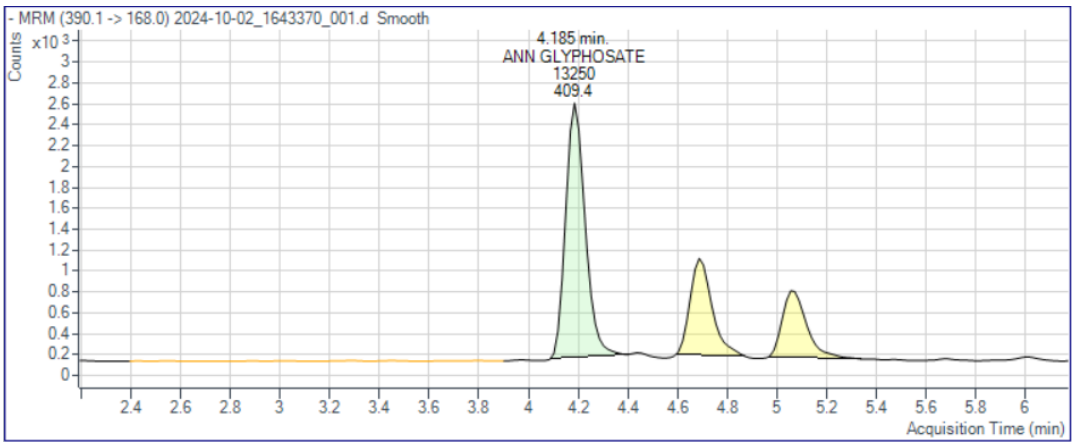
$$\text{Concentration } (\mu g\, L^{-1}) = \frac{\left(\frac{\text{Sample Response}}{\text{Sample ISTD Response}}\right)}{\left(\frac{\text{Standard Response}}{\text{Standard ISTD Response}}\right)} \times \text{Standard Concentration } (\mu g\, L^{-1})$$

Figure A1 - Typical chromatograms

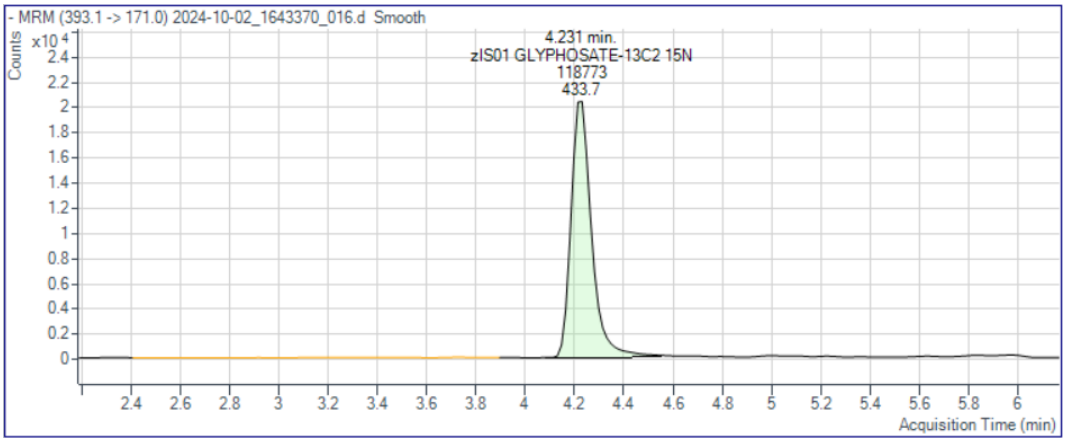
Glyphosate, 0.100 µg L^{-1}, Selected Reaction Monitoring (SRM) transition, 390.1>168.0,



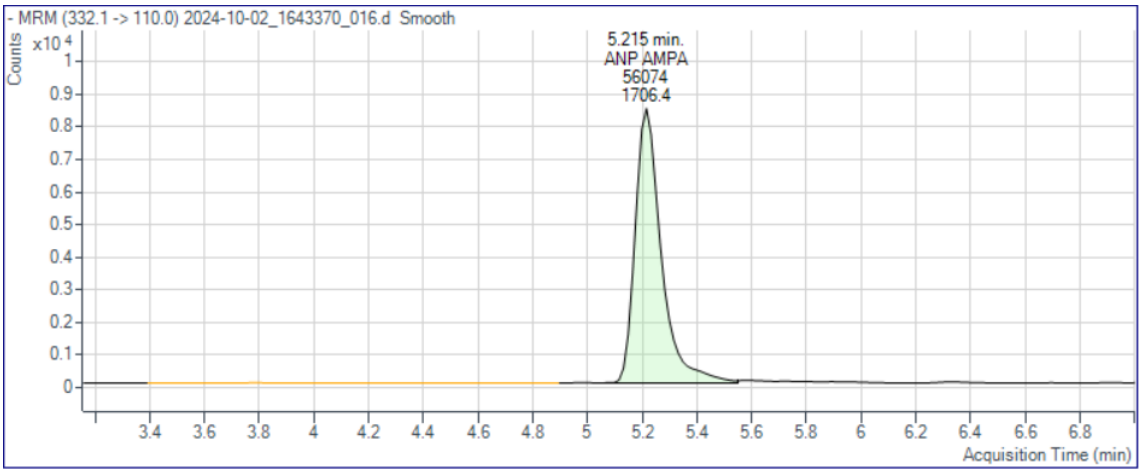
Glyphosate, 0.010 µg L^{-1}, Selected Reaction Monitoring (SRM) transition, 390.1>168.0,



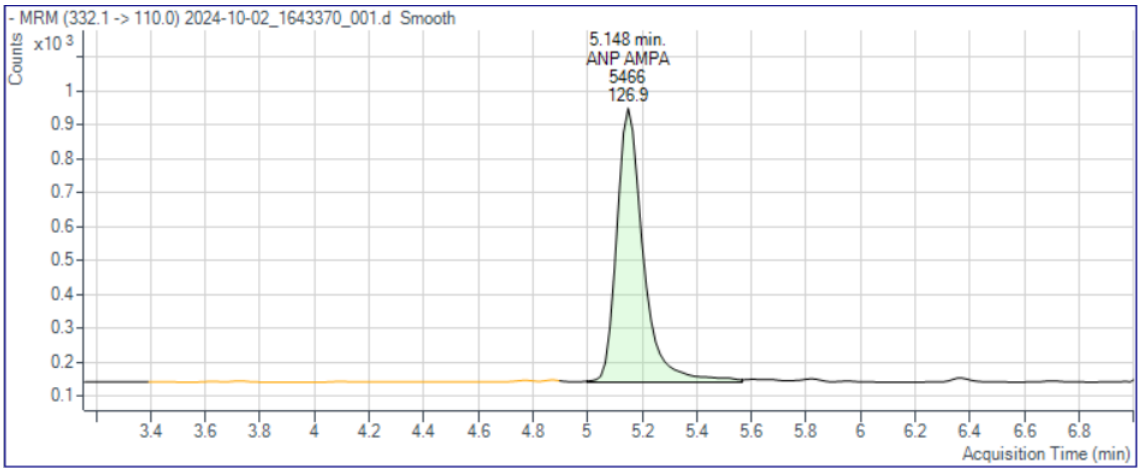
Glyphosate 13C2 15N, 0.100 µg L^{-1}, Selected Reaction Monitoring (SRM) transition, 393.1>171.0,



AMPA, 0.100 µg L^{-1}, Selected Reaction Monitoring (SRM) transition, 332.1>110.0,



AMPA, 0.010 µg L^{-1}, Selected Reaction Monitoring (SRM) transition, 332.1>110.0,



AMPA 13C 15N, 0.100 µg L^{-1}, Selected Reaction Monitoring (SRM) transition, 334.1>112.0,

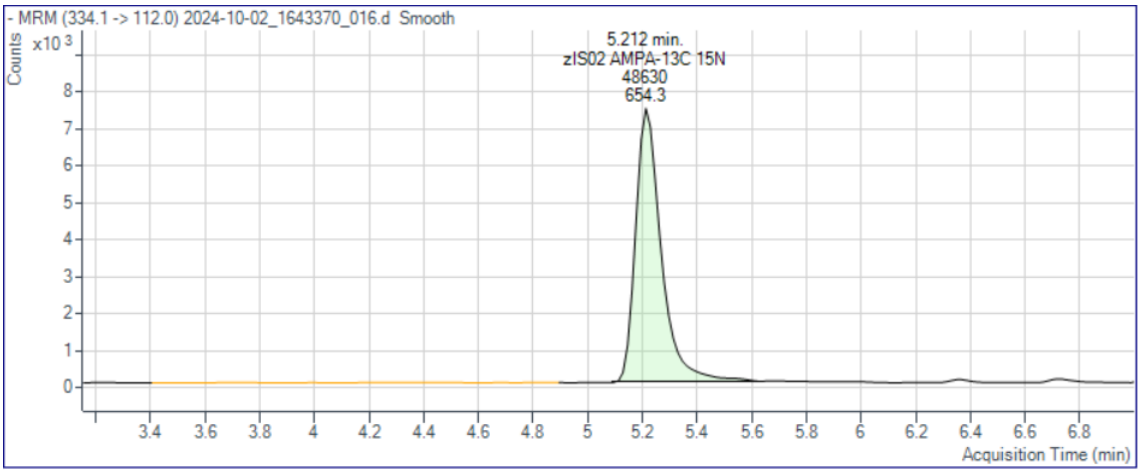


Table A1 - Method performance data

Method performance data provided by ALS.

Determinand	LoQ	UoM	Direct Standards				Elvington Treated (Hard)	
			Low Standard		High Standard		PCV Spike	
	(ng L^{-1})	(%)*	Bias (%)	RSD (%)	Bias (%)	RSD (%)	Bias (%)	RSD (%)
Glyphosate	5	6.60	-1.10	2.30	-0.50	1.60	-0.40	3.20
AMPA	6	7.30	-0.60	3.50	-0.80	2.40	0.10	2.50

* Uncertainty of Measurement, UoM, data from method validation exercise.

Uncertainty Of Measurement

The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2, which gives a level of confidence of approximately 95%.

Uncertainty of measurement is calculated on an ongoing basis according to Standing Committee of Analysts Blue Book 'Estimation of Uncertainty of Measurement for Chemical and Physico-chemical Determinands in Drinking Water 2018' and Table A3 of The Water Supply (Water Quality) (Amendment) Regulations 2018.

Below is an example of a year's rolling UoM value and its target threshold for Glyphosate (Data provided by ALS).

Analyte	UoM (%) from method validation exercise	Yearly rolling UoM (%) (1/4/2024 – 31/3/2025)
Glyphosate	6.6%	18.0%
AMPA	7.3%	15.4%

B The Determination of Glyphosate, AMPA and Glufosinate in Raw and Potable Water by automated multi-purpose sampler (MPS) FMOC derivatisation and analysis by Liquid Chromatography with Mass Spectrometry Detection

B1 Performance characteristics of the method

B1.1	**Substances determined**	Glyphosate (N-(phosphonomethyl)glycine), AMPA (Aminomethyl)phosphonic acid), Glufosinate-ammonium.
B1.2	**Type of sample**	Raw waters (ground and surface), potable waters.
B1.3	**Basis of method**	Samples are derivatised with 9-fluorenylmethyl chloroformate. This is all automated using a stand-alone MPS type system and the aqueous portion of the extract is analysed by liquid chromatography with mass spectrometric detection.
B1.4	**Range of application**	Typically up to 0.300 µg L^{-1}
B1.5	**Standard deviation**	See Table B1**.**
B1.6	**Limit of Quantification**	Glyphosate 0.015 µg L^{-1}, Glufosinate 0.014 µg L^{-1} and AMPA 0.016 µg L^{-1}, based on a Low Level LOD / LOQ standard (0.002 µg L^{-1}). See Table B1.
B1.7	**Bias**	See Table B1.

B2 Principle

Using an automated Multipurpose Sampler (MPS) station, water samples are treated with 100 µL of internal standard, 200 µL of EDTA, 250 µL of borate buffer, 625 µL of FMOC (9-fluorenylmethyl chloroformate) derivatising reagent, 63 µL of formic acid and 300 µL of dichloromethane for a clean-up step. The aqueous portion of the extracts are then analysed by high performance liquid chromatography (HPLC) coupled to detection by triple quadrupole mass spectrometry in negative ion electrospray mode with multiple reaction monitoring. The analytes are separated by an LC column and are identified and quantified with mass spectrometric detection (MSD) in selected reaction monitoring mode. Quantitation is based on an internal standardisation procedure.

This automated method reduces the need for a solid phase extraction, a concentration step, has smaller sample sizes and bottles, and a reduction in manual preparation steps and lower consumables costs.

B3 Interferences

HPLC-MS/MS is an extremely selective technique, and interferences are expected to occur only rarely. Any interfering compound would have to display the identical SRM transition at the same retention time as the analyte; this is extremely unlikely in potable water samples. However, any compound, which passes through the extraction procedure, and has a similar liquid chromatographic retention time and mass spectrometric properties to the compound of interest, will cause interference. Samples containing high humic or fulvic content have been shown not to cause significant ion suppression.

B4 Hazards/COSHH

Analysts using this method should familiarise themselves with the COSHH and risk assessments for the analysis. See Safety Data Sheet (SDS) provided by the chemical manufacturer/supplier.

B4.1 **Acetonitrile** (CH_3CN)
CAS No. 75-05-8

B4.2 **Dichloromethane** (CH_2Cl_2)
CAS No. 75-09-2

B4.3 **Methanol** (CH_3OH)
CAS No. 67-56-1

B4.4 **2-Propanol (**C_3H_8O)
CAS No. 67-63-0

B4.5 **9-Fluorenylmethylchloroformate** ($C_{15}H_{11}ClO_2$)
CAS No. 28920-43-6

B4.6 **Formic acid** (CH_2O_2)
CAS No. 64-18-6

B4.7 **di-Sodium tetraborate, decahydrate** ($Na_2B_4O_7.10H_2O$)
CAS No. 1303-96-4

B4.8 **5% DMDCS in Toluene (Sylon CT)**
CAS No. 75-78-5; 108-88-3

B5 Reagents

All reagents should be of sufficient purity that they do not give rise to interferences during the analysis and distilled, deionised or similar grade water should be used throughout. A procedural blank should be run with each batch of samples to check for interferences. All solutions should be mixed well prior to use.

B5.1 **Acetonitrile**, LC-MS grade

B5.2 **Dichloromethane**, alcohol free

B5.3 **Methanol**, LC-MS grade

B4.4 **2-Propanol,** HPLC Grade

B5.5 **Formic acid**, LC-MS grade

B5.6 **Sodium thiosulfate pentahydrate**, AR grade

B5.7 **di-Sodium tetraborate, decahydrate**, AR grade

B5.8 **9-Fluorenylmethyl chloroformate**, HPLC grade

B5.9 **Ethylenediaminetetraacetic acid, disodium salt, dihydrate**, AR grade

B5.10 **Agilent Infinity Lab Deactivator Additive,** AR grade.
(Methylenediphosphonic Acid also known as Medronic Acid)

B5.11 **5% DMDCS in Toluene (Sylon CT),** GC derivatization grade

B5.12 Water – Pesticide free e.g. De-ionised MilliQ water or HPLC grade

Ultrapure grade water should contain no measurable amounts of the compounds of interest. Millipore 'MilliQ SP' water has been found to be satisfactory. This water is used to prepare aqueous stock, intermediate and working solutions.

B5.13 Mineral Water, Medium Hardness – Pesticide free, e.g., Buxton or Brecon Carreg or equivalent.

Matrix water should contain no measurable amounts of the compounds of interest. This 'real' water is used to prepare the calibration / AQC matrix water.

B5.14 Sodium thiosulfate solution, 3.00% $^{w}/_{v}$

Prepared by dissolving 46.0 ± 0.1g of sodium thiosulfate pentahydrate in 1 L of ultrapure water in a volumetric flask. This is used as preservative solution for all samples and to produce the calibration and AQC matrix water. Store at room temperature for no longer than 6 months.

Larger or smaller volumes can be prepared if required, by increasing or reducing the volumes used.

B5.15 di-Sodium tetraborate, decahydrate solution, 50 g L^{-1}

Prepared by dissolving 50 ± 1 g di-sodium tetraborate, decahydrate in ultrapure water in a 1000 mL volumetric flask. Use a magnetic stirrer to aid in preparation. Store at ambient room temperature for no longer than 3 months.

B5.16 9-Fluorenylmethyl chloroformate, (FMOC) working solution, 2.5 g L^{-1} in acetonitrile.

Prepared by dissolving 2.5 ± 0.1 g FMOC in acetonitrile in a 1000 mL volumetric flask. Mix thoroughly by inversion. Store under refrigeration at 5 ± 3 °C for no longer than 3 months.

Note: Once decanted into the MPS reagent reservoir, allow the FMOC to reach room temperature before initiating the run. This will need replacing every 3 days due to lack of refrigeration.

B5.17 Ethylenediaminetetraacetic acid, disodium salt, dihydrate solution, 0.1 M (aq) (Purchased from Honeywell or equivalent)

B5.18 Certified reference materials

Certified reference materials are stored at room temperature in the dark or under refrigeration at 3 ± 2 °C or in a freezer at less than -17 °C according to the manufacturer's instruction. The expiry date is recorded on the certificate when supplied. Calibration and AQC certified reference materials are sourced from separate independent suppliers where possible (only if this is not possible – then as a minimum – different lot numbers from the same supplier are used). It is good practice to ensure that the calibration stock reference material is ISO 17034 accredited.

B5.19 Certified reference stock solution, Glyphosate, 100 mg L^{-1} in ultrapure water.

Certified reference stock solutions are purchased from approved analytical suppliers, e.g., LGC, part number DRE- XA14050000WA. Shelf life: as stated by supplier. Store in the dark under refrigeration at 5 ± 3 °C.

Alternatively, a neat reference powder can be used. See below.

Stock Glyphosate solution from solid reference material
Glyphosate, part number DRE-C14050000 available from LGC or equivalent. Refer to manufacturers' guidelines for storage conditions and expiry as stipulated on the certificate.

Into a 25 mL volumetric flask containing approximately 5 mL deionised water add approximately 30 mg of glyphosate reference material. Record the exact weight added to the solution to ± 0.01 mg and make a note of the purity of the material. Make up to volume with deionised water and calculate the concentration in mg L^{-1} accordingly:

Concentration (mg L^{-1}) = Weight (mg) x 40 x (% Purity/100)

For example, a solution prepared from 31.13 mg of reference material with 99.7% purity:

Concentration (mg L^{-1}) = 31.13 x 40 x (99.7/100)
= 31.13 x 40 x 0.997
= 1241 mg L^{-1}

Store in a fridge at 5 ± 3 °C and renew every year.

Intermediate Glyphosate Standard, 100 mg L^{-1} in ultrapure water.

Once a stock glyphosate solution has been prepared from a solid reference material calculate the volume of stock solution required to produce a 100

mg L^{-1} intermediate solution in 10 mL deionised water. Spike an exact amount of the solution into a volumetric flask containing approximately 8 mL deionised water – the actual amounts are likely to be approximately 833 µL and should be spiked with a tolerance of 1% (see example below). Make up to the volume with deionised water and store at 5 ± 3 °C. Renew every 6 months:

Volume for spiking solution (µL) = 1 000 000 / Conc. reference stock (mg L^{-1})

For example, using the 1241 mg L^{-1} solution noted

Volume of spiking solution (µL) = 1 000 000 / 1241
= 806 µL spiked and made up to 10 mL.

B5.20 **Certified reference stock solution, AMPA**, 100 mg L^{-1} in ultrapure water.

Certified reference stock solutions are purchased from approved analytical suppliers, e.g., LGC, part number DRE-XA10205000WA. Shelf life: as stated by supplier. Store in the dark under refrigeration at 5 ± 3 °C.

B5.21 **Certified reference stock solution, Glufosinate**, 100 mg L^{-1} in ultrapure water.
Certified reference stock solutions are purchased from approved analytical suppliers, e.g., HPC Standards, part number 678211. Shelf life: as stated by supplier. Store in the dark under refrigeration at 5 ± 3 °C.

B5.22 **Certified reference stock solution, internal standard, Glyphosate 1,2-$^{13}C_2$ ^{15}N**, 100 mg L^{-1} in ultrapure water

Certified reference stock solutions are purchased from approved analytical suppliers, e.g., LGC, part number DRE-XA14050100WA. Shelf life: as stated by supplier. Store in the dark under refrigeration at 5 ± 3 °C. Alternatively a neat reference powder can be used.

B5.23 **Certified reference stock solution, internal standard, Aminomethyl phosphonic acid (AMPA) ^{13}C ^{15}N**, 100 mg L^{-1} in ultrapure water

Certified reference stock solutions are purchased from approved analytical suppliers, e.g., LGC, part number DRE-XA10205100WA. Shelf life: as stated by supplier. Store in the dark under refrigeration at 5 ± 3 °C.

B5.24 **Intermediate Mixed Calibration Spiking Solution**, 500 µg L^{-1} in ultrapure water

Using a micropipette transfer 250 µL of each of the 100 µg L^{-1} Glyphosate, Glufosinate-ammonium and AMPA solutions to a 50 mL volumetric flask containing ultrapure water. Transfer to 100 mL Azlon PE/HD bottle and store in the dark under refrigeration at 5 ± 3 °C for no longer than 2 weeks. Alternatively, you can reduce volumes to prolong the use of the stocks. In this instance, reduce all volumes listed above by a factor of 5.

B5.25 **Daily Working Mixed Calibration Spiking Solution**, 5 µg L^{-1} in ultrapure water

Using a micropipette transfer 500 µL of the 500 µg L^{-1} Intermediate Mixed Calibration solution to a 50 mL volumetric flask containing ultrapure water. Transfer to 100 mL Azlon PE/HD bottle and store in the dark under refrigeration at 5 ± 3 °C. Shelf life: 1 day.

B5.26 **Working Spiking Internal Standard Solution**, 200 µg L^{-1} in ultrapure water

Using a micropipette transfer 100 µL each of 100 mg L^{-1} glyphosate 2-13C15N and 100 mg L^{-1} AMPA 13C15N solutions into a 50 mL volumetric flask containing 50 mL ultrapure water. Transfer to 100 mL Azlon PE/HD bottle and store in the dark under refrigeration at 5 ± 3 °C for no longer than 2 weeks.

Note: When operating the MPS, pour the internal standard into the designated labelled 10 mL amber vial and cap. Allow to reach room temperature before initiating the run. Renew with each run.

B5.27 **Daily Calibration working solutions**, 0 (Blank), 0.010, 0.025, 0.050, 0.100, 0.200 and 0.300 µg L^{-1} in medium hardness mineral water.

Remove the daily mixed working calibration spiking solution from refrigeration and allow to equilibrate to room temperature. On each occasion a batch of samples is analysed prepare calibration working solutions by adding the volume of calibration spiking solution in the table below into 10 mL screw cap clear vials of 5 mL medium mineral water. Cap with magnetic screw caps lids and shake briefly to mix. Then follow the analytical procedure from **B8.3.**

Working Calibration Level	Volume of Medium Mineral Water (mL) in 10 mL vial	Working Calibration Spiking Solution Added (µL)	Concentration (µg L^{-1})
Cal Level 0	5	0	0.000
Cal Level 1	5	10	0.010
Cal Level 2	5	25	0.025
Cal Level 3	5	50	0.050
Cal Level 4	5	100	0.100
Cal Level 5	5	200	0.200
Cal Level 6	5	300	0.300

Alternatively, the above spiking protocol may be programmed into the MPS software and automated.

B5.28 **AQC stock solutions**, 100 mg L^{-1} in ultrapure water.

Analytical Quality Control standards are analysed with each batch of samples.

AQC Solutions are made up in house, by a separate analyst who made the calibration standards. AQC solutions must have a different LOT number to the solutions used for standards. Alternatively, a separate solution can be obtained from a different manufacturer.

Equivalent reference materials as to the calibration standards may be used (with separate lot numbers). Refer to manufacturer's guidelines for storage conditions and expiry as stipulated on the certificates.

B5.29 **Intermediate Mixed AQC Spiking Solution**, 500 µg L^{-1} in ultrapure water

Using a micropipette transfer 250 µL of each of the 100 µg L^{-1} Glyphosate, Glufosinate-ammonium and AMPA AQC solutions to a 50 mL volumetric flask containing ultrapure water. Transfer to 100 mL Azlon PE/HD bottle and store in the dark under refrigeration at 5 ± 3 °C for no longer than 2 weeks. Alternatively, you can reduce volumes to prolong the use of the stocks. In this instance, reduce all volumes listed above by a factor of 5.

5.30 **Daily Working Mixed AQC Spiking Solution**, 5 µg L^{-1} in ultrapure water

Using a micropipette transfer 500 µL of the 500 µg L^{-1} Intermediate Mixed AQC solution to a 50 mL volumetric flask containing ultrapure water. Transfer to 100 mL Azlon PE/HD bottle and store in the dark under refrigeration at 5 ± 3 °C for no longer than 1 day.

B5.31 **Daily AQC working solution**, 0.100 µg L^{-1} in medium matrix / mineral water.

Remove the daily mixed working AQC spiking solution from refrigeration and allow to equilibrate to room temperature. On each occasion a batch of samples is analysed prepare AQC working spikes by adding the volume of AQC spiking solution in the table below into 10 mL screw cap clear vials of 5 mL medium mineral water. Cap with magnetic screw caps lids and shake briefly to mix.

Working AQC Level	Volume of Medium Mineral Water (mL) in 10 mL vial	Working AQC Spiking Solution Added (µL)	Concentration (µg L^{-1})
AQC Blank	5	0	0.000
AQC Spike	5	100	0.100

Alternatively, the above spiking protocol may be programmed into the MPS software and automated.

B5.32 **Mobile Phase A**

Dispense 1000 mL of ultrapure water into a 1000 mL measuring cylinder and add 1 mL of Agilent Infinity Lab Deactivator Additive. Spike 100 µL of formic acid. Stopper and invert to mix.

Alternatively, you may wish to reduce volumes for shorter runs. In this instance, reduce all volumes listed above by half. Store at room temperature and renew with each analytical run.

Note: failure to add formic acid will result in later peak elution, poorer peak shapes and sensitivity and response loss on AMPA.

B5.33 **Mobile Phase B**

Dispense 1000 mL of LCMS grade Methanol into a 1000 mL measuring cylinder and add 1 mL of Agilent Infinity Lab Deactivator Additive. Stopper and invert to mix.

Alternatively, you may wish to reduce volumes for shorter runs. In this instance, reduce all volumes listed above by half. Store at room temperature and renew weekly.

B6 Apparatus

In addition to normal laboratory glassware and apparatus the following are required.

B6.1 Refrigerator and freezer, set at 5 ± 3 °C and less than -17 °C, respectively with valid calibration status.

B6.2 Analytical Balance, five decimal place of a gram, for the preparation of stock solutions.

B6.3 Top Pan Balance, two decimal places of a gram.

B6.4 Magnetic stirrer

B6.5 Pasteur pipettes, glass, disposable

B6.6 Micropipettes, positive displacement, with valid calibration status, various sizes

B6.7 Vial 10 mL Clear screw 18 mm

B6.8 Stainless steel screw cap magnetic thread

B6.9 Flasks, volumetric, glass, various sizes

B6.10 Measuring Cylinder, stoppered, 1000 mL capacity, used for mobile phase preparation

B6.11 Sample bottles: 500 mL, Glass, Amber bottles, for sample collection add sodium thiosulfate 3.00% $^{w}/_{v}$ solution for sample preservation.

B6.12 Agilent Zorbax Eclipse Plus C18 Rapid Resolution HD, 50 mm x 2.1 mm, 1.8 µm particles, part number 959757-902.

B6.13 Auto-sampler vials, snap, amber, glass, 2 mL capacity and pre-slit snap caps

B6.14 Gerstel Multipurpose Sampler MPS Robotic Pro or equivalent, with various object modules

1 CF200 solvent safe centrifuge
2 Robotic head with 100 µL syringe
3 Solvent modules
(Disodium tetraborate buffer, MilliQ Water, HPLC Methanol, 2.5 g L^{-1} FMOC in Acetonitrile, Dichloromethane)
4 QMix (Shaker)
5 Three drawer Peltier cooled stack (10°C) with 2 mL vial trays
6 Large washes
(EDTA 0.1 M, formic acid 98/100%)
7 2 x 10-CVM 10 mL vial trays
8 10 mL vial tray for Internal standard / spiking solutions
9 Robotic Pro head with 1 mL syringe
10 Small waste pot (250 mL) with secondary overspill containment
11 Agitator

B6.15 1 mL syringe for Robotic MPS

B6.16 100 µL syringe for Robotic MPS

B6.17 Liquid Chromatograph Mass Spectrometer

A high-performance liquid chromatograph triple quadrupole mass spectrometer system fitted with binary and quaternary pumps, auto-sampler and column oven connected to a triple quadrupole mass spectrometer capable of unit mass resolution and operating in multiple reaction

monitoring (MRM) mode, with an appropriate data station, e.g., Agilent, 1290 Infinity 2 series liquid chromatograph and 6470 triple quadrupole mass spectrometer.

The following instrument conditions have been used and found to be satisfactory.

Typical Operating Conditions for the Liquid Chromatograph

Binary Pump, Analytical	
Binary Pump	Agilent 1290 Infinity Series
Analytical Column	Agilent Zorbax Eclipse C18 Rapid Resolution, 50 mm x 2.1 mm, 1.8 µm particles, part number 959757-902
Temperature	40 °C
Injection Volume	50 µL
Mobile Phase A Mobile Phase B	Water, 0.01% formic acid, 0.1% Agilent Infinity Lab Deactivator Additive Methanol, 0.1% Agilent Infinity Lab Deactivator Additive
Flow Rate	0.400 mL minute^{-1}

Binary Pump, Analytical Gradient Program		
Time (minutes)	Flow Rate (mL minute^{-1})	Mobile Phase B (%)
0.00	0.400	5
8.50	0.400	80
9.00	0.400	100
10.00	0.600	100

Typical Operating Conditions for the Mass Spectrometer

Triple Quadrupole Mass Spectrometer	
Mass Spectrometer	Agilent 6470
Ionisation Source	Jet Stream Electrospray Ionisation
Polarity	Negative

Ionisation Source Parameters	
Drying Gas Temperature	225 °C
Drying Gas Flow	8 L minute^{-1}
Nebulizer	45 psi
Sheath Gas Temperature	350 °C
Sheath Gas Flow	11 L minute^{-1}
Nebulizer Voltage	1000 V
Capillary Voltage	4500 V

Time Segment 1 – 3:				
Time Segment #	Time	Div Valve	Delta EMV (V)	Store
1	0.00	To Waste	0	No
2	5.00	To MS	500	Yes
3	10.00	To Waste	0	No

MRM								
Compound Name	Prec. Ion	MS1 Res	Prod. Ion	MS2 Res	Dwell	Frag. (V)	CE (V)	Cell Acc (V)
Glufosinate	402.1	Wide	206.0	Wide	50	100	10	7
Glufosinate	402.1	Unit	180.0	Wide	50	100	4	7
Glyphosate 13C2 15N	393.0	Unit	171.0	Wide	100	105	8	3
Glyphosate	390.0	Unit	168.0	Wide	100	105	8	3
Glyphosate	390.0	Unit	150.0	Wide	100	105	28	3
AMPA 13C 15N	334.0	Unit	138.0	Wide	100	75	12	3
AMPA	332.0	Unit	136.0	Wide	100	75	12	3
AMPA	332.0	Unit	110.0	Wide	100	75	2	3

Typical Retention Times and SRM Transition Information

Compound Name	Rt (min)	Quantifier SRM		Qualifier SRM		Qualifier SRM Ratio (%)
		Precursor	Product	Precursor	Product	
Glyphosate 13C2 15N	6.45	393.0	171.0	-	-	-
Glyphosate	6.45	390.0	168.0	390.0	150.0	20
AMPA 13C 15N	6.79	334.0	138.0	-	-	-
AMPA	6.79	332.0	110.0	332.0	136.0	20
Glufosinate	7.21	402.1	206.0	402.1	180.0	20

All compound retention times will vary depending on the condition of the analytical column therefore they should only be used as a guide. Compound elution order will remain unchanged.

B7 Sample collection and preservation

Sampling, samples are taken in 500 mL amber glass bottles, with screw tops (lined with an appropriate inert material) containing 0.5 mL sodium thiosulfate solution, 3.00% w/v. Samples are stored in the refrigerator at 1-5°C and are extracted within the no of days from sampling as per section 3.

B8 Analytical Procedure

Sample Preparation:

B8.1 Homogenise the 500 mL glass sample bottle by inverting several times.

B8.2 Accurately dispense 5 mL of the sample into a 10 mL screw top clear vial and cap.

Automated Instrument Extraction on MPS:

B8.3 Place extract collection 2 mL Amber Snap Cap Vials into the VT98 MPS Tray, up to the required number of samples, calibrations and AQC's.

B8.4 Place prepared samples, calibrations and AQC's onto the MPS sequence trays.

B8.5 Load the default prep sequence or previously used one and modify with sample vial ranges.

B8.6 Check all eluting solvents, waste and check solvent filling station levels.

B8.7 Initiate the run.

Program the control software the perform the following actions and processes.

Care should be taken in initial setup on object alignments and adjusting MPS arm settings to reduce any air bubbles in syringes. The draw, fill, viscosity delay and eject speed will need to be configured for the system and each reagent and solvents.

It is advised that the 100 µL syringe (which is used for the internal standard spiking and the formic acid reagent) is silanised to reduce active sites on the glass syringe barrel.

Over time the consistency and response recovery on the LC-QQQ will show variability. It is recommended that the syringe is silanised using the 5% DMDCS in Toluene (Sylon CT) reagent every 2 to 3 months or when deterioration is seen, whichever is sooner.

The 100 µL syringe and internal standard solution vessel on the MPS are silanised by rinsing with the reagent, leaving them to stand filled with the reagent for approximately 12.5 ± 2.5 seconds, followed by rinsing with clean hexane, and then acetone or methanol. A typical procedure is outlined in the product information certificate.

A summary of the automated extraction on the MPS is below. This may be run as a batch process of 6 vials and actions at a time to enable automation overlap and speed up the prep ahead function in the control software to reduce extraction time and improve efficiency. A rough idea of extraction time for 30 vials is 7 hours 30 minutes and 60 vials is 15 hours. During this time analysts can carry out other tasks and analysis as the MPS can be left to complete.

- Spike 100 µL of Internal Standard (200 µg L^{-1} of glyphosate 2-13C15N and AMPA 13C15N) into 10 mL vial.
- Spike 200 µL of EDTA (0.1M) into 10 mL vial.
- Spike 250 µL of Disodium Tetraborate Buffer Solution into 10 mL vial.
- Spike 625 µL of FMOC (2.5 g L^{-1} in acetonitrile) into 10 mL vial.
- Move 10 mL sample vial to the Agitator for 10 minutes at 60 °C
- (derivatisation process).
- Move 10 mL vial back to sample tray.
- Spike 63 µL formic acid (quench reaction) into 10 mL vial.
- Spike 300 µL of dichloromethane (cleanup stage) into 10 mL vial.
- Move 10 mL sample vial to QMIX at 2000 rpm for 0.25 minutes.
- Move 10 mL sample vial to centrifuge at 3000 rpm for 0.5 minutes.
- Move 10 mL vial back to sample tray.
- Transfer 1 mL of the sample extract to the 2 mL snap cap amber vials held in the refrigerated MPS chiller tray (set at 10 °C).
- The extract is now ready for analysis on the LC-QQQ.
- Same day or next day, the sample vials are removed and placed on the LC-QQQ autosampler and run.
- Extracts can be kept in the fridge for up to 4 days prior to running on the LC-QQQ based on in-house in-vial stability data.

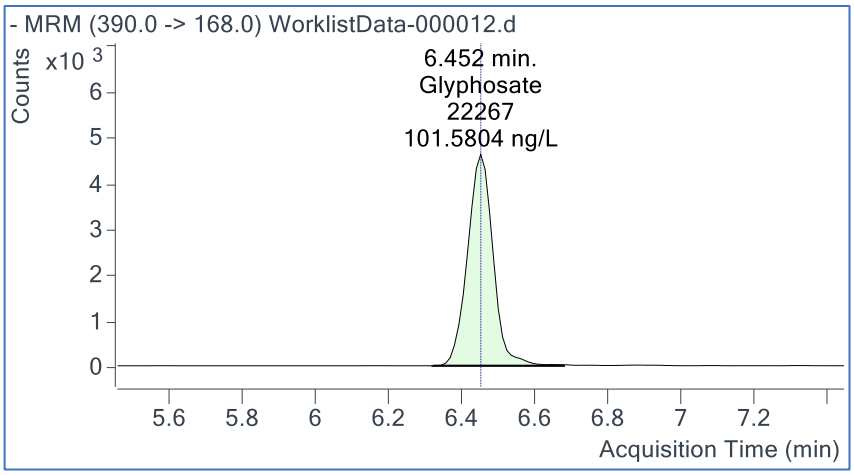
B9 Calculation

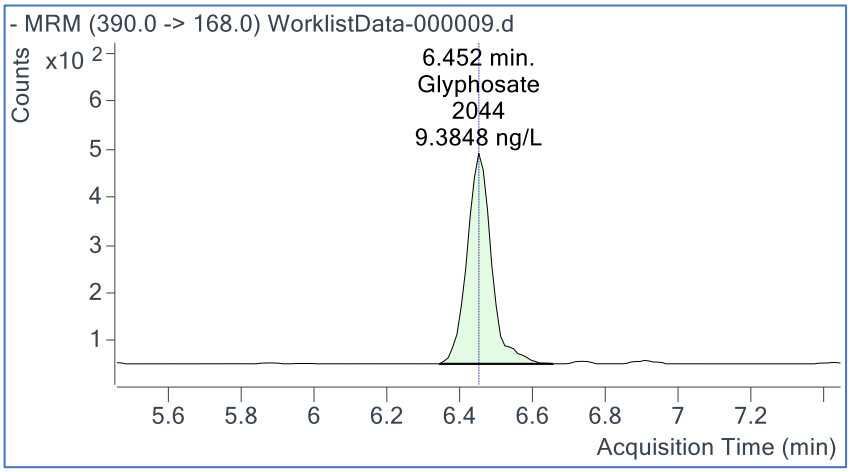
Results for target compounds are calculated using the following equation:

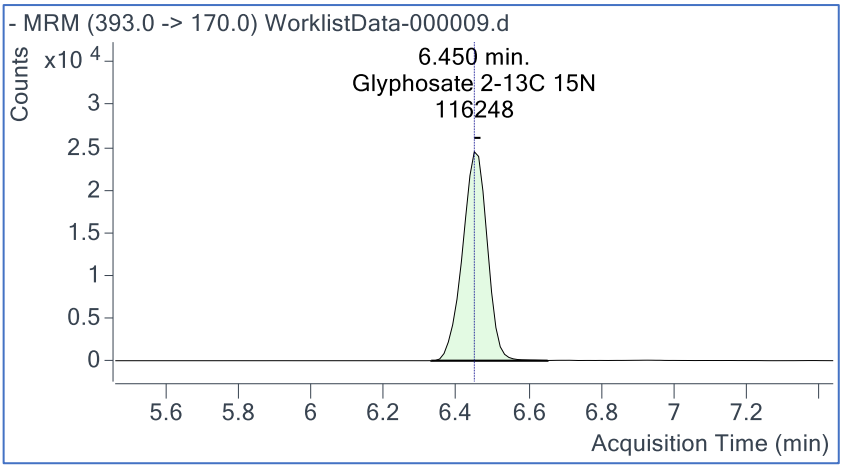
$$\text{Sample concentration } (\mu g\ L^{-1}) = \frac{\text{Sample area}}{\text{Std area}} \times \frac{\text{Std concentration}}{\text{Sample Internal Std Area/Std Internal Std Area}} \times \text{Multiplier}$$

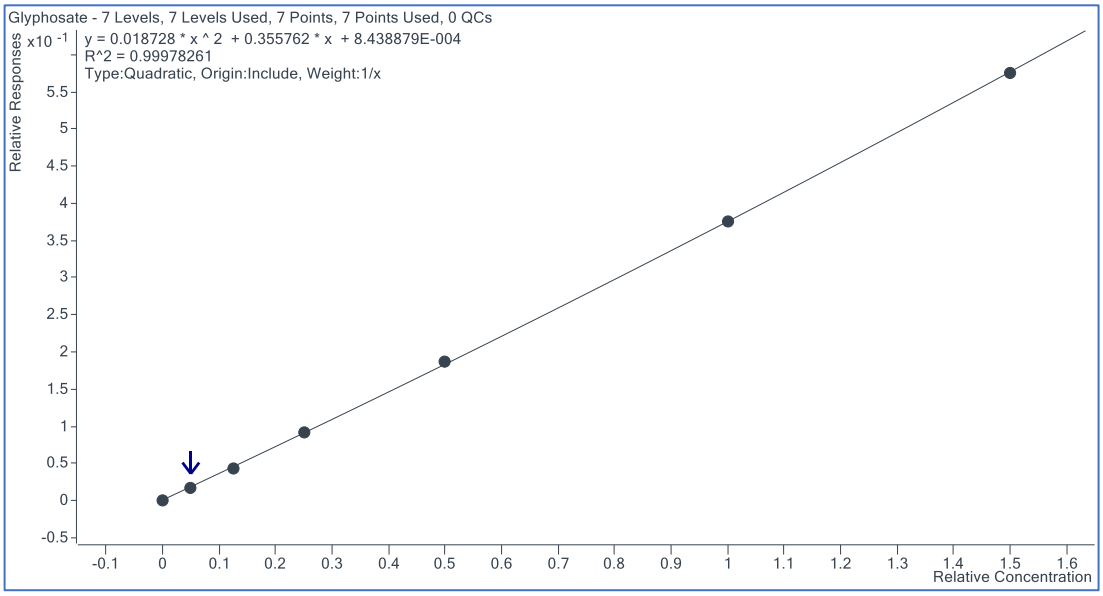
Figure B1 - Typical chromatograms and calibration graphs

Glyphosate

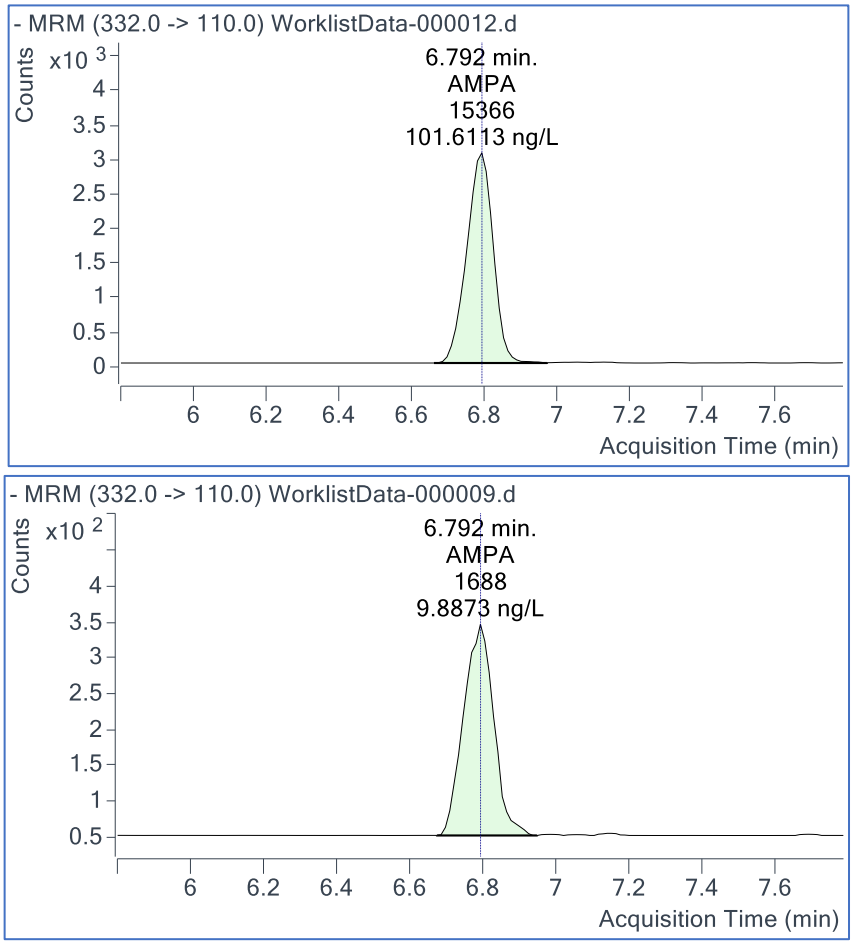


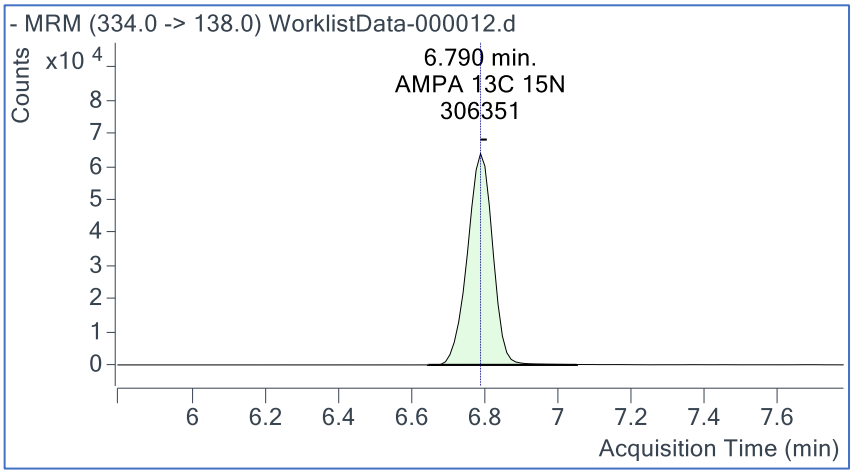


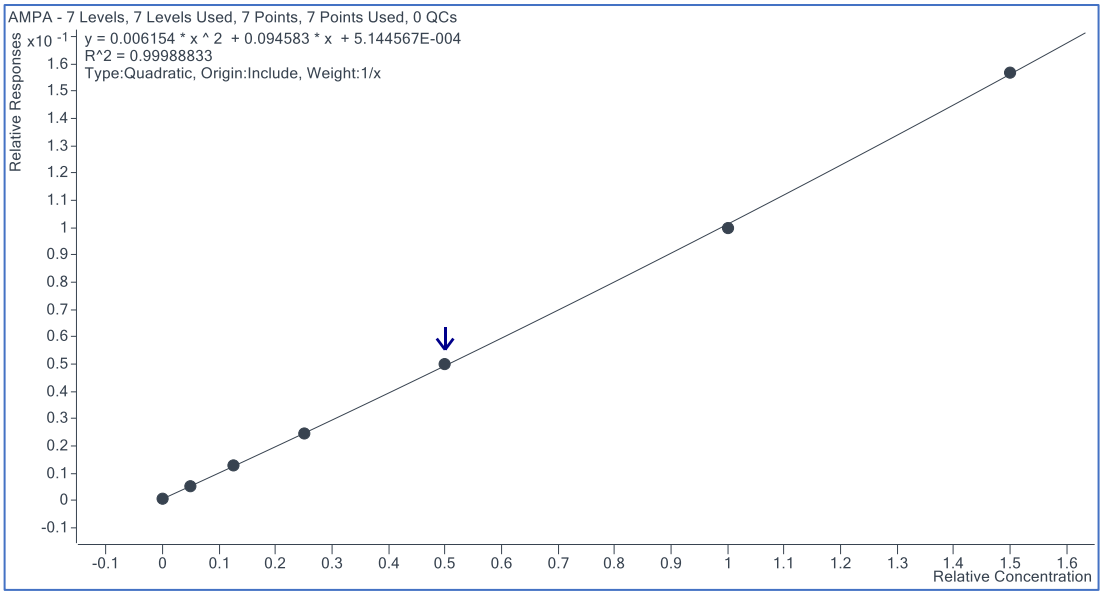



Glyphosate - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs
y = 0.018728 * x ^ 2 + 0.355762 * x + 8.438879E-004
R^2 = 0.99978261
Type:Quadratic, Origin:Include, Weight:1/x
Relative Responses
x10 -1
Relative Concentration

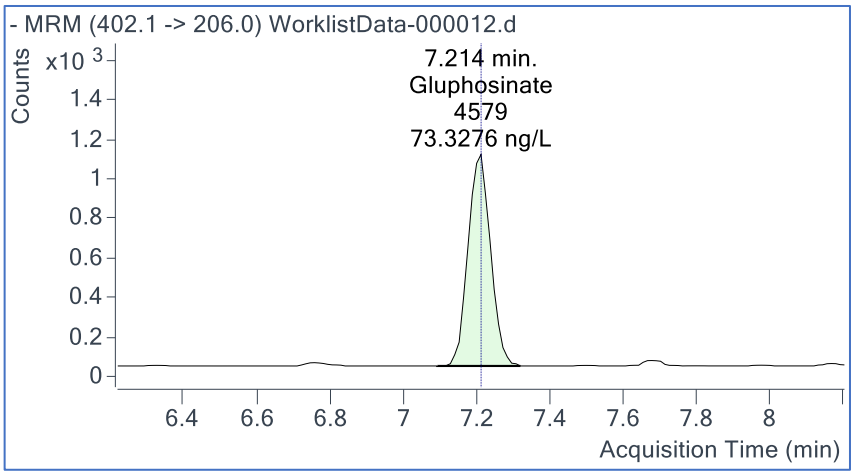
AMPA

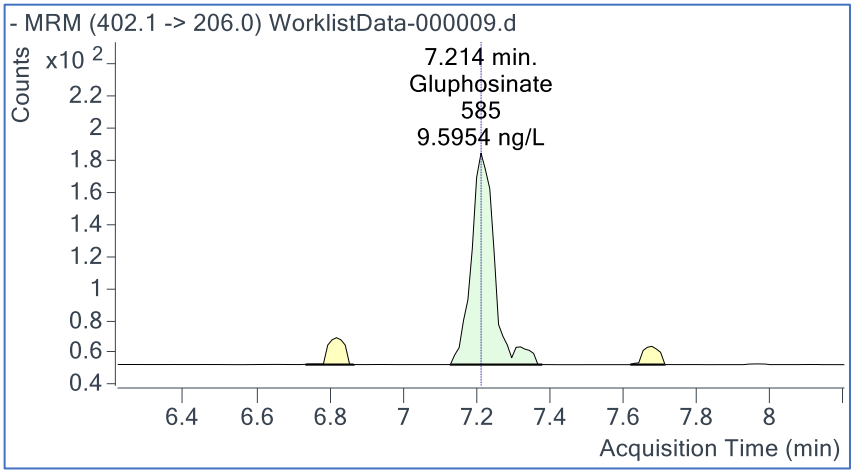

- MRM (332.0 -> 110.0) WorklistData-000012.d
Counts
x10 3
6.792 min.
AMPA
15366
101.6113 ng/L
Acquisition Time (min)
- MRM (332.0 -> 110.0) WorklistData-000009.d
Counts
x10 2
6.792 min.
AMPA
1688
9.8873 ng/L
Acquisition Time (min)

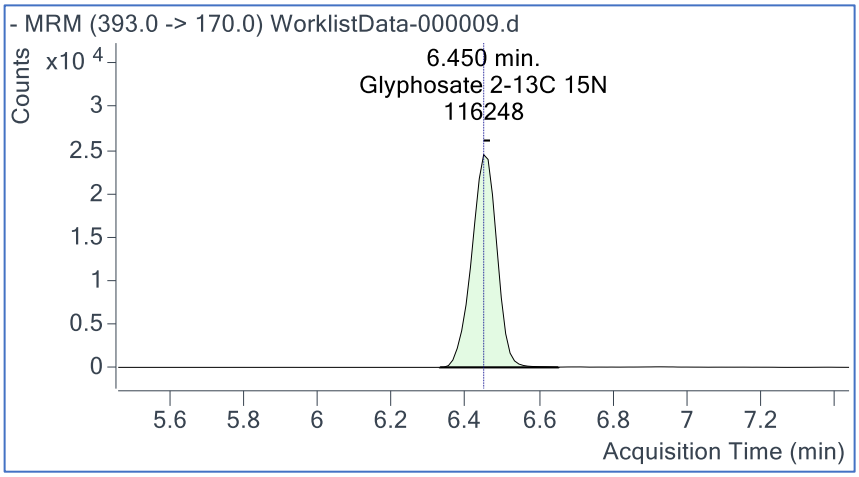

- MRM (334.0 -> 138.0) WorklistData-000012.d
Counts
x10 4
6.790 min.
AMPA 13C 15N
306351
Acquisition Time (min)


