Direct Aqueous Determination of Glyphosate and Related Compounds by Liquid Chromatography/ Tandem Mass Spectrometry using Reversed-Phase and Weak Anion-Exchange Mixed-mode Column

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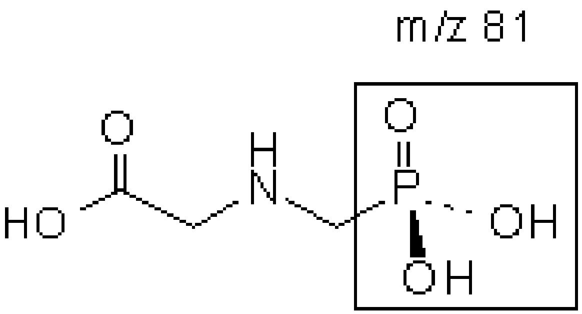
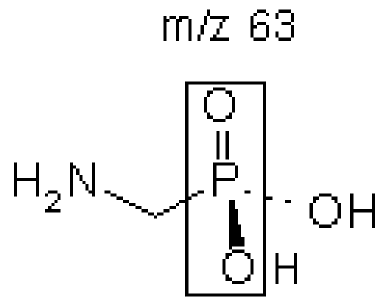
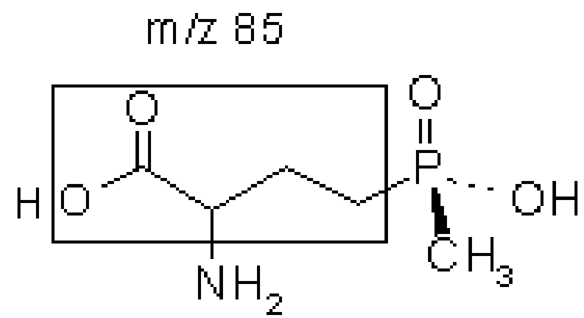
Glyphosate and Related Compounds



glyphosate (MW 169)



AMPA (MW 111)



glufosinate (MW 181)

AMPA: aminomethylphosphonic acid

Glyphosate – A global herbicide

- Non-selective, broad-spectrum herbicide to kill weeds
- Marketed under names Roundup, Touchdown, Vision, Tumbleweed, Wipeout, etc.
- Most used herbicide (5–8 million pounds on lawns/yards & 85–90 million pounds in agriculture yearly in the USA)
- Relatively low in toxicity
- U.S. EPA regulation: 700 µg/L
- Ontario Regulation 169/03: 280 µg/L

U.S. EPA: United States Environmental Protection Agency

Aminomethylphosphonic acid (AMPA)

- Metabolite/degradation product of glyphosate: an indicator for the occurrence of glyphosate
- Detected more frequently and occurred at similar or higher concentrations than the parent compound – United States Geological Survey report 2007-5122
- Other possible sources of AMPA in the environment:

Name of compound	Abbreviation	Main use
Amino tris(methylenephosphonate)	ATMP	Industrial boilers/cooling
Ethylenediamine tetra(methylenephosphonate)	EDTMP	Laundry detergents
Hexamethylenediamine tetra(methylenephosphonate)	HDTMP	Industrial boilers/cooling
Diethylenetriamine penta(methylenephosphonate)	DTPMP	Detergents

Glufosinate

- Non-selective, broad-spectrum herbicide
- Marketed under names Basta, Rely, Finale, Challenge and Liberty, etc.
- Similar structure as glyphosate, different mode-of-action: glyphosate resistance encountered in problematic weeds, such as rye grass, can be overcome by applying glufosinate
- Usage expected to increase due to recent development of genetically modified glufosinate-tolerant crops

Aqueous Samples Analysis Review

Challenge: high polarity, high water solubility, low volatility, lack of chromophore or fluorophore in molecular structures

Monsanto Method

- anion exchange column extraction
- ion chromatography/post-column derivatization/fluorescence detection

Zeneca Ag Method (*J. Agric. Food Chem.;* **1994**; *42:* 2751)

- rotary-evaporation, derivatization
- gas chromatography/mass spectrometry analysis

Ontraio Ministry of the Environment method E3415 *(J. AOAC*; **2001;** 84: 1770)

- rotary-evaporation, derivatization
- LC/isotope-dilution MS analysis

Hanke Method *(Anal. Bioanal. Chem.* **2008;** 391: 2265)

- acidification, derivatization & solid phase extraction of sample
- LC-MS/MS analysis

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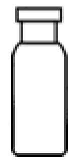
Direct Aqueous Injection (DAI) Analysis

1 mL of sample

+ internal standard



all standards and samples ready for analysis contained 100 µg/L of 13C,15N-glyphosate

Shimadzu Prominence/20 series HPLC system
+
Applied Biosystems 4000 Q-trap mass spectrometer

Instrument detection limits (signal-to-noise ratios ≥ 5) for glyphosate, AMPA and glufosinate: 1.0, 2.0 and 0.9 µg/L

Quantification range: ~10 to 1000 µg/L

Liquid Chromatography Parameters for Target Compounds

Column: Acclaim Mix-mode WAX-1 (reversed-phase/weak anion-exchange), 50 x 3 mm , 3 μ m

Solvent A: 50:50 methanol:water (v:v)

Solvent B: 300 mM ammonium acetate in 50:50 methanol:water (v:v)

Column temperature: 30° C

Injection volume: 70 μL

Time (min)	Solvent B (%)	Flow (μL/min)
0.0	40.0	400
4.0	100.0	400
6.0	100.0	400
6.5	40.0	400
12.0	40.0	400

Mass Spectrometry Parameters for Target Compounds

Compound	Formula	CAS #	Q1 Mass	Q3 Mass	Quantification/ Confirmation	Collision Energy (eV)
Glyphosate	$C_3H_8NO_5P$	1071-83-6	168	63	Quantification	-30
			168	81	Confirmation	-20
AMPA	CH_6NO_3P	1066-51-9	110	63	Quantification	-35
			110	81	Confirmation	-20
Glufosinate	$C_5H_{12}NO_4P$	51276-47-2	180	63	Quantification	-50
			180	85	Confirmation	-30
^{13}C,^{15}N-Glyphosate	-	-	170	63	IS	-30
			170	81	Confirmation	-20

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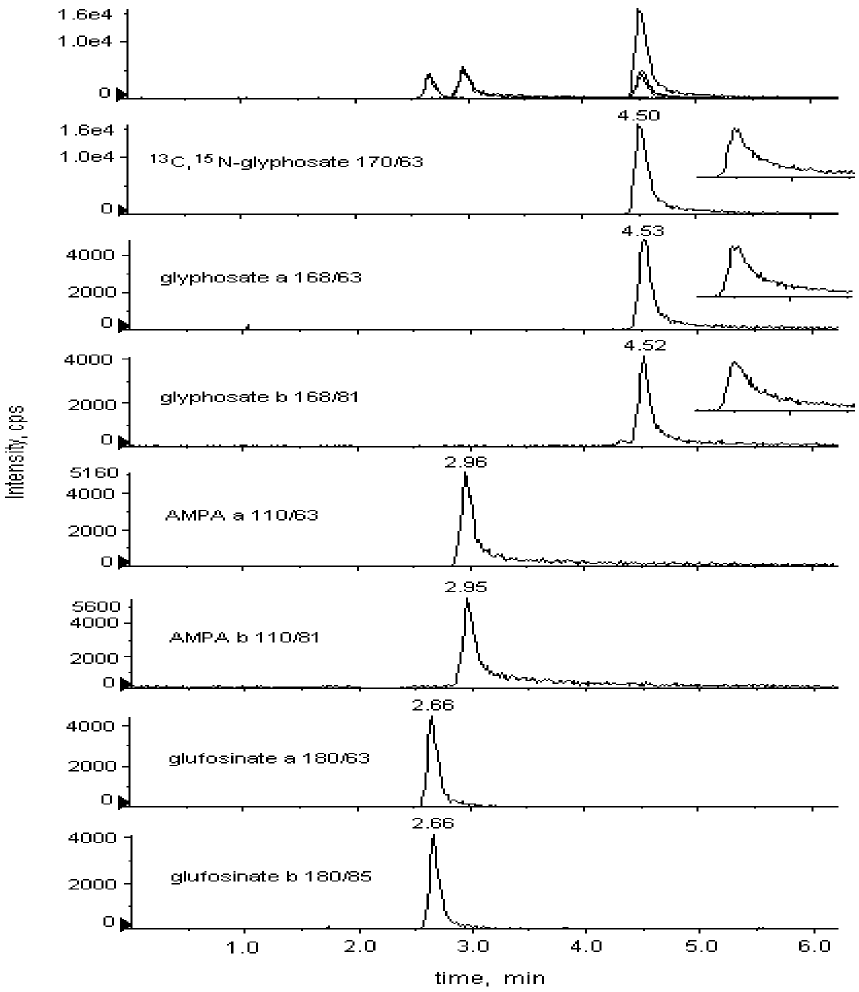
Chromatograms for Target Compounds



peak degradation due to metal ions accumulation: ethylenediaminetetra acetic acid disodium salt ($EDTANa_2$) wash

Instrument Performance, Within Run (N = 10; June 2010)

Compounds	Conc. µg/L	%Average Accuracy	%STD	%RSD
Glyphosate (a)	100	101.3	2.3	2.3
Glyphosate (b)	100	100.2	2.1	2.1
AMPA (a)	200	93.8	5.8	6.2
AMPA (b)	200	91.9	6.0	6.5
Glufosinate (a)	91.4	93.7	8.5	9.1
Glufosinate (b)	91.4	93.8	8.0	8.5

STD: standard deviation
RSD: relative standard deviation

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Instrument Linearity (N=6)

ug/L		gly a	gly b	AMPA a	AMPA b	glu a	glu b	IS
~10	Avg Acc %	96.3	100	102	101	94.0	95.8	
	Avg Area	3.32E+04	1.67E+04	3.25E+04	3.80E+04	1.70E+04	1.55E+04	2.32E+05
~20	Avg Acc %	96.5	98.5	99.2	98.6	96.3	96.9	
	Avg Area	4.64E+04	2.54E+04	5.30E+04	6.04E+04	2.55E+04	2.30E+04	2.39E+05
~50	Avg Acc %	101	98.0	97.8	98.6	102	101	
	Avg Area	1.45E+05	9.00E+04	1.91E+05	2.07E+05	8.68E+04	7.65E+04	2.57E+05
~100	Avