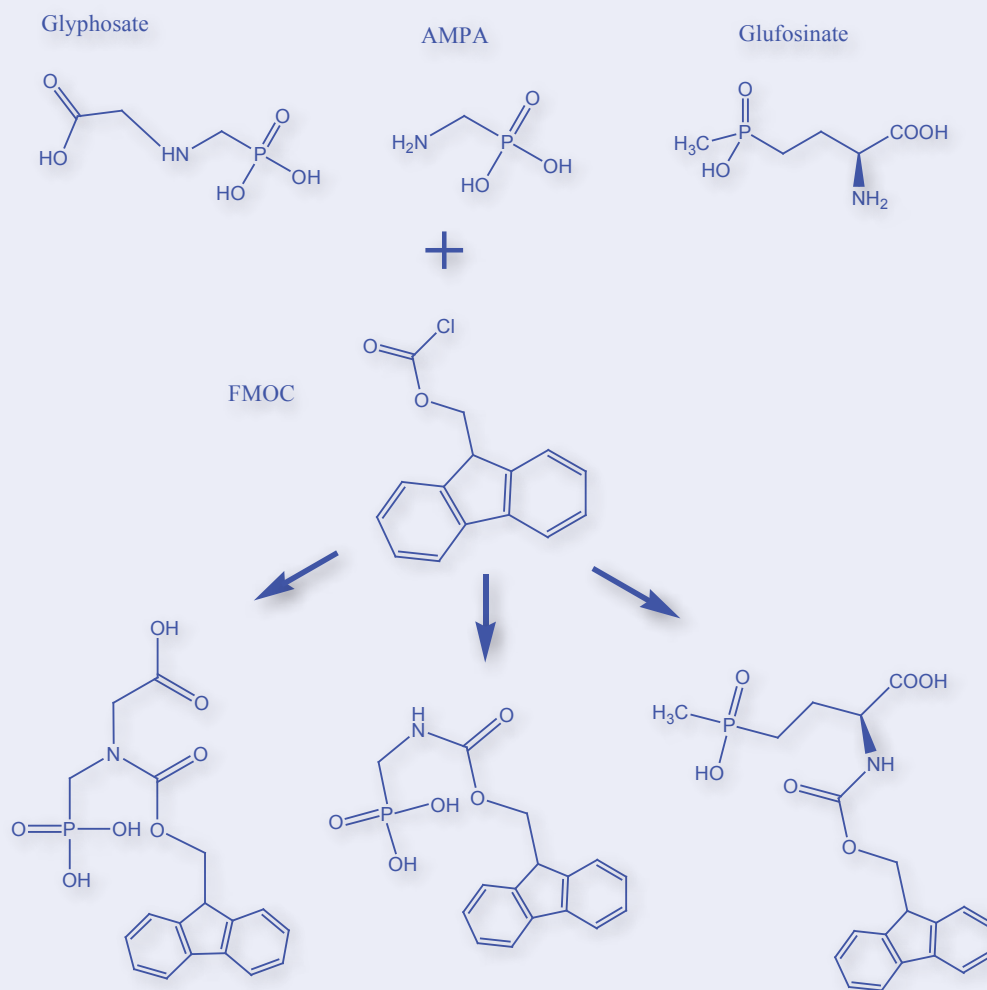


Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water by Isotope Dilution and Online Solid-Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry



Techniques and Methods 5–A10

Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water by Isotope Dilution and Online Solid-Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry

By Michael T. Meyer, Keith A. Loftin, Edward A. Lee, Gary H. Hinshaw, Julie E. Dietze, and Elisabeth A. Scribner

Techniques and Methods 5–A10

**U.S. Department of the Interior
U.S. Geological Survey**

U.S. Department of the Interior
KEN SALAZAR, Secretary

U.S. Geological Survey
Suzette M. Kimball, Acting Director

U.S. Geological Survey, Reston, Virginia: 2009

For more information on the USGS—the Federal source for science about the Earth, its natural and living resources, natural hazards, and the environment, visit <http://www.usgs.gov> or call 1-888-ASK-USGS

For an overview of USGS information products, including maps, imagery, and publications, visit <http://www.usgs.gov/pubprod>

To order this and other USGS information products, visit <http://store.usgs.gov>

Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Although this report is in the public domain, permission must be secured from the individual copyright owners to reproduce any copyrighted materials contained within this report.

Suggested citation:

Meyer, M.T., Loftin, K.A., Lee, E.A., Hinshaw, G.H., Dietze, J.E., Scribner, E.A., 2009, Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water by Isotope Dilution and Online Solid-Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry: U.S. Geological Survey Techniques and Methods, book 5, chap. A10, 32p.

Contents

Abstract.....	1
Introduction.....	1
Purpose and Scope	2
Analytical Method.....	3
1. Scope and Application.....	3
2. Summary of Method	3
3. Safety Precautions	4
4. Interferences	4
5. Apparatus and Instrumentation	4
6. Reagents and Consumable Materials	5
7. Sampling Methods.....	5
8. Standard and Reagent Solutions	5
9. Sample Preparation.....	6
10. Automated Solid-Phase Extraction.....	6
11. Liquid Chromatography/Tandem Mass Spectrometry Preparation.....	6
Evaluation of Instrument Performance	7
12. Compound Identification and Quantitation.....	7
13. Calculation of Results	8
14. Reporting of Results	9
Method Performance	9
15. Comparison of Isotope Dilution and Standard Addition	9
16. Matrix Performance	10
Method Detection Limits.....	12
Compound Stability in Underivatized and Derivatized Water Samples	14
Conclusions.....	14
Acknowledgments.....	15
References Cited.....	15

Figures

1. Diagram showing chemical structure of glyphosate, aminomethylphosphonic acid, glufosinate, and their 9-fluorenylmethyl-chloroformate derivatized compounds2
2. Graph showing linear regressions of concentrations of glyphosate, aminomethylphosphonic acid, and glufosinate calculated by isotope dilution compared to standard addition for surface-water and groundwater samples analyzed between April 5, 2004 and June, 2006.....10

Tables

1. Molecular weights, Chemical Abstracts Service Registry Numbers, National Water Information System parameter codes for glyphosate, aminomethylphosphonic acid, glufosinate and their fluorenylmethylchloroformate derivatized compounds.....4
2. Summary of the liquid chromatography conditions for the mobile phase gradients and flow rates

3. Summary of the Multiple Reaction Monitoring deprotonated molecule and daughter-ion transition pairs, retention times, optimized cone voltages, and collision cell parameters for derivatized glyphosate, aminomethylphosphonic acid, glufosinate, and internal standards.....	8
4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.....	18
5. Statistical summary of average concentrations for six sets of duplicate deionized-distilled-water, groundwater, and surface-water samples spiked at 0.05 µg/L analyzed between March 30 and April 30, 2007.....	11
6. Statistical summary of the average concentration of glyphosate, aminomethylphosphonic acid, and glufosinate normalized as a percentage of the expected spiked concentration (normalized concentration) for six sets of duplicate deionized-distilled water, groundwater and surface-water samples spiked at three concentration levels.....	13
7. Statistical summary of mean percent difference between glyphosate, aminomethylphosphonic acid, and glufosinate concentrations determined by isotope dilution and multiple-point regressed standard curves for spiked deionized-distilled-water, groundwater, and surface-water samples.....	13
8. Statistical summary of glyphosate and aminomethylphosphonic acid concentrations in filtered raw water samples and derivatized water samples with time normalized to beginning concentrations.....	14

Conversion Factors, Abbreviated Water-Quality Units, and Other Abbreviations

Inch/Pound to SI

Multiply	By	To obtain
Length		
mile (mi)	1.609	kilometer (km)
inch (in.)	25.4	millimeter (mm)
inch (in.)	25400	micrometer (μm)
Area		
acre	4,047	square meter (m^2)
Volume		
gallon (g)	3.785	liter (L)
gallon (g)	3785411.784	microliter (μL)
ounce, fluid (oz)	0.02957	liter (L)
Flow rate		
square mile (mi^2)	2.590	square kilometer (km^2)
Mass		
ounce(oz)	$2.834952313 \times 10^{10}$	nanogram (ng)
pound avoirdupois (lb)	453.6	gram (g)

SI to Inch/Pound

Multiply	By	To obtain
Length		
millimeter (mm)	0.03937	inch (in.)
kilometer (km)	0.6214	mile (mi)
Area		
square meter (m^2)	0.0002471	acre
Volume		
liter (L)	33.82	ounce, fluid (fl. oz)
micrometer (μm)	3.937×10^{-5}	inch (in.)
liter (L)	0.2642	gallon (gal)
microliter (μL)	2.642×10^{-7}	gallon (gal)
Flow rate		
square kilometer (km^2)	0.3861	square mile (mi^2)
Mass		
gram (g)	0.0022	pound avoirdupois (lb)
nanogram (ng)	3.527×10^{-11}	ounce (oz)

Temperature can be converted to degrees Celsius ($^{\circ}\text{C}$) or degrees Fahrenheit ($^{\circ}\text{F}$) by the following equations:

$$^{\circ}\text{C}=(^{\circ}\text{F}-32)/1.8$$

$$^{\circ}\text{F}=(1.8\times^{\circ}\text{C})+32$$

Abbreviated Water-Quality Units:

gram (g)
 liter per hour (L/hr)
 microgram per liter ($\mu\text{g/L}$)
 microgram per milliliter ($\mu\text{g/mL}$)
 microliter per minute ($\mu\text{L/min}$)
 milligram (mg)
 milligram per liter (mg/L)
 milligram per milligram (mg/mg)
 milligram per milliliter (mg/mL)
 milliliter (mL)
 milliliter per minute (mL/min)
 millimeter (mm)
 millimolar (mM)
 nanogram per microliter (ng/ μL)
 nanogram per milliliter (ng/mL)

Other Abbreviations or Acronyms Used in This Report:

%	percent
ACN	Acetonitrile
ACS	American Chemical Society
AMPA	Aminomethylphosphonic Acid
CAS	Chemical Abstracts Service
DI	Deionized-Distilled Laboratory Water
ESI	Electrospray Ionization
FMOG	9-fluorenylmethylchloroformate
HPLC	High-performance liquid chromatogram
V	voltage
IS	internal standard
LC/MS/MS	liquid chromatography/tandem mass spectrometry
MDL	method detection limit
MRL	method reporting level
MRM	multiple reaction monitoring
m/z	mass-to-charge ratio
M-H	molecule- hydrogen (deprotonated molecule)
NCP	Normalized Concentration Percent
prsd	percent relative standard deviation
psi	pound per square inch
r^2	correlation coefficient
rsd	relative standard deviation
SPE	solid-phase extraction
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
\pm	plus or minus
v/v	volume-to-volume

Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water by Isotope Dilution and Online Solid-Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry

By Michael T. Meyer, Keith A. Loftin, Edward A. Lee, Gary Hinshaw, Julie E. Dietze, and Elisabeth A. Scribner

Abstract

The U.S. Geological Survey method (0-2141-09) presented is approved for the determination of glyphosate, its degradation product aminomethylphosphonic acid (AMPA), and glufosinate in water. It was validated to demonstrate the method detection levels (MDL), compare isotope dilution to standard addition, and evaluate method and compound stability. The original method USGS analytical method 0-2136-01 was developed using liquid chromatography/mass spectrometry and quantitation by standard addition. Lower method detection levels and increased specificity were achieved in the modified method, 0-2141-09, by using liquid chromatography/tandem mass spectrometry (LC/MS/MS). The use of isotope dilution for glyphosate and AMPA and pseudo isotope dilution of glufosinate in place of standard addition was evaluated. Stable-isotope labeled AMPA and glyphosate were used as the isotope dilution standards. In addition, the stability of glyphosate and AMPA was studied in raw filtered and derivatized water samples.

The stable-isotope labeled glyphosate and AMPA standards were added to each water sample and the samples then derivatized with 9-fluorenylmethylchloroformate. After derivatization, samples were concentrated using automated online solid-phase extraction (SPE) followed by elution in-line with the LC mobile phase; the compounds separated and then were analyzed by LC/MS/MS using electrospray ionization in negative-ion mode with multiple-reaction monitoring. The deprotonated derivatized parent molecule and two daughter-ion transition pairs were identified and optimized for glyphosate, AMPA, glufosinate, and the glyphosate and AMPA stable-isotope labeled internal standards.

Quantitative comparison between standard addition and isotope dilution was conducted using 473 samples analyzed between April 2004 and June 2006. The mean percent difference and relative standard deviation between the two quantitation methods was 7.6 plus or minus 6.30 ($n = 179$), AMPA 9.6 plus or minus 8.35 ($n = 206$), and glufosinate 9.3 plus or minus 9.16 ($n = 16$).

The analytical variation of the method, comparison of quantitation by isotope dilution and multipoint linear regressed

standard curves, and method detection levels were evaluated by analyzing six sets of distilled-water, groundwater, and surface-water samples spiked in duplicate at 0.0, 0.05, 0.10 and 0.50 microgram per liter and analyzed on 6 different days during 1 month. The grand means of the normalized concentration percentage recovery for glyphosate, AMPA, and glufosinate among all three matrices and spiked concentrations ranged from 99 to 114 plus or minus 2 to 7 percent of the expected spiked concentration. The grand mean of the percentage difference between concentrations calculated by standard addition and linear regressed multipoint standard curves ranged from 8 to 15 plus or minus 2 to 9 percent for the three compounds. The method reporting levels calculated from all the 0.05-microgram per liter spiked samples were 0.02 microgram per liter for all three compounds.

Compound stability experiments were conducted on 10 samples derivatized four times for periods between 136 to 269 days. The glyphosate and AMPA concentrations remained relatively constant in samples held up to 136 days before derivatization. The half life of glyphosate varied from 169 to 223 days in the underivatized samples. Derivatized samples were analyzed the day after derivitization, and again 54 and 64 days after derivatization. The derivatized samples analyzed at days 52 and 64 were within 20 percent of the concentrations of the derivatized samples analyzed the day after derivatization.

Introduction

Glyphosate (N-(phosphonomethyl)glycine, fig. 1), a non-selective, post-emergence herbicide, has been widely used since it was released commercially in 1974, and is one of the world's most widely used agrochemical herbicides (Monsanto Company, 2002; Cox, 2004). In 2004, glyphosate usage estimates indicated that between 103 and 113 million pounds were applied annually to crops in the United States (Cox, 2004; Kiely and others, 2004). Glyphosate usage substantially increased with the introduction of genetically modified glyphosate-resistant soybean and corn cultivars in 1997 and 1998, respectively, known as Roundup Ready™ (Iowa

2 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

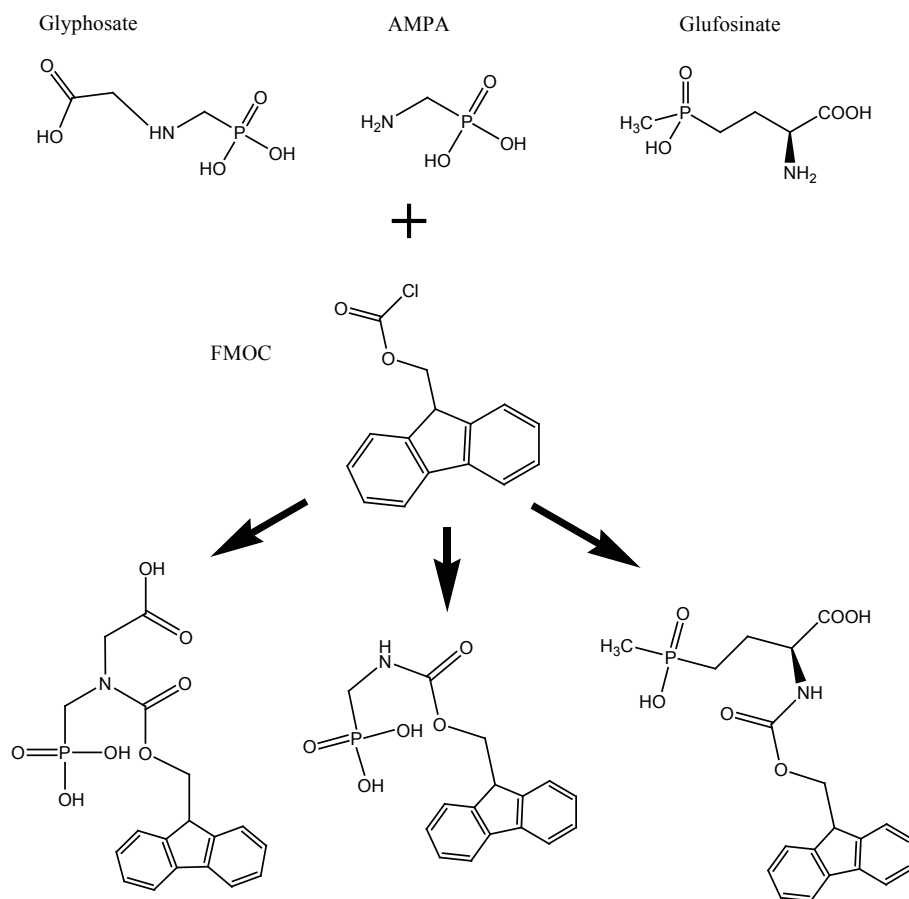


Figure 1. Chemical structure of glyphosate, aminomethylphosphonic acid, glufosinate, and their 9-fluorenylmethyl-chloroformate derivatized compounds.

State University, 1997). Glyphosate is degraded primarily by microbial metabolism producing aminomethylphosphonic acid (AMPA; Rueppel and others, 1977). Glufosinate is similar to glyphosate in chemical structure and use (Cox, 1996; fig 1). The three compounds are polar and extremely soluble in water, and require derivatization for chromatographic separation.

Because of the difficulty of the analysis with past technology, the transport of glyphosate in surface and groundwater is not well studied; however, the original method by Lee and others (2002, USGS method 0-2136-01) determined that glyphosate and AMPA are detectable in many streams throughout the country (Battaglin and others, 2005; Scribner and others, 2007). A 2004 collaborative study between the U.S. Geological Survey (USGS) National Water Quality Program (NAWQA) Indiana Agricultural Chemicals Team (ACT) study unit and the USGS Toxic Substances Hydrology Program, using a LC/MS/MS modification to the original method, developed at the USGS Organic Geochemistry Research Laboratory, Lawrence, Kansas, indicated that glyphosate and AMPA contaminate stream water by overland flow runoff and tile drains. Glyphosate and AMPA also were detected in wet deposition samples (Scribner and others, 2007). It also was determined that without the lower reporting levels, there would have been about 30 percent fewer detections. The

original method and the modified method have improved our understanding of glyphosate and its degradation products in the environment as shown in recent studies by the USGS OGRL in which the modified method was used (Battaglin and others, 2005; Kolpin and others, 2006; Stone and others, 2006; Scribner and others, 2007). Documentation and validation data for the modified glyphosate method are needed to promote continued use in environmental studies.

Purpose and Scope

Since the introduction of glyphosate tolerant corn and soybeans glyphosate has become the most widely used herbicide in the world. However, because of past analytical limitations the analysis of glyphosate was problematic. Thus, little is known about its occurrence, fate, and transport in groundwater, surface water, and the atmosphere relative to other widely used herbicides such as atrazine. In 2001 the USGS Kansas Water Science Center, Organic Geochemistry Research Laboratory developed a method to analyze glyphosate, its degradation, AMPA, and glufosinate in filtered water. Data generated from this method showed that glyphosate and AMPA were

commonly transported in surface water from agricultural and urban sources. However, minimum reporting levels (MRL) of 0.1 µg/L made it difficult to assess their occurrence in environments (e.g. groundwater) or at times (e.g. late Fall) when concentrations would be expected to be low. To obtain lower MRL's the method was adapted for Tandem Mass Spectrometry in 2003. Data obtained from this method in a collaborative study with the National Water Quality Assessment Program (NAWQA) showed that the detection frequency would have been about 30 percent less with the higher MRL of 0.1 µg/L instead of 0.02 µg/L. Also a stable-isotope labeled analog for glyphosate became commercially available in 2004 offering the possibility to quantitate glyphosate and AMPA by isotope dilution. Because only a stable isotope analog of AMPA was previously available, quantitation was performed using the method of standard addition, which required an unspiked and spiked analysis for each environmental sample. With isotope dilution the need for the spiked analysis for each sample would not be necessary. Thus, to lower the MRL and also reduce the number of analyses per sample this method (0-2121-09) was developed.

The purpose of this report is to document the approved USGS method 0-2141-09 for the analysis of glyphosate (N-(phosphonomethyl)glycine), its degradation product AMPA (aminomethylphosphonic acid), and the herbicide glufosinate (4-((RS)-hydroxy(methyl)phosphinoyl)-DL-homoalanine) in filtered water. This method (0-2141-09) was modified from USGS method 0-2136-01 (Lee and others, 2002). The original methods uses derivatization, on-line SPE and liquid chromatography/mass spectrometry, and quantitation by the method of standard addition. The modified method, 0-2141-09 uses derivitization, on-line SPE and liquid chromatography/tandem mass spectrometry (LC/MS/MS) for analysis and isotope dilution for the quantitation of glyphosate and AMPA and pseudo isotope dilution for glufosinate. This report documents the equivalence of quantitation by isotope dilution compared to standard addition from the analysis of 473 environmental samples; assessment of analytical variation from six sets of distilled-water, groundwater, and surface-water samples analyzed in duplicate at three concentration levels; comparison of quantitation using isotope dilution and a linear regressed multi-point standard curve and calculation of the method detection levels (MDLs) of the modified method; and the stability of derivatized and underivatized environmental samples with time using. The method of analysis described in this report has been assigned a USGS method number (0-2141-9) and OGRL code of LCGY. These unique codes represent the automated method of analysis as it is described in the report, and can be used to identify the method.

Analytical Method

Molecular weights, chemical abstracts service registry numbers, and U.S. Geological Survey National Water

Information System (NWIS) parameter codes for glyphosate, its degradation product AMPA, glufosinate, and their FMOC-derivatized compounds are shown in table 1.

1. Scope and Application

This method is suitable for the determination of sub microgram per liter (µg/L) concentrations for glyphosate analysis, its degradation product AMPA, and glufosinate in water samples (table 1). Because suspended particulate matter is removed from the samples by filtration, the method only is suitable for analysis of these compounds in the dissolved phase. The quantitation range for the method is from 0.02 to 5.0 µg/L.

2. Summary of Method

All surface-and groundwater samples collected for analysis are filtered and shipped at 2–4 °C to the USGS OGRL in Lawrence, Kansas. Water samples are derivatized to prepare for analysis within 5 days after they are received. From each water sample, 10 milliliter (mL) aliquots are pipetted into labeled, 19-mL, screw-capped plastic test tubes. Then 200 microliter (µL) of a 50 nanogram per milliliter (ng/mL) internal standard solution is added to each sample, the sample is adjusted to pH 9.0 by adding a 5 percent borate buffer, and then 1500 microliters (µL) of 2.5 millimolar (mM) 9-fluorenylmethylchloroformate (FMOC) in acetonitrile (ACN) is added to each sample. Derivatization is carried out in the dark in a water bath at 40 °C for approximately 24 hours. The reaction then is stopped and stabilized by adding 600 µL of 2 percent phosphoric acid to each sample. The samples are then stored in the test tubes and refrigerated in the dark until analysis.

A 5.5-mL aliquot of each derivatized sample and 5.5-mL of deionized-distilled laboratory water (DI) water are added to glass autosampler vials that subsequently are capped and placed in the tray of the on-line SPE autosampler. The SPE cartridge is conditioned with 2 mL of acetonitrile and then 2 mL of DI water; 10 mL of the sample is then loaded onto the cartridge. The SPE cartridge is then rinsed with 500 µL of DI water, and the cartridge is placed into the flow path of the liquid chromatography (LC) mobile-phase stream. The conditions and gradient of the mobile phase described in this report are set to elute the compounds of interest and minimize the elution of excess FMOC reagent from the SPE cartridge. The compounds are separated on a liquid chromatograph using a gradient separation and analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS) with electrospray ionization in negative-ion mode using multiple reaction monitoring (MRM). The compounds are identified by comparing their retention times to the internal standards in each sample, and comparing the ratio of the quantitation MRM daughter-ion to the confirming MRM daughter-ion for each compound. The concentration of each identified compound was calculated

4 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

Table 1. Molecular weights, Chemical Abstracts Service Registry Numbers, National Water Information System parameter codes for glyphosate, aminomethylphosphonic acid, glufosinate and their fluorenylmethylchloroformate derivatized compounds.

[CAS, chemical abstract service; NWIS, National Water Information System; USGS, U.S. Geological Survey; FMOC, 9-fluorenylmethylchloroformate; --, not applicable]

Compound name	Molecular weight (atomic mass units)	CAS number	USGS NWIS parameter codes	USGS method number
Glyphosate	169.1	107836	62722T	0-2141-09
Glyphosate-FMOC	392.3	--	--	--
Isotope Labeled +3 - glyphosate	171.1	--	--	--
Isotope Labeled +3 - glyphosate-FMOC	393.3	--	--	--
Aminomethylphosphonic acid	111.0	--	62649T	0-2141-09
Aminomethylphosphonic acid- FMOC	333.3	--	--	--
Isotope labeled +4- aminomethylphosphonic acid	115.0	--	--	--
Isotope labeled +4- aminomethylphosphonic acid-FMOC	337.3	--	--	--
Isotope labeled +2- aminomethylphosphonic acid	113.0	--	--	--
Isotope labeled +2- aminomethylphosphonic acid-FMOC	335.3	--	--	--
Glufosinate	181.1	77182-82-2	62721T	0-2141-09
Glufosinate-FMOC	402.3	--	--	--

This report contains CAS Registry Numbers®, which is a Registered Trademark of the American Chemical Society. CAS recommends the verification of the CASRNs through CAS Client Servicessm.

by determining the ratio of the area response produced by the quantitation daughter-ion of the analyte to the area response produced by the quantitation daughter-ion of the corresponding internal standard.

3. Safety Precautions

- 3.1 Perform all steps that require using organic solvents and strong acids and bases in a well-vented fume hood.
- 3.2 Use appropriate personal protective equipment during the handling of any reagents and chemicals, including eye protection, gloves, and protective clothing.
- 3.3 Ensure that the electrospray waste exhaust and the vacuum pump exhaust is vented through a laboratory hood system.
- 3.4 Take precaution when handling columns or working with the spray chamber of the mass spectrometer as temperatures are in excess of 300 °C; allow these areas to cool before touching them.

4. Interferences

Samples and field collection equipment that are handled improperly might become contaminated; therefore, sample-collection protocols and cleaning procedures for

field equipment (Webb and others, 1999) should be followed closely.

5. Apparatus and Instrumentation

- 5.1 *Analytical balance*—capable of accurately weighing approximately 0.0500 grams (g) ± 0.0001 gram.
- 5.2 *Autopipettes*—10- to 10,000-µL, variable-volume autopipettes with disposable plastic tips.
- 5.3 *Plastic Bottles*—plastic bottles should be HDPE, Teflon, or some other material to which glyphosate, AMPA, and glufosinate will not adsorb.
- 5.4 *Plastic test tube rack*—40 holes for tubes.
- 5.5 *Plastic Test Tubes*—19-mL plastic round bottom screw-capped test tubes.
- 5.6 *Black Permanent Marker*—for labeling the project code and sample identification number on the sides of the plastic tubes.
- 5.7 *Water bath*—capable of holding a steady temperature of 40 °C.
- 5.8 *Analytical column*—Luna 150-x 3.0-millimeters (mm), 3-micrometer (µm) C-18(2) column, Phenomenex (Torrance, California).

- 5.9 Autosampler**—Triathlon, type 900 (Spark-Holland, The Netherlands) equipped with a 10-mL syringe, 10-mL teflon sample loop, and eight type C sample trays. Each tray holds four, 20-mm, 10-mL vials.
- 5.10 Automated online SPE instrument**—Prospekt II or Symbiosis (Spark-Holland, The Netherlands) consisting of a high pressure dispenser (HPD) and an automated cartridge exchange unit (ACE).
- 5.11 LC/MS/MS**—Agilent (Santa Clara, California) model 1100 Series 2 High Performance Liquid Chromatogram (HPLC) with autoinjector and Waters (Milford, Massachusetts) Quattro Micro atmospheric pressure ionization (API) triple-stage quadrupole (tandem) mass spectrometer system with electrospray-ionization probe.
- 5.12 HPLC-Online SPE**—system computer with Agilent ChemStation (Santa Clara, California) and Spark-Holland (Netherlands) software SparkLink software.
- 5.13 Tandem Mass Spectrometry (MS/MS)**—system computer with MassLynx software (Waters Corporation, Milford, Massachusetts) to operate the mass spectrometer and acquire and store data. QuanLynx software (Waters Corporation, Milford, Massachusetts) for quantitation of compounds.

6. Reagents and Consumable Materials

- 6.1 Sample bottles**—baked 4-ounce (oz) amber glass bottles (Boston round) with Teflon-lined lids.
- 6.2 Sample filters**—nominal 0.7- μm glass-fiber filters.
- 6.3 SPE cartridges**—Oasis HLB extraction, Prospekt (10 mm x 2 mm) cartridges (Waters, Milford, Massachusetts).
- 6.4 Analytical Standards**—glyphosate, aminomethylphosphonic acid (AMPA), glufosinate, 99, 99, and 95 percent purity, respectively. (Chem Service, West Chester, Pennsylvania, obtained as powders).
- 6.5 Stable Isotope-labeled standards**—glyphosate isotope ($^{13}\text{C}_2$ (99 percent), ^{15}N (98 percent), (glyphosate 3+)) aminomethylphosphonic acid isotope (^{13}C (99 percent), ^{15}N (98 percent), methylene- D_2 (98 percent) – (AMPA 4+)) (Cambridge Isotopes Laboratories, Woburn, Massachusetts), aminomethylphosphonic acid isotope (^{13}C , ^{15}N (AMPA 2+)) (Dr. Ehrenstorfer GmbH, Germany), purity 98, 98, and 97, respectively. All of the standards were obtained as 100 $\mu\text{g}/\text{mL}$ (micrograms per milliliter) solutions.
- 6.6 9-Fluorenylmethylchloroformate (FMOC) derivatizing agent**—American Chemical Society (ACS) grade.
- 6.7 Sodium tetraborate**—ACS grade, powder.
- 6.8 Phosphoric Acid**—ACS grade
- 6.9 tetra sodium (Na)-EDTA**—ACS grade
- 6.10 Sodium Hydroxide**—ACS grade.
- 6.11 Ammonia Acetate**—ACS grade
- 6.12 Acetonitrile (ACN)**—HPLC grade or better.
- 6.13 Methanol**—HPLC grade or better.
- 6.14 DI water**—generated by purification of tap water through activated charcoal filter and deionization with a high-purity, mixed-bed resin, followed by another activated charcoal filtration, and finally distillation in a Barnstead autostill (Dubuque, Iowa) referred to as deionized/distilled (DI) water.
- 6.15 Gas for mass spectrometer**—high purity nitrogen; high-purity argon

7. Sampling Methods

Samples analyzed for this study were collected using USGS methods (Webb and others, 1999) and filtered through a 0.7- μm pore-size baked glass-fiber filter, using an aluminum plate-filter holder and a ceramic-piston fluid-metering pump with all Teflon tubing. Filters are leached with about 200 mL of sample before sample collection. The filtered water is collected in baked 4-oz amber glass bottles with Teflon-lined lids. The remainder of the water in the compositing container was used for various onsite measurements such as specific conductance, pH, and water temperature (Wilde and others, 1998). Samples were chilled immediately and shipped to the USGS OGRL in Lawrence, Kansas. At the USGS OGRL, samples were assigned identification numbers, logged into a database, and refrigerated at 4 $^{\circ}\text{C}$ until derivatization and analysis.

8. Standard and Reagent Solutions

- 8.1 Primary standard solutions**—individual 1 milligram per milliliter (mg/mL) (corrected for purity) stock solutions of glyphosate, AMPA, and glufosinate are prepared by weighing, to the nearest 0.0001 g, 50 mg of standard into a 50-mL volumetric flask and adding DI water. The solutions are stored at 4 $^{\circ}\text{C}$.
- 8.2 Intermediate standard mix**—a 10- $\mu\text{g}/\text{mL}$ solution containing all three standards, glyphosate, AMPA, and glufosinate is prepared in a plastic container by adding 1 mL of each 1 mg/mL standard to 97 gram (g) of DI water. The intermediate standard mix is prepared monthly and stored at 4 $^{\circ}\text{C}$.
- 8.3 Working standard mix**—a 50- $\mu\text{g}/\text{L}$ solution of glyphosate, AMPA, and glufosinate is prepared in a plastic bottle by pipetting 300 μl of intermediate standard mix and adding DI water to a final weight of 60 g and stored at 4 $^{\circ}\text{C}$.

6 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

- 8.4 Working stable-isotope labeled standard mix**—a 50- $\mu\text{g/L}$ solution of the three stable-isotope labeled standards is prepared in a 100-mL plastic bottle by weighing 60 g of DI grade water and adding 30 μL of each of the three 100- $\mu\text{g/mL}$ stable-isotope labeled standard solutions, glyphosate 3+, Ampa 4+, and AMPA 2+, and stored at 4 °C.
- 8.5 Working FMOC solution**—a 2.5-mM FMOC solutions prepared by weighing 0.2587 g of FMOC into 200 mL of ACN and stored at 4 °C.
- 8.6 Sodium tetraborate buffer solution**—a 5-percent (weight/volume) solution is prepared by weighing 10 g of sodium tetraborate (or borate) into 200 mL of DI water and stored at 4 °C.
- 8.7 2-percent phosphoric acid solution**—a 2-percent (volume/volume) in DI (to adjust sample pH after derivatization) and stored at 4 °C.
- 8.8 0.1-percent phosphoric acid**—a 0.1-percent phosphoric acid (volume/volume) in DI water (autosampler rinse solution).
- 8.9 Mobile Phase A and B**—5 mM ammonia acetate in DI water and ACN, respectively.

9. Sample Preparation

The samples are derivatized within 5 days after arrival at the laboratory and then stored in a refrigerator in the dark until analysis. Up to 40 environmental, duplicate, matrix spiked, DI blank, and DI spiked samples can be derivatized at one time. A typical sample set consists of 30 environmental, 3 duplicate, 3 matrix spiked, 4 blank DI water, and 4 spiked DI water samples. For each sample, a plastic screw-capped tube is labeled with the laboratory identification number and 10 mL of sample are dispensed into each tube. The appropriate amount of working standard mix is added to the samples selected for matrix spikes and matrix spiked DI water samples. Environmental matrix spiked samples are prepared at 1 $\mu\text{g/L}$, and 100 μL of the isotope-labeled glyphosate internal standard solution is added to all the tubes; then 500 μL of 5-percent sodium borate solution is added to all the tubes. All tubes are mixed by vortexing. Finally, 1,500 μL of 2.5-mM FMOC in ACN are added to all tubes and mixed by inverting at least three times. All tubes are placed in a 40 °C water bath in the dark for 24 hours \pm 1 hour. The tubes are removed, and 600 μL of 2-percent phosphoric acid in DI water are added to each tube. Tubes are mixed by inversion at least three times. The derivatized samples then are stored at 4 °C and in the dark until analysis. Experimental results in this document indicate that derivatized solutions are stable at least 60 days. Before analysis, 5.5 mL of derivatized sample from each tube is diluted with 5.5 mL of DI water in the 10-mL autosampler vial.

10. Automated Solid-Phase Extraction

The autosampler, automated cartridge exchange unit, and high pressure dispenser pump that comprise the automated on-line SPE instrument are programmed to prepare, load, and elute the SPE cartridge and rinse the sample lines. Each sample is loaded into the sample tray of the autosampler, and the SPE instrument is loaded with cartridges. A cartridge automatically is placed in the ACE loading clamp and the cartridge is conditioned with 2-mL of methanol and then 2 mL of DI water. Ten mL of sample is then loaded onto the cartridge from the autosampler at a rate of 2 mL/min and the cartridge is then washed with 1 mL of DI water. The loaded SPE cartridge is then transferred to the ACE elution clamp, and placed in the flow path of the LC binary pump mobile phase when the LC sends an inject signal to the on-line SPE and tandem mass spectrometry (MS/MS) instruments to start the analysis. The binary mobile phase mixture elutes the SPE cartridge for 1 minute at 0.1 mL/min. The binary pump mobile phase mixture is diluted using a stainless steel “T” fitting for the first 5 minutes of the sample analysis at 0.35 mL/min with an aqueous mobile phase delivered by an isocratic pump to focus the compounds onto the head of the LC column. Thus, as one sample is being analyzed, another is being extracted.

11. Liquid Chromatography/Tandem Mass Spectrometry Preparation

The eluted compounds are separated on an Agilent 1100 model D series LC system with a Luna 150 x 3-mm, 3- μm C-18(2) analytical column (Phenomenex, California). The LC column is equilibrated with the mobile phase for 2 hours before analysis. Mass spectral analysis is conducted using a Waters Quattro Micro API benchtop triple quadrupole (tandem) MS system, with electrospray ionization (ESI) in negative-ion mode using MRM.

11.1 Sample analysis—The LC/MS/MS mobile phases for the isocratic and binary pumps used for the analysis of glyphosate, AMPA, and glufosinate are listed below and the mobile phase gradient conditions are shown in table 2.

11.2 LC conditions:

11.2.1 LC column oven conditions: 45 °C.

11.3 LC mobile phase:

11.3.1 A, 5 mM ammonium acetate

11.3.2 B, acetonitrile

11.3.3 isocratic, 5 mM ammonium acetate

11.4 MS/MS source parameters:

11.4.1 MS ionization mode: electrospray ionization in negative-ion mode.

11.4.2 Capillary voltage: 2,000 volts (V).

- 11.4.3 Extractor voltage: 1 V.
 11.4.4 Radio frequency (RF) lens voltage: 0.1 V.
 11.4.5 Source temperature: 120 °C.
 11.4.6 Desolvation temperature: 400 °C.
 11.4.7 Cone gas flow: 15 L/hr.
 11.4.8 Desolvation gas flow: 500 liters per hour (L/hr).

11.5 Quadrupole 1 parameters:

- 11.5.1 Low mass (LM 1) resolution: 13.
 11.5.2 High mass (HM 1) resolution: 14.
 11.5.3 Ion energy: 1.0.

11.6 Collision Cell parameters:

- 11.6.1 Entrance: 0.
 11.6.2 Exit : 3.

11.7 Quadrupole 2 parameters:

- 11.7.1 Low mass (LM 2) resolution: 13.
 11.7.2 High mass (HM 2) resolution: 13.
 11.7.3 Ion energy: 2.0.
 11.7.4 Photo Multiplier: 650 V.

11.8 *Data acquisition and processing*—The data are acquired using MassLynx and quantified using Waters QuanLynx data analysis program (Waters Corp., Milford, Massachusetts).

Table 2. Summary of the liquid chromatography conditions for the mobile phase gradients and flow rates.

[mL, milliliter]

Isocratic pump mobile phase conditions		
Time (minutes)	Mobile phase (percent)	Flow (mL/minutes)
0	100	0.350
5.00	100	.350
5.01	--	0
Binary pump mobile phase conditions		
Time (minutes)	Mobile phase B (percent)	Flow (mL/minutes)
0	20	0.100
5.00	20	.100
5.01	20	.400
11.50	60	.400
11.51	100	.400
14.00	100	.650
14.01	15	.400
17.00	15	.400

Evaluation of Instrument Performance

Peak shape, system pressure, and check standards are used to evaluate LC/MS/MS performance before each analytical run. If peak shape deteriorates (diminished response and peak tailing), the columns may need to be cleaned or replaced. If the pressure reading is high (overpressures), there may be a clog in the mobile-phase flow path.

For the MS/MS, a static, scanning, and scan speed compensation mass calibration of the quadrupoles is performed every 6 months unless mass “drift” has been identified as a problem. The MS/MS method source parameters optimized for this method are listed in section 11. Liquid Chromatography/Tandem Mass Spectrometry Preparation. The MRM transition parameters (cone voltage and collision cell energy) needed to maximize the response of the deprotonated molecule (molecule – hydrogen, M-H) ion and daughter-ion transitions for each compound are listed in table 3. The MS/MS source and MRM transition parameters are optimized for each compound by infusing 50 milligram per liter (mg/L) of each of the pre-derivatized compound (80/20 ammonia acetate/ACN) solutions at 10 microliter per minute (μL/min) using a syringe pump into a “T” fitting into which a 50/50 mixture of mobile phases A and B (table 2) are pumped at 0.36 mL/min. The MS/MS tune parameters (source and quadrupole 1 and 2 parameters in section 11. Liquid Chromatography/Tandem Mass Spectrometry Preparation) for the source and Q1 were adjusted to optimize the response of the FMOc deprotonated molecule (M-H) for glyphosate, AMPA, glufosinate and the collision cell, and Q2 parameters are adjusted to identify and optimize response of the daughter ions. The mass spectrometer performance is evaluated before each sample run by analyzing 0.02- and 1.0-μg/L spiked DI water samples and assessing daughter ion abundances. The ion-abundances on check standards are evaluated during each analytical run to assess if the general source tune parameters and compound specific (Q1, Q2) tune parameters need to be reoptimized.

12. Compound Identification and Quantitation

The isotope-labeled standards for glyphosate and AMPA are used as a retention time reference and for quantitation. The relative retention time (RRT_c) is calculated for each selected compound in the calibration samples or in a sample as follows:

$$RRT_c = RT_c / RT_i \quad (1)$$

where

$$RT_c = \text{uncorrected retention time of the selected compound; and}$$

$$RT_i = \text{uncorrected retention time of the internal standard.}$$

The expected retention time (RT) of the peak of the selected compound needs to be within ± 5 percent of the expected

8 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

Table 3. Summary of the Multiple Reaction Monitoring deprotonated molecule and daughter-ion transition pairs, retention times, optimized cone voltages, and collision cell parameters for derivatized glyphosate, aminomethylphosphonic acid, glufosinate, and internal standards.

[FMOC, 9-fluorenylmethylchloroformate; M-H, deprotonated molecule]

FMOC compounds	Molecular weight M-H ion	Quantitation daughter-ion	Confirming daughter-ion	Retention time (minutes)	Cone voltage	Collision cell energy
Glyphosate-FMOC	389.9	168	150	9.48	15	13, 25
Aminomethylphosphonic acid-FMOC	331.9	110	136	12.07	11	8, 17
Glufosinate-FMOC	401.9	180	206	10.73	15	11, 15
Isotope Labeled +3 - glyphosate-FMOC (glyphosate 3+)	391.9	170	152	9.48	15	13, 25
Isotope labeled +4- aminomethylphosphonic acid-FMOC (AMPA 4+)	335.9	114	140	12.07	11	8, 17
Isotope labeled +2- aminomethylphosphonic acid-FMOC (AMPA 2+)	333.9	112	138	12.07	11	8, 17

retention time on the basis of the RRT_c . The expected retention time is calculated as follows:

$$RT = (26RRT_c) (RT_i) \quad (2)$$

where

- RT = expected retention time of the selected compound;
 RRT_c = relative retention time of the selected compound; and
 RT_i = uncorrected retention time of the internal standard.

The dilution factor (DF) of the processed sample is calculated using equation 3 shown below:

$$DF = (2610/(10-V_{np})) (2610/(10-V_a)) \quad (3)$$

where

- DF = dilution factor;
 V_{np} = volume not pumped = milliliters not pumped through the SPE column; and
 V_a = volume added = milliliters of distilled water added to a sample that contained less than 10 mL.

The DF is incorporated into the calculation for determining final concentrations of samples.

13. Calculation of Results

13.1 Qualitative Identification—Identification and quantitation of compounds is performed on the raw data files using MassLynx with the QuanLynx data analysis package. A

compound is not correctly identified unless the correct M-H to daughter ion transitions are detected, the relative ratio of the quantitation to confirming daughter-ions is within ± 25 percent of the average ratio obtained from the spiked reagent-water samples, and the relative retention time is within tolerance.

13.2 Quantitation—Samples analyzed between April 2004 and June 2006 were quantitated using standard addition and isotope dilution. The following equation was used to calculate concentrations by standard addition:

$$C = (Rus/(Rsp-Rus)) Csp \quad (4)$$

where

- C = concentration of the analyte in the unspiked sample;
 Rus = area ratio of the quantitation-ion of the analyte to the area of the quantitation-ion of the internal standard in the unspiked sample;
 Rsp = area ratio of the quantitation-ion of the analyte to the area of the quantitation-ion of the internal standard in the spiked sample; and
 Csp = the concentration of the analytes in the spiked sample because of the spike.

Samples were quantitated by isotope dilution using the following equation:

$$C = ((Ac/Ai) (Rf) (Ci) (DF)), \text{ in micrograms per liter} \quad (5)$$

where

- C = concentration of the selected compound in the sample, in micrograms per liter;
 Ac = peak area of the quantitation ion for the selected compound;
 Ai = peak area of the quantitation ion for the stable-isotope labeled standard;
 Rf = response factor based on response difference between area of stable-isotope labeled and unlabeled compound analyzed at equivalent concentrations;
 Ci = concentration of stable-isotope labeled standard;
 DF = dilution factor calculated using equation 3; and,

The six sets of duplicate DI-water, groundwater, and surface-water spiked samples and unspiked samples were quantitated by isotope dilution (equation 5) and by linear regressed seven-point standard curves constructed from 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, and 1.0 $\mu\text{g/L}$ spiked DI water samples with each set. The correlation coefficient (r^2) for each standard curve has to be greater than or equal to 0.99 to be accepted. If a selected compound has passed the qualitative identification criteria, the concentration in the sample is calculated as follows:

$$C = ((Ac/Ai) (m) + b) (DF) \quad (6)$$

where

- C = concentration of the selected compound in the sample, in micrograms per liter;
 Ac = area of the quantitation ion for the selected compound;
 Ai = area of the quantitation ion for the internal standard;
 m = slope of calibration curve using extracted standards for the selected compound and the internal standard from the analytical run;
 b = intercept of calibration curve for the selected compound and the internal standard from the analytical run; and
 DF = dilution factor calculated using equation 3.

14. Reporting of Results

Glyphosate, AMPA, and glufosinate are reported in concentrations ranging from 0.02 to 5.0 $\mu\text{g/L}$. If the concentration is greater than 5.00 $\mu\text{g/L}$ and estimated to be less than 10 $\mu\text{g/L}$, a part of the original derivatized sample is diluted appropriately with DI water and reanalyzed; if the sample is greater than 10 $\mu\text{g/L}$, the raw water sample is diluted and rederivatized.

Method Performance

Comparison of quantitation by standard addition (the approved quantitation procedure for U.S. Geological Survey method 0-2136-01; Lee and others, 2002) and isotope dilution was conducted on 473 samples analyzed between April 2004 and June 2006. This was done because standard addition requires an unspiked and a spiked sample to be analyzed to calculate the concentration of the detected analytes. With the acquisition of stable-isotope labeled standards for AMPA, along with the stable-isotope labeled glyphosate, it was determined that sample analysis could be reduced by approximately 40 percent if quantitation could be done using isotope dilution.

The overall method performance for precision, accuracy, and evaluation of two quantitation methods was assessed by analyzing six sets of unspiked and spiked samples between March 30, 2007 and April 30, 2007. Each sample set contained DI water, groundwater samples collected from Pennsylvania, and surface-water samples collected from Marion Lake, Kansas. Two, 10-mL sample aliquots from each matrix were spiked at concentrations of 0.0, 0.05, 0.10, and 0.50 $\mu\text{g/L}$. Method detection levels were determined using replicate 0.05 $\mu\text{g/L}$ spiked sample matrices of all six sets. All three sample matrices and concentration levels from all six sets were used to assess method accuracy and precision, and also were used to assess the comparability of quantitation by isotope dilution and linear regressed multiple-point standard curves.

15. Comparison of Isotope Dilution and Standard Addition

Concentrations determined by standard addition and isotope dilution for glyphosate, AMPA, and glufosinate determined in 473 samples along with the average percent difference and percent relative standard deviation (prsd) between concentrations calculated by standard addition and isotope dilution are shown in table 4 (at the back of this report). For samples that were diluted because of elevated concentrations, the diluted concentrations were reported in table 4 and used for the comparison. The percent difference between standard addition and isotope dilution for each sample was determined using the following equation:

$$\text{percent difference} = ((C_{id} - C_{sa}) / ((C_{id} + C_{sa}) / 2)) 100 \quad (7)$$

where

- C_{id} = sample concentration determined by isotope dilution; and
 C_{sa} = sample concentration determined by standard addition.

The mean percent difference \pm relative standard deviation (rsd) was determined from all the samples for which the compounds were detected. The data set in table 4 represents more than 2 years of data from surface- and groundwater samples collected

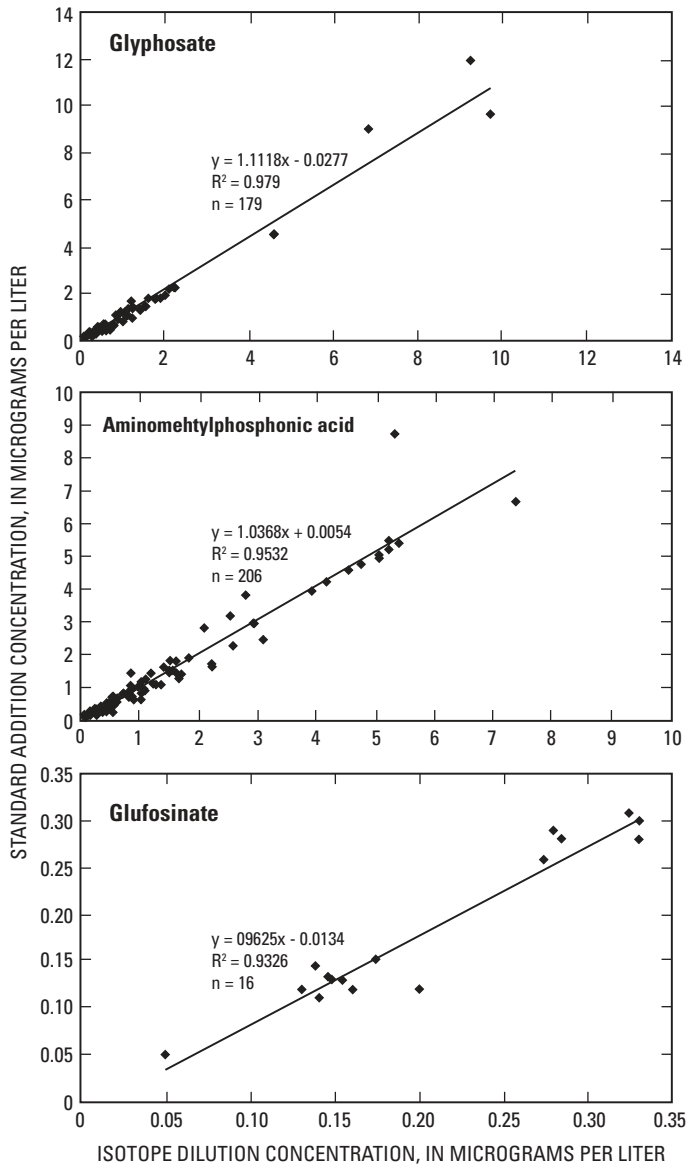


Figure 2. Linear regressions of concentrations of glyphosate, aminomethylphosphonic acid, and glufosinate calculated by isotope dilution compared to standard addition for surface water and groundwater samples analyzed between April, 2004 and June, 2006.

throughout the United States. Glyphosate was detected in 180 (38 percent), AMPA in 207 (44 percent), and glufosinate in 16 (3 percent) of 473 samples. The mean percent difference and \pm rsd between the two quantitation methods, was 7.6 ± 6.30 for glyphosate, 9.6 ± 8.35 for AMPA, and 9.3 ± 9.16 for glufosinate. The percent difference between the two quantitation methods was less than 20 percent for most of the samples in which there were detections (table 4). Linear regressions (fig. 2) indicated a good correlation between sample concentrations calculated by standard addition and isotope dilution, with r^2 values of 0.98, 0.95, and 0.93 and slopes of 1.11, 1.04, and 0.96 for glyphosate, AMPA, and glufosinate, respectively. The data also indicate that above 5 $\mu\text{g/L}$ there was greater variation

between the concentrations determined by the two methods of quantitation. For regressions of samples with concentrations less than or equal to 5.0 $\mu\text{g/L}$, the r^2 was 0.98 for glyphosate and AMPA and the slopes were 1.01 and 1.04 for glyphosate and AMPA, respectively. There also was 100 percent agreement between isotope dilution and standard addition in samples with concentrations less than the reporting limit (0.02 $\mu\text{g/L}$) for all three compounds (table 4). The data from table 4 and figure 2 indicate that there is good agreement between the two quantitation methods for glyphosate and AMPA from 0.02 through 5 $\mu\text{g/L}$.

16. Matrix Performance

To evaluate general method performance, 10-mL sample aliquots of each of the three samples matrices were spiked with glyphosate, AMPA, and glufosinate in duplicate at concentrations of 0.05, 0.10, and 0.50 $\mu\text{g/L}$ and analyzed on 6 different days between March 30, 2007, and April 30, 2007. Thus, the analysis of different matrices and concentrations included bias from day-to-day variation. Unspiked samples of each matrix were extracted and analyzed to determine background concentrations of the glyphosate, AMPA, and glufosinate. All samples were analyzed at the USGS OGRL using one on-line SPE LC/MS/MS system.

A statistical summary of six sets of duplicate spiked DI-water, groundwater, and surface-water samples is given in table 5. The concentration of glyphosate, AMPA, and glufosinate in each sample was calculated by isotope dilution and linear regression using a seven-point standard curve constructed from DI-water samples spiked at 0.01, 0.02, 0.05, 0.1, 0.20, 0.50, and 1.0 $\mu\text{g/L}$, extracted, and analyzed with each of the six sample sets. The average calculated concentration within each sample matrix and each concentration level generally was within 20 percent of the spiked concentration for each of the three compounds and the prsd's ranged from approximately 9 to 24 percent for the 0.05 and 0.10 $\mu\text{g/L}$ spiked samples, and from 3 to 18 percent for the 0.50 $\mu\text{g/L}$ spiked samples. The prsd was less than 24 percent among all three matrices within each concentration level for all three compounds.

The normalized concentration, the calculated concentration divided by the theoretical spiked concentration expressed as a percentage, within each sample matrix and among all sample matrices within each concentration level and also the grand mean among all sample matrices and all concentration levels is shown in table 6. These data indicate the average normalized concentration varied from approximately 92 to 122 percent of the expected concentration within each sample matrix and concentration level. Within each concentration level and among all sample matrices, the NCP varies from approximately 95 to 116 percent and the percent rsd's ranged from less than 1 to 3 percent. The grand mean NCP among all sample matrices and all concentration levels ranged from 99 to 114 percent, with percent rsd's ranging from approximately

Table 5. Statistical summary of average concentrations for six sets of duplicate deionized-distilled-water, groundwater and surface-water samples spiked at 0.05 µg/L analyzed between March 30 and April 30, 2007.

[AMPA, aminomethylphosphonic acid; µg/L, microgram per liter; n, number of samples]

	0.05 µg/L spiked samples					
	Isotope dilution quantitation			Standard curve quantitation		
	Glyphosate	AMPA	Glufosinate	Glyphosate	AMPA	Glufosinate
	Reagent water (n=12)					
Average concentration (µg/L)	0.05	0.05	0.05	0.05	0.05	0.05
Standard deviation (µg/L)	.010	.011	.006	.006	.007	.005
Relative standard deviation (percent)	19.5	20.2	12.3	11.6	13.3	9.7
Method detection limit (µg/L)	.032	.033	.020	.019	.021	.016
	Groundwater (n=12)					
Average concentration (µg/L)	.05	.05	.06	.05	.06	.06
Standard deviation (µg/L)	.008	.006	.005	.007	.005	.007
Relative standard deviation (percent)	16.3	11.0	9.0	13.0	9.1	11.2
Method detection limit (µg/L)	.025	.019	.017	.022	.016	.021
	Surface water (Marion Lake, n=12)					
Average concentration (µg/L)	.05	.06	.06	.06	.05	.06
Standard deviation (µg/L)	.008	.007	.003	.006	.007	.006
Relative standard deviation (percent)	16.0	13.0	5.5	10.7	13.1	9.9
Method detection limit (µg/L)	.025	.023	.010	.018	.021	.018
	All samples (n =36)					
Grand mean concentration (µg/L)	.05	.05	.06	.05	.05	.06
Standard deviation (µg/L)	.009	.008	.006	.006	.007	.007
Relative standard deviation (percent)	17.2	14.9	11.2	11.6	12.9	11.8
Method detection limit (µg/L)	.024	.022	.017	.017	.019	.018
	0.10 µg/L spiked samples					
	Isotope dilution quantitation			Standard curve quantitation		
	Glyphosate	AMPA	Glufosinate	Glyphosate	AMPA	Glufosinate
	Reagent water (n=12)					
Average concentration (µg/L)	.10	.09	.11	.09	.10	.10
Standard deviation (µg/L)	.014	.015	.016	.018	.008	.011
Relative standard deviation (percent)	14.6	16.2	14.8	19.9	7.5	10.7
	Groundwater (n=12)					
Average concentration (µg/L)	.10	.10	.12	.10	.11	.11
Standard deviation (µg/L)	.010	.014	.013	.022	.008	.014
Relative standard deviation (percent)	11.2	13.6	11.1	22.1	7.2	11.9
	Surface water (Marion Lake, n=12)					
Average concentration (µg/L)	.10	.12	.11	.10	.12	.11
Standard deviation (µg/L)	.009	.020	.015	.022	.028	.015
Relative standard deviation (percent)	9.0	16.5	13.2	23.2	23.7	12.3
	All samples (n =36)					
Grand mean concentration (µg/L)	.10	.11	.11	.10	.11	.11
Standard deviation (µg/L)	.012	.019	.016	.021	.019	.014
Relative standard deviation (percent)	11.9	18.3	13.9	21.6	17.1	13.3

Table 5. Statistical summary of average concentrations for six sets of duplicate deionized-distilled-water, groundwater and surface-water samples spiked at 0.05 µg/L analyzed between March 30 and April 30, 2007.—Continued

[AMPA, aminomethylphosphonic acid; µg/L, microgram per liter; n, number of samples]

	0.50 µg/L spiked samples					
	Isotope dilution quantitation			Standard curve quantitation		
	Glyphosate	AMPA	Glufosinate	Glyphosate	AMPA	Glufosinate
	Reagent water (n=12)					
Average concentration (µg/L)	0.49	0.54	0.53	0.51	0.50	0.48
Standard deviation (µg/L)	.025	.012	.032	.018	.018	.058
Relative standard deviation (percent)	5.0	2.2	6.1	3.6	3.6	12.1
	Groundwater (n=12)					
Average concentration (µg/L)	.49	.54	.61	.51	.51	.55
Standard deviation (µg/L)	.021	.028	.077	.030	.021	.099
Relative standard deviation (percent)	4.4	5.2	12.6	5.9	4.1	17.8
	Surface water (Marion Lake, n=12)					
Average concentration (µg/L)	.49	.55	.60	.52	.51	.54
Standard deviation (µg/L)	.020	.018	.053	.031	.028	.079
Relative standard deviation (percent)	4.1	3.3	8.8	5.9	5.4	14.7
	All samples (n =36)					
Grand mean concentration (µg/L)	.49	.54	.58	.51	.51	.52
Standard deviation (µg/L)	.022	.021	.068	.026	.023	.087
Relative standard deviation (percent)	4.5	3.9	11.8	5.1	4.4	16.5

2 to 7 percent. These data indicate that the method provides adequate quantitation among a range of matrices and concentrations. The data from tables 5 and 6 also indicate that quantitation by isotope dilution and regressed standard curves provide similar results; this is shown in more detail in table 7.

The mean percent difference and percent rsd between the average calculated concentration among all samples matrices within each concentration level and among all samples is shown in table 7. The mean percent difference varied from approximately 5 to 22 percent and the percent rsd's ranged from approximately 2 to 19 percent within each concentration level. The largest percent differences and percent rsd's were for glyphosate at the 0.05 and 0.1 µg/L concentration levels. Among all samples, the percent difference between the two quantitation levels ranged from approximately 8 to 15 percent with percent rsd's that ranged from approximately 2 to 9 percent. These data also indicate that quantitation using isotope dilution or multi-point standard curves is similar among all matrices and at all concentration levels with larger variation observed for glyphosate at lower concentration levels.

Generally, the data from tables 3–6 indicate that quantitation by standard addition, isotope dilution, and multiple regressed standard curves provides accurate and similar results in multiple matrices from 0.02 to 5.0 µg/L. The method has a demonstrated stability since it was transferred to the LC/MS/MS in 2004 (table 4).

Method Detection Limits

A method detection limit (MDL) is defined as the minimum concentration of a substance that can be identified, measured, and reported with a 99-percent confidence that the compound concentration is greater than zero. MDLs were calculated according to procedures outlined by the U.S. Environmental Protection Agency (USEPA) (1992). The replicate 0.05-µg/L samples from the six data sets in table 5 were used to calculate the method detection levels. The MDL was calculated using the following equation:

$$\text{MDL} = (\text{SD}) \cdot (t_{(n-1, 1-\alpha=0.99)}) \quad (8)$$

where

- SD = standard deviation of replicate analysis, in micrograms per liter, at the spiked concentration;
- $(t_{(n-1, 1-\alpha=0.99)})$ = student's t-value for the 99-percent confidence level with n-1 degrees of freedom (U.S. Environmental Protection Agency, 1992); and
- n = number of replicate analyses.

Method detection levels were calculated for each of the three matrices and also among the three matrices (table 5). MDL's were calculated for the isotope dilution and regressed standard

Table 6. Statistical summary of the average concentration of glyphosate, aminomethylphosphonic acid, and glufosinate normalized as a percentage of the expected spiked concentration (normalized concentration) for six sets of duplicate deionized-distilled water, groundwater and surface-water samples spiked at three concentration levels.

[$\mu\text{g/L}$; microgram per liter; AMPA, aminomethylphosphonic acid; prsd, percent r squared]

	Isotope dilution			Standard curve		
	Glyphosate	AMPA	Glufosinate	Glyphosate	AMPA	Glufosinate
0.05 $\mu\text{g/L}$ spiked samples						
Deionized water	106	105	103	107	102	103
Groundwater	99.2	109	122	109	116	120
Surface water	99.8	112	117	111	103	116
Mean percent all matrices	102	109	114	109	107	113
prsd	3.76	3.51	9.85	2.00	7.81	8.89
0.10 $\mu\text{g/L}$ spiked samples						
Deionized water	99.6	92.3	107	91.8	101	101
Groundwater	97.4	102	121	98.3	108	115
Surface water	95.9	120	111	96.8	120	105
Mean percent all matrices	97.6	105	113	95.1	110	107
prsd	1.86	12.73	7.21	3.40	9.61	7.21
0.50 $\mu\text{g/L}$ spiked samples						
Deionized water	98.5	108	105	103	101	95.2
Groundwater	97.1	108	122	102	103	111
Surface water	98.7	111	121	103	102	108
Mean percent all matrices	98.2	109	116	103	102	105
prsd	.872	1.73	9.54	0.58	1.00	2.12
All spiked samples						
Grand mean	99.3	108	114	102	106	108
prsd	2.39	2.31	1.53	6.97	4.04	4.16

curve quantitated data. The estimated MDL for glyphosate, AMPA, and glufosinate ranged from 0.010 to 0.032 $\mu\text{g/L}$ within the individual matrices and from 0.017 to 0.024 $\mu\text{g/L}$ among the three matrices. According to the USEPA procedure, the spiked concentrations should be no more than five times the estimated MDL. Thus, the 0.05- $\mu\text{g/L}$ spiked samples were well within the US EPA recommended spiked levels. The MDL was set at 0.02 $\mu\text{g/L}$ for each of the three compounds,

based on the statistical derivation of the MDL's (table 5), and evaluation of the peak-to-peak signal-to-noise ratios calculated by the Quan-Lynx software of the 0.01- and 0.02- $\mu\text{g/L}$ DI- water samples analyzed as part of the standard curves with each of the six sample sets. The signal-to-noise ratio of the 0.02- $\mu\text{g/L}$ standards ranged from 3 to 5, 3 to 6, and 4 to 7 for glyphosate, AMPA, and glufosinate, respectively.

Table 7. Statistical summary of mean percent difference between glyphosate, aminomethylphosphonic acid, and glufosinate concentrations determined by isotope dilution and multiple-point regressed standard curves for spiked deionized-distilled-water, groundwater, and surface-water samples.

[AMPA, aminophosphonic acid; $\mu\text{g/L}$, microgram per liter; rsd, relative standard deviation; n, number of samples]

	Mean \pm rsd 0.05 $\mu\text{g/L}$, n = 36	Mean \pm rsd 0.10 $\mu\text{g/L}$, n = 36	Mean \pm rsd 0.50 $\mu\text{g/L}$, n = 36	Grand Mean \pm rsd All Samples
Glyphosate	18.3 \pm 12.63	21.6 \pm 19.49	4.90 \pm 3.326	14.9 \pm 8.832
AMPA	10.7 \pm 7.369	9.89 \pm 10.85	7.02 \pm 3.224	9.20 \pm 1.925
Glufosinate	6.32 \pm 4.245	7.05 \pm 6.566	11.8 \pm 11.94	8.39 \pm 2.982

Table 8. Statistical summary of glyphosate and aminomethylphosphonic acid concentrations in filtered raw water samples and derivatized water samples with time normalized to beginning concentrations.

[AMPA, Aminophosphonic Acid; ≤, less than or equal to; ±, plus or minus; ≥, greater than or equal to]

	Normalized percent glyphosate	Number of samples	Normalized percent AMPA	Number of samples
Stability of raw water samples with time				
≤85 days	98±7.3	5	100±22.1	6
≤136 days	93±18.3	12	100±23.4	13
≥148 days	65±25.4	12	89±32.6	11
Stability of derivatized water samples with time				
52 days	94±10.3	8	112±8.2	9
64 day	103±19.0	8	103±13.1	8
All days	101±20.0	18	111±24.8	21

Compound Stability in Underivatized and Derivatized Water Samples

The stability of glyphosate and AMPA was evaluated in raw filtered water samples with time. Ten raw water samples were derivatized at four to five different times from 136 to 269 days (table 8). The concentrations of glyphosate and AMPA detected in each of raw samples derivatized and analyzed after the initial (day 1) analysis were normalized to the day-one analysis for that sample. For the 6 samples that had analyses at 85 days and 10 samples that had analyses at 136 days or less the mean normalized values ranged from 93 to 100 percent ± 7 to 23 percent of the initial analyzed concentrations. The normalized concentrations for all the analyses in the 10 samples analyzed between 148 and 269 days were 65 to 89 percent ± 25 to 33 percent of the initial analyzed concentrations. The half-life of glyphosate varied from 169 to 223 days calculated from four samples with log linear declines ($r^2 > 0.95$) in concentration. The AMPA concentrations in several of the samples remained constant, whereas glyphosate concentrations decreased indicating that glyphosate and AMPA degrade at a similar rate. These data indicate that derivatization of raw filtered water samples within 5 to 6 days of collection should result in minimal loss of glyphosate because of degradation. The half-life data also indicate that the maximum glyphosate loss during a 14-day holding time would be approximately 8 percent.

Between October 2001 and May 2002 water samples from the same 10 sites that were used in the underivatized stability study also were used to assess the stability of glyphosate and AMPA in derivatized water samples. Aliquots from each sample were derivatized and analyzed on day 1, and then again after 52 days. The samples then were derivatized again and analyzed on day 1 and then again after 64 days. Three of the samples also were derivatized on day 1, and analyzed on days 22, 34, and 75, respectively. The concentrations of the derivatized samples that were analyzed at the end of the

holding times were normalized to the concentrations of the derivatized sample that were analyzed on day 1. During the experiment one of the 64-day samples did not extract properly and was discarded. The mean NCP for all the samples was 101 ± 20.3 and 111 ± 24.8 percent for glyphosate and AMPA, respectively. These data indicate that derivatized samples can be held at least for up to 60 days before analysis.

Conclusions

This on-line SPE-LC/MS/MS method (O-2141-09) provides for routine analysis of glyphosate, AMPA, and glufosinate in samples collected from ground- and surface-water. Equivalence between isotope dilution and standard addition was demonstrated with more than 2 years of data. The compounds also showed good precision, generally less than 24 percent relative standard deviation within each matrix by isotope dilution and linear regressed standard curves. Method detection limits of 0.02 µg/L were established for all three analytes in multiple matrices, and the mean variation from expected spiked concentrations generally was less than 20 percent for all three compounds. This study also indicated that holding times of 5 days for raw filtered samples would result in minimal loss of glyphosate and that derivatized samples are stable for at least 60 days.

Information about the fate and transport of glyphosate, and its degradation product, AMPA, and glufosinate in water can be acquired from the analysis of ground and surface water. This method was an important breakthrough and is contributing to an improved understanding of the occurrence, persistence, and transport of glyphosate and its degradation products in the environment.

Acknowledgments

Support for this project was provided by the U.S. Geological Survey Toxic Substances Hydrology Program.

References Cited

- Battaglin, W.A., Kolpin, D.W., Scribner, E.A., Kuivila, K.M., and Sandstrom, M.W., 2005, Glyphosate, other herbicides, and transformation products in Midwestern Streams, 2002: *Journal of the American Water Resources Association*, v. 41 no. 2, p. 323–332.
- Cox, Caroline, 1996, Glyphosate Herbicide Fact Sheet: *Journal of Pesticide Reform*, Winter 2004, v. 24, p. 10–15.
- Cox, Caroline, 2004, Glyphosate Herbicide Fact Sheet: *Journal of Pesticide Reform*, Winter 2004, v. 24, p. 10–15.
- Iowa State University, 1997, Glyphosate, accessed September 23, 2005, at URL: <http://www.agron.iastate.edu/~weeds/Ag317/manage/herbicide/glyphosate.html>
- Kiely, T., Donaldson, D., and Grube, A., 2004, Pesticides industry sales and usage: 2000 and 2001 market estimates: U.S. Environmental Protection Agency. Office of Prevention, Pesticides, and Toxic Substances, Office of Pesticide Programs, Biological and Economic Analysis Division, accessed July 11, 2006, at URL: http://www.epa.gov/opp-bead1/pestsales/01pestsales/tab_e_of_contents2001.htm
- Kolpin, D.W., Thurman, E.M., Lee, E.A., Meyer, M.T., Furlong, E.T., and Glassmeyer, S.T., 2006, Urban contributions of glyphosate and its degradate AMPA to streams in the United States: *Science of the Total Environment*, v. 354, p. 191–197.
- Lee, E.A., Strahan, A.P., and Thurman, E.M., 2002, Methods of analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of glyphosate, aminomethylphosphonic acid, and glufosinate in water using online solid-phase extraction and high-performance liquid chromatography/mass spectrometry: U.S. Geological Survey Open-File Report 01–454, 13 p.
- Monsanto Company, 2002, Backgrounder: History of Monsanto's glyphosate herbicides: accessed July 11, 2006, at URL: www.monsanto.com/monsanto/layout/sci_tech/crop_chemicals/default.asp
- Rueppel, M.L., Brightwell, B.B., Schaeffer, J., Marvel J.T., 1977, Metabolism and degradation of glyphosate in soil and water: *Journal of Agricultural and Food Chemistry*, v. 25, p. 517–528.
- Scribner, E.A., Battaglin, W.A., Dietze, J.E., Thurman, E.M., 2003, Reconnaissance data for glyphosate, other selected herbicides, their degradation products, and antibiotics in 51 streams in nine Midwestern states, 2002: U.S. Geological Survey Open-File Report 03–217, pp. 101.
- Scribner, E.A., Battaglin, W.A., Gilliom, R.J., and Meyer, M.T., 2007, Concentrations of glyphosate, its degradation product, aminomethylphosphonic acid, and glufosinate inground- and surface-water, rainfall, and soil samples collected in the United States, 2001–06: U.S. Geological Survey Scientific Investigations Report 2007–5122, 111 p.
- Stone, W.W., and Wilson, J.T., 2006, Preferential flow estimates to an agricultural tile drain with implications for glyphosate transport: *Journal of Environmental Quality*, v.35, p. 1,825–1,835.
- U.S. Department of Agriculture, 2004, National Agricultural Statistics Service (NASS) agriculture chemical use database: accessed February 6, 2006, at URL: <http://www.pestmanagement.info/nass/>
- U.S. Environmental Protection Agency, 1992, Guidelines establishing test procedures for the analysis of pollutants (appendix B, part 136, Definition and procedures for the determination of the method detection limit): U.S. Code of Federal Regulations, Title 40, revised as of July 1, 1992, p. 565–567.
- Webb, W.E., Radatke, D.B., and Iwatsubo, R.T., 1999, Surface-water sampling collection methods at flowing-water and still-water sites: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A4, section 4.1, p. 23–59.
- Wilde, F.D., Radtke, D.B., Gibs, Jacob, and Iwatsubo, R.T., 1998, National field manual for the collection of water-quality data—preparations for water sampling: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A1–A5 (variously paged).

Table 4

18 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Surface water									
District of Columbia									
03/14/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
03/14/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
03/14/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
03/14/05	1.06	1.14	4.79	.14	.21	25	<.02	<.02	--
03/24/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
03/24/05	.33	.30	6.64	.40	.41	.99	<.02	<.02	--
03/24/05	.32	.33	.82	.15	.15	0	<.02	<.02	--
03/24/05	.44	.60	19	<.02	<.02	--	<.02	<.02	--
03/08/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
03/08/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
03/08/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
03/08/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
03/08/06	<.02	<.02	--	.11	.09	13	<.02	<.02	--
04/05/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/05/06	.12	.12	1.11	.12	.12	4.53	<.02	<.02	--
Florida									
06/07/04	<.02	<.02	--	.10	.07	26	<.02	<.02	--
06/07/04	<.02	<.02	--	.09	.06	27	<.02	<.02	--
06/08/04	.02	.01	29	.08	.06	24	<.02	<.02	--
06/08/04	.01	.02	22	.08	.04	47	<.02	<.02	--
09/06/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/06/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/07/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/07/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/07/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
Iowa									
04/13/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/13/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/13/05	.04	.05	5.84	.09	.09	1.49	<.02	<.02	--
04/13/05	<.02	<.02	--	.91	1.04	8.70	<.02	<.02	--
04/13/05	.04	.05	8.70	<.02	<.02	--	<.02	<.02	--

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Surface water—Continued									
Iowa—Continued									
06/08/05	9.25	12	17	2.47	3.18	16	<0.02	<0.02	--
06/08/05	.12	.12	1.70	.20	.18	7.69	<.02	<.02	--
06/08/05	.10	.10	1.34	.19	.17	5.59	<.02	<.02	--
06/08/05	.53	.56	2.79	.76	.75	.88	<.02	<.02	--
06/08/05	.09	.09	1.48	<.02	<.02	--	<.02	<.02	--
06/08/05	<.02	<.02		<.02	<.02	--	<.02	<.02	--
05/24/06	1.52	1.54	.74	2.87	2.87	0	<.02	<.02	--
05/24/06	1.53	1.44	4.12	2.92	2.92	0	<.02	<.02	--
05/24/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
05/31/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
05/31/06	<.02	<.02	--	.01	.01	0	<.02	<.02	--
06/01/06	<.02	<.02	--	.01	.01	0	<.02	<.02	--
06/01/06	<.02	<.02	--	.02	.02	0	<.02	<.02	--
06/01/06	<.02	<.02	--	.01	<.02	--	<.02	<.02	--
Kansas									
04/29/02	.12	.16	18	<.02	<.02	--	<.02	<.02	--
05/05/02	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
05/07/02	.97	1.23	15	5.21	5.41	2.57	<.02	<.02	--
05/07/02	.43	.47	6.14	1.64	1.24	20	<.02	<.02	--
05/10/02	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/20/02	.44	.42	2.96	1.17	1.21	2.17	<.02	<.02	--
04/16/03	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/16/03	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/23/03	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/23/03	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
05/12/03	.33	.33	1.21	1.00	1.03	2.16	<.02	<.02	--
05/12/03	.62	.59	3.09	1.49	1.55	2.79	<.02	<.02	--
05/12/03	1.08	1.02	3.59	3.04	2.49	14	<.02	<.02	--
05/24/03	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
05/24/03	<.02	<.02	--	.32	.26	15	<.02	<.02	--
Maryland									
04/05/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/05/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/05/06	.05	.06	9.30	<.02	<.02	--	<.02	<.02	--
04/05/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--

20 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Surface water—Continued									
Michigan									
08/12/05	0.05	0.04	13	<0.02	<0.02	--	<0.02	<0.02	--
08/12/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
Mississippi									
10/13/04	.45	.59	18	.99	1.12	7.92	<.02	<.02	--
10/14/04	.16	.13	13	.68	.79	9.87	<.02	<.02	--
11/03/04	.33	.35	3.92	1.50	1.83	13	<.02	<.02	--
11/04/04	.15	.13	8.35	.57	.48	12	<.02	<.02	--
12/07/04	.28	.28	.73	1.16	1.11	2.95	<.02	<.02	--
12/08/04	.14	.12	6.25	.51	.41	15	<.02	<.02	--
01/04/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
01/04/05	.13	.12	3.23	.82	1.46	34	<.02	<.02	--
03/21/05	.10	.11	7.03	<.02	<.02	--	<.02	<.02	--
03/23/05	.10	.12	7.83	.25	.26	3.40	<.02	<.02	--
04/21/05	.34	.38	7.23	.24	.29	13	<.02	<.02	--
05/02/05	.86	.85	.31	1.69	1.50	8.46	.14	<.02	--
05/24/05	.22	.24	5.74	.64	.77	11	<.02	<.02	--
05/26/05	.37	.40	4.82	1.81	1.91	3.62	<.02	<.02	--
05/31/05	.95	1.25	18	2.06	2.83	20	<.02	<.02	--
06/07/05	.96	1.13	11	2.76	3.85	21	<.02	<.02	--
06/09/05	<.02	<.02	--	.52	.61	9.66	<.02	<.02	--
06/20/05	1.90	1.82	3.00	5.3	8.70	30	<.02	<.02	--
06/22/05	.07	.08	7.90	.55	.52	3.78	<.02	<.02	--
07/05/05	1.23	1.73	21	5.04	5.04	.07	<.02	<.02	--
07/07/05	<.02	<.02	--	<.02	.70	--	<.02	<.02	--
07/20/05	1.13	1.13	0	3.87	3.87	0	<.02	<.02	--
07/20/05	1.24	1.49	12	4.51	4.55	.59	<.02	<.02	--
07/25/05	.12	.13	3.66	1.07	1.06	.38	<.02	<.02	--
08/08/05	1.60	1.75	5.96	5.00	4.91	1.16	<.02	<.02	--
08/09/05	2.20	2.25	1.61	5.00	5.00	0	<.02	<.02	--
08/10/05	.17	.19	7.09	.98	.80	14	<.02	<.02	--
08/23/05	1.29	1.39	4.87	7.36	6.66	6.77	<.02	<.02	--
08/24/05	1.01	.84	12	4.14	4.14	0	<.02	<.02	--
09/06/05	.61	.53	9.40	2.17	1.66	19	<.02	<.02	--

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Surface water—Continued									
Mississippi—Continued									
09/07/05	0.14	0.13	4.05	0.81	0.95	9.76	<0.02	<0.02	--
09/15/05	<.02	<.02	--	.07	.06	6.15	<.02	<.02	--
09/15/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/15/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/20/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
Nebraska									
04/12/04	.07	.06	13	.11	.15	22	.14	.15	3.72
04/12/04	.05	.05	1.34	.24	.16	28	<.02	<.02	
04/12/04	.15	.15	1.76	.31	.31	0	.14	.11	17
04/27/04	.12	.15	16	.50	.31	33	.13	.12	5.41
05/03/04	.08	.08	.83	.19	.19	1.39	.20	.12	36
05/08/04	.81	.65	15	1.36	1.60	11	.16	.13	13
05/10/04	.30	.24	16	.78	.76	1.65	.15	.13	6.83
05/10/04	.28	.27	2.68	.82	1.1	19	.18	.15	9.60
05/13/04	.35	.26	20	<.02	.76	--	.15	.13	9.29
05/13/04	.25	.20	15	.51	.56	6.52	.16	.12	20
05/18/04	.05	.06	18	.61	.63	2.25	<.02	<.02	--
05/22/04	.16	.22	19	.48	.66	20	<.02	<.02	--
05/22/04	.08	.10	13	.37	.50	19	<.02	<.02	--
05/22/04	.04	.05	19	.29	.40	21	<.02	<.02	--
05/22/04	.13	.17	16	.53	.74	21	<.02	<.02	--
05/22/04	.14	.17	12	.47	.62	18	<.02	<.02	--
05/25/04	.07	.09	18	.45	.58	16	<.02	<.02	--
05/25/04	.09	.09	.76	.55	.59	5.33	<.02	<.02	--
06/01/04	.28	.28	.48	.28	.38	19	<.02	<.02	--
06/01/04	.27	.27	.49	.71	.82	9.49	<.02	<.02	--
06/02/04	.06	.06	3.28	.33	.34	2.78	<.02	<.02	--
06/08/04	.17	.20	11	.55	.70	16	<.02	<.02	--
06/15/04	.23	.26	7.17	.53	.64	13	<.02	<.02	--
06/16/04	.55	.65	11	.50	.70	21	<.02	<.02	--
06/16/04	.54	.70	16	.43	.52	12	<.02	<.02	--

22 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Surface water—Continued									
Nebraska—Continued									
06/22/04	0.15	0.18	11	0.43	0.52	12	<0.02	<0.02	--
06/22/04	.62	.50	15	1.57	1.86	11	<.02	<.02	--
06/29/04	1.23	.97	16	.98	1.18	12	<.02	<.02	--
06/29/04	1.25	1.37	6.02	.97	1.02	3.05	<.02	<.02	--
06/29/04	<.02	<.02	--	.11	.09	11	<.02	<.02	--
06/29/04	.10	.08	14	.35	.36	2.25	<.02	<.02	--
06/29/04	.07	.06	14	.37	.42	7.58	<.02	<.02	--
07/06/04	.68	.53	18	1.35	1.13	12	<.02	<.02	--
07/06/04	.65	.53	14	1.26	1.13	7.22	<.02	<.02	--
07/07/04	4.55	4.55	0	5.36	5.36	0	<.02	<.02	--
07/07/04	1.21	.97	15	1.64	1.37	12	<.02	<.02	--
07/07/04	9.72	9.72	0	5.21	5.21	0	<.02	<.02	--
07/09/04	2.14	2.10	1.14	.58	.58	0	<.02	<.02	--
07/09/04	.09	.08	4.08	.22	.21	2.51	<.02	<.02	--
07/09/04	1.13	1.36	12	1.59	1.45	6.41	<.02	<.02	--
07/20/04	.40	.37	5.09	.99	1.12	7.92	<.02	<.02	--
07/21/04	.28	.25	6.45	.11	.12	5.13	<.02	<.02	--
07/22/04	<.02	<.02	--	.08	.08	2.53	<.02	<.02	--
07/22/04	1.41	1.36	2.23	2.53	2.30	6.42	<.02	<.02	--
07/22/04	<.02	<.02	--	.02	.01	9.09	<.02	<.02	--
07/25/04	.18	.16	7.62	.53	.51	2.33	<.02	<.02	--
07/27/04	.36	.35	2.45	1.09	1.28	10	<.02	<.02	--
07/27/04	.35	.35	.96	1.01	.63	33	<.02	<.02	--
08/04/04	.27	.27	.25	.87	.77	8.46	<.02	<.02	--
08/04/04	.26	.23	9.13	1.06	.90	11	<.02	<.02	--
08/05/04	<.02	<.02	--	.03	.03	2.25	<.02	<.02	--
08/05/04	1.10	1.21	6.02	1.19	1.16	1.60	<.02	<.02	--
08/05/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/05/04	.26	.26	.77	.92	.97	3.43	<.02	<.02	--
08/06/04	<.02	<.02	--	.02	.02	6.90	<.02	<.02	--
08/10/04	.32	.32	1.05	1.45	1.45	.14	<.02	<.02	--
08/12/04	.85	1.06	14	.02	.02	0	<.02	<.02	--
08/12/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/17/04	.11	.11	.61	.49	.43	8.61	<.02	<.02	--
08/24/04	.06	.06	1.12	.50	.43	10	<.02	<.02	--

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Surface water—Continued									
Nebraska—Continued									
08/24/04	1.19	<0.02	--	0.03	0.03	4.55	<0.02	<0.02	--
08/24/04	2.01	1.86	5.13	.02	.03	2.70	<.02	<.02	--
08/25/04	.24	.32	19	2.18	1.72	16	<.02	<.02	--
08/30/04	<.02	<.02	--	.45	.44	.76	<.02	<.02	--
08/31/04	.12	.12	2.25	.09	.09	1.49	<.02	<.02	--
09/07/04	<.02	<.02	--	.37	.35	4.29	<.02	<.02	--
09/23/04	<.02	<.02	--	.29	.33	8.86	<.02	<.02	--
09/23/04	<.02	<.02	--	.41	.49	12	<.02	<.02	--
New York									
08/10/05	.06	.07	7.92	.24	.29	12	<.02	<.02	--
South Dakota									
07/29/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/31/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/01/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/02/04	<.02	<.02	--	.04	.04	3.28	<.02	<.02	--
08/04/04	<.02	<.02	--	.02	<.02	--	<.02	<.02	--
08/04/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/13/05	<.02	<.02	--	.33	.32	1.23	<.02	<.02	--
06/13/05	<.02	<.02	--	.27	.21	19	<.02	<.02	--
06/13/05	.07	.07	1.91	<.02	<.02	--	<.02	<.02	--
06/13/05	<.02	.10	--	<.02	<.02	--	<.02	<.02	--
06/13/05	<.02	<.02	--	.29	.26	7.41	<.02	<.02	--
06/14/05	.11	.10	5.25	<.02	<.02	--	<.02	<.02	--
06/14/05	.08	.09	2.38	<.02	<.02	--	<.02	<.02	--
06/14/05	.04	.04	1.70	<.02	<.02	--	<.02	<.02	--
06/15/05	.16	.16	.85	.07	.08	6.09	<.02	<.02	--
06/15/05	.38	.39	.52	1.17	1.50	16	<.02	<.02	--
06/15/05	.09	.08	1.59	.11	.14	13	<.02	<.02	--
06/15/05	.07	.07	6.51	.05	.04	4.44	<.02	<.02	--
06/15/05	.55	.66	12	.36	.34	3.50	<.02	<.02	--
06/15/05	.08	.08	4.05	.36	.38	2.87	<.02	<.02	--
06/16/05	<.02	<.02	--	.28	.29	.70	<.02	<.02	--
06/16/05	.29	.40	20	.29	.32	7.37	<.02	<.02	--
06/16/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/16/05	1.81	1.80	.26	4.74	4.70	.50	<.02	<.02	--
06/16/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--

24 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Surface water—Continued									
South Dakota—Continued									
06/16/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
06/16/05	.07	.07	3.94	<0.02	<0.02	--	<0.02	<0.02	--
06/16/05	.15	.13	8.17	<0.02	<0.02	--	<0.02	<0.02	--
06/16/05	.15	.19	14	.20	.20	2.31	<0.02	<0.02	--
06/16/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
06/16/05	.18	.18	.37	.32	.36	7.18	<0.02	<0.02	--
Vermont									
08/10/05	.04	.04	1.84	<0.02	<0.02	--	<0.02	<0.02	--
08/10/05	<0.02	<0.02	--	.06	.06	3.45	<0.02	<0.02	--
08/10/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
06/26/06	.09	.08	7.60	.05	.05	0	<0.02	<0.02	--
06/26/06	.06	.06	2.16	.03	.03	0	<0.02	<0.02	--
06/26/06	.03	.03	4.21	<0.02	<0.02	--	<0.02	<0.02	--
06/26/06	.60	.62	1.75	.09	.09	0.72	<0.02	<0.02	--
06/27/06	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
Washington									
10/13/04	.05	.06	17	.12	.14	13	<0.02	<0.02	--
10/13/04	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
10/13/04	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
11/10/04	.06	.05	15	.14	.15	1.38	<0.02	<0.02	--
11/10/04	.11	.10	9.18	.11	.11	.63	<0.02	<0.02	--
12/14/04	.07	.07	1.84	.14	.15	4.67	<0.02	<0.02	--
01/12/05	<0.02	<0.02	--	.13	.17	17	<0.02	<0.02	--
05/24/05	.07	.08	5.48	<0.02	<0.02	--	<0.02	<0.02	--
05/24/05	.06	.06	5.62	.08	.07	6.54	<0.02	<0.02	--
06/01/05	.47	.53	7.30	.11	.12	5.87	<0.02	<0.02	--
06/08/05	.16	.18	7.38	<0.02	<0.02	--	<0.02	<0.02	--
06/15/05	.08	.08	.86	.12	.11	4.78	<0.02	<0.02	--
06/21/05	.04	.05	11	.10	.11	1.27	<0.02	<0.02	--
06/21/05	.04	.05	10	.09	.10	8.00	<0.02	<0.02	--
06/28/05	.08	.07	1.79	.10	.09	8.27	<0.02	<0.02	--
Wyoming									
06/28/05	.06	.06	0	<0.02	<0.02	--	<0.02	<0.02	--
07/13/05	<0.02	<0.02	--	.07	.07	2.00	<0.02	<0.02	--
07/13/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
07/20/05	<0.02	<0.02	--	.09	.07	10	<0.02	<0.02	--
07/20/05	.02	.02	0	.09	.07	15	<0.02	<0.02	--

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Surface water—Continued									
Wyoming—Continued									
07/25/05	0.03	0.03	6.67	0.13	0.11	10	<0.02	<0.02	--
08/10/05	<.02	<.02	--	.18	.20	8.87	<.02	<.02	--
08/16/05	<.02	<.02	--	.10	.09	4.90	<.02	<.02	--
08/23/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/06/05	.56	.45	15	.27	.33	14	<.02	<.02	--
09/20/05	.09	.09	1.54	.15	.18	13	<.02	<.02	--
07/13/04	.02	.02	3.39	.24	.27	7.69	<.02	<.02	--
07/13/04	.04	.04	6.45	.48	.66	20	<.02	<.02	--
07/18/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/18/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/16/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/16/04	<.02	<.02	--	<.02	<.02	--	.05	.05	0
04/19/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/19/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/19/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
05/05/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
05/05/05	.02	.02	5.71	<.02	<.02	--	<.02	<.02	--
05/05/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
05/24/05	.04	.06	14	<.02	<.02	--	<.02	<.02	--
05/24/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
05/31/05	.02	.02	9.09	<.02	<.02	--	<.02	<.02	--
05/31/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/15/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/15/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/27/05	.07	.08	6.72	.04	.06	29	<.02	<.02	--
08/09/05	.06	.06	4.35	.03	.04	15	<.02	<.02	--
08/09/05	.06	.07	10	.04	.05	22	<.02	<.02	--
08/15/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/15/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/21/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
04/21/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/26/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/26/06	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--

26 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Groundwater									
California									
07/12/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
07/12/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/20/05	.02	.02	3.64	<.02	<.02	--	<.02	<.02	--
07/20/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/02/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/02/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/02/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/02/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/03/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/03/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/03/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/04/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/04/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/04/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/16/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/16/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/16/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/17/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/17/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/17/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/17/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/17/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/20/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
Idaho									
06/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/22/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/22/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/22/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/23/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/27/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/27/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Groundwater—Continued									
Idaho—Continued									
06/28/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
06/28/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/28/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/28/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/29/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/29/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/29/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/29/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/30/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/30/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/30/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/30/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/30/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/30/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/20/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/20/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/20/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/25/05	<.02	<.02	--	.02	.02	11	<.02	<.02	--
07/25/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/25/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26 /05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/31/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--

28 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Groundwater—Continued									
Illinois									
07/11/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
07/12/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/12/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/13/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/13/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/14/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/25/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/27/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/27/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/28/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/01/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/01/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/02/05	<.02	<.02	--	<.02	.16	--	<.02	<.02	--
08/02/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/02/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/03/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/03/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/04/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/04/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/08/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/09/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/09/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/09/05	.05	.06	8.54	<.02	<.02	--	<.02	<.02	--
08/10/05	<.02	<.02	--	.33	.35	3.28	<.02	<.02	--
08/10/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/07/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/07/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/07/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Groundwater—Continued									
Iowa									
12/20/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
12/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
12/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
12/22/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
12/22/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
12/22/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
12/22/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
12/22/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
12/23/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
12/29/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
12/29/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
12/30/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/01/06	.04	.04	0	<.02	<.02	--	<.02	<.02	--
Nebraska									
05/10/04	.13	.13	1.02	.23	.28	13	.33	.28	11
05/11/04	.18	.18	1.47	.22	.32	23	.33	.30	6.45
05/11/04	.03	.02	18	.08	.09	7.69	.32	.31	2.97
05/11/04	.04	.03	27	.05	.07	21	.28	.28	.95
05/11/04	.04	.04	0	.09	.07	21	.28	.29	2.33
05/11/04	.02	.02	6.45	.11	.08	25	.27	.26	3.53
05/18/04	.03	.03	9.30	.07	.08	8.70	<.02	<.02	--
05/18/04	.03	.04	14	.16	.15	4.35	<.02	<.02	--
05/24/04	.15	.14	4.65	.23	.25	6.04	<.02	<.02	--
05/24/04	.02	.02	6.45	.08	.09	6.11	<.02	<.02	--
05/24/04	.02	.03	14	.09	.10	4.08	<.02	<.02	--
05/24/04	.01	.01	6.90	.03	.04	20	<.02	<.02	--
05/24/04	.02	.02	14	.05	.06	9.30	<.02	<.02	--
05/24/04	.01	.01	6.90	.03	.03	9.30	<.02	<.02	--
06/14/04	<.02	<.02	--	.41	.30	21	<.02	<.02	--
06/14/04	<.02	<.02	--	.33	.29	8.37	<.02	<.02	--
06/14/04	<.02	<.02	--	.17	.19	7.27	<.02	<.02	--
06/14/04	.05	.04	8	.43	.33	19	<.02	<.02	--
06/14/04	.07	.06	14	.33	.26	16	<.02	<.02	--
06/14/04	<.02	<.02	--	.12	.12	0	<.02	<.02	--

30 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Groundwater—Continued									
Nebraska—Continued									
06/22/04	0.23	0.21	6.75	0.86	0.62	23	<0.02	<0.02	--
07/06/04	<.02	<.02	--	.22	<.02	--	<.02	<.02	--
07/06/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/06/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/06/04	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/06/04	<.02	<.02	--	.17	.17	1.56	<.02	<.02	--
07/23/04	.10	.10	2.63	.31	.31	.43	<.02	<.02	--
08/19/04	<.02	<.02	--	.06	.06	2.20	<.02	<.02	--
08/19/04	<.02	<.02	--	.04	.04	6.45	<.02	<.02	--
08/19/04	<.02	<.02	--	.13	.12	5.41	<.02	<.02	--
04/12/05	<.02	<.02	--	.02	.02	0	<.02	<.02	--
04/12/05	<.02	<.02	--	.41	.46	7.52	<.02	<.02	--
New Jersey									
06/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
06/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/10/05	<.02	<.02	--	.23	.28	13	<.02	<.02	--
08/11/05	.03	.03	6.90	.15	.20	16	<.02	<.02	--
08/11/05	<.02	<.02	--	.12	.16	20	<.02	<.02	--
08/15/05	<.02	<.02	--	.10	.13	16	<.02	<.02	--
08/15/05	<.02	<.02	--	.16	.18	6.86	<.02	<.02	--
08/16/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/16/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/17/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/17/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/18/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/18/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/22/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/22/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/23/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/23/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/23/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/23/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/24/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Groundwater—Continued									
New Jersey—Continued									
08/24/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
08/24/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/25/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/25/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/25/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/30/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/30/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/31/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/31/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/31/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/01/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/20/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/20/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
09/21/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
Washington									
07/07/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/07/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/07/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/25/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/25/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/27/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/28/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/28/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
07/29/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/01/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/02/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/02/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/03/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/03/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/04/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/04/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/05/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--

32 Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water

Table 4. Comparison of glyphosate, aminomethylphosphonic acid, and glufosinate quantitation using standard addition and isotope dilution for 473 water samples collected between April, 2004 and June, 2006.—Continued

[<, less than; --, not applicable]

Collection date	Glyphosate isotope dilution	Glyphosate standard addition	Percent difference	Amino-methyl-phosphonic acid isotope dilution	Aminomethyl-phosphonic acid standard addition	Percent difference	Glufosinate isotope dilution	Glufosinate standard addition	Percent difference
Groundwater—Continued									
Washington—Continued									
08/08/05	<0.02	<0.02	--	<0.02	<0.02	--	<0.02	<0.02	--
08/08/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/09/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/09/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/09/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/10/05	<.02	<.02	--	.29	.31	4.56	<.02	<.02	--
08/10/05	.03	.03	2.60	.26	.31	12	<.02	<.02	--
08/11/05	<.02	<.02	--	.16	.21	19	<.02	<.02	--
08/11/05	<.02	<.02	--	.15	.19	16	<.02	<.02	--
08/11/05	<.02	<.02	--	.11	.13	11	<.02	<.02	--
08/11/05	<.02	<.02	--	.10	.14	20	<.02	<.02	--
08/12/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/12/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
08/26/05	<.02	<.02	--	<.02	<.02	--	<.02	<.02	--
			Glyphosate	Aminomethylphosphonic Acid		Glufosinate			
Average percent difference			7.644	9.589		9.280			
Percent relative standard deviation			6.304	8.354		9.160			
Number of samples			179	206		16			
Percent detection			37.84	43.55		3.38			

Publishing support provided by:

Rolla Publishing Service Center

For additional information concerning this publication, contact:

Director, USGS Kansas Water Science Center

4821 Quail Crest Place, Lawrence, KS (785) 842-9909

Or visit the Kansas Water Science Center Web Site at:

<http://ks.water.usgs.gov>