AMPA - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs
y = 0.006154 * x ^ 2 + 0.094583 * x + 5.144567E-004
R^2 = 0.99988833
Type:Quadratic, Origin:Include, Weight:1/x
Relative Responses
x10 -1
Relative Concentration

Glufosinate







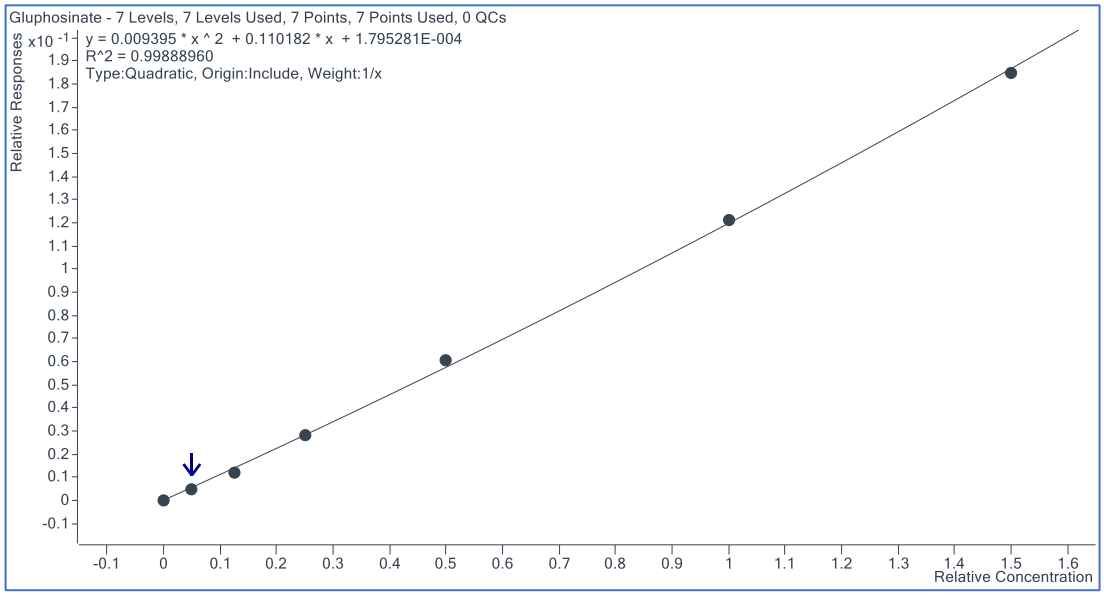

Gluphosinate - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs
y = 0.009395 * x ^ 2 + 0.110182 * x + 1.795281E-004
R^2 = 0.99888960
Type:Quadratic, Origin:Include, Weight:1/x
Relative Responses
x10 -1
Relative Concentration

Table B1 - Method performance data

Method performance data provided by Severn Trent Water (STW).

Analyte	LoQ µg L^{-1}	Low Std 0.0600 µg L^{-1}		High Std 0.2400 µg L^{-1}	
		Bias (%)	RSD (%)	Bias (%)	RSD (%)
Glyphosate	0.015	10.62	6.67	5.02	8.43
AMPA	0.016	3.22	5.62	3.05	5.30
Glufosinate	0.014	9.46	10.12	5.72	9.72
LoQ derived statistically from worse performing water matrix (Raw Surface) from a 11 x 2 replicate 0.002 µg L^{-1} spiking exercise. Improvements to LoQ can be due to the chosen Raw water source in from water companies' catchment area. The raw surface chosen water in this instance gave some very high blank values for analytes of interest which has affected the statistical data, raising some LoQ values.					

Matrix	Spike	Recovery %	RSD %
Soft Water			
Glyphosate	60 ng L^{-1}	109.33	9.04
	240 ng L^{-1}	105.28	6.69
AMPA	60 ng L^{-1}	102.24	5.05
	240 ng L^{-1}	101.72	4.38
Glufosinate	60 ng L^{-1}	101.79	8.77
	240 ng L^{-1}	99.90	7.31
Medium Water			
Glyphosate	60 ng L^{-1}	110.39	6.67
	240 ng L^{-1}	104.97	8.43
AMPA	60 ng L^{-1}	102.76	5.62
	240 ng L^{-1}	102.93	5.30
Glufosinate	60 ng L^{-1}	109.07	10.12
	240 ng L^{-1}	105.62	9.72
Hard Water			
Glyphosate	60 ng L^{-1}	110.76	6.23
	240 ng L^{-1}	106.75	6.94
AMPA	60 ng L^{-1}	104.78	6.48
	240 ng L^{-1}	103.58	5.91
Glufosinate	60 ng L^{-1}	106.95	9.36
	240 ng L^{-1}	105.50	8.04
Surface Water			
Glyphosate	60 ng L^{-1}	108.43	8.2
	240 ng L^{-1}	108.04	7.69
AMPA	60 ng L^{-1}	72.02	15.63
	240 ng L^{-1}	94.50	9.24
Glufosinate	60 ng L^{-1}	132.35	15.08
	240 ng L^{-1}	136.51	15.97

Uncertainty Of Measurement

The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2, which gives a level of confidence of approximately 95%.

Uncertainty of measurement is calculated on an ongoing basis according to Standing Committee of Analysts Blue Book 'Estimation of Uncertainty of Measurement for Chemical and Physico-chemical Determinands in Drinking Water 2018' and Table A3 of The Water Supply (Water Quality) (Amendment) Regulations 2018.

Individual analyte UoM values are calculated using a spreadsheet on a daily rolling basis, and take into account stock solutions, AQC data and performance, matrix recovery, glassware used, and any syringes or pipettes used during the whole analysis process.

Below is an example of a year's rolling UoM value and its target threshold for Glyphosate (Data supplied March-2025 by STW).

Note: A UoM value for Glyphosate only is monitored as that analyte is the only accredited analyte in the test method. AMPA and Glufosinate are non-accredited screening analytes only and results supplied to clients for investigational purposes or upon request.

Analyte	UoM Indicator from validation exercise (*)	Yearly rolling UoM (current data)	UoM agreed Target threshold
Glyphosate	27%	26%	45%

* The Indicator UoM was calculated from the validation precision and bias data and is for indication purposes only.

Generally, the rolling UoM data, as outlined in 'Estimation of Uncertainty of Measurement for Chemical and Physico-chemical Determinands in Drinking Water 2018', is taken as a representation of the method and UoM performance.

Members assisting with these methods.

Without the good will and support given by these individuals and their respective organisations SCA would not be able to continue and produce the highly valued and respected blue book methods.

Project Leader(s):

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Sarah Roberts	UKAS
Shaun Jones	mua Water
Steven Jones	Severn Trent Water
Stuart Collins	South West Water
Toni Hall	Wessex Water
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Correspondence

However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts:

secretary@standingcommitteeofanalysts.co.uk

Amendment History

The Determination of Glyphosate, Aminomethylphosphonic acid and Glufosinate in Raw and Potable Waters is a new book. Therefore, there isn't an amendment history on this occasion.

standing
committee of
analysts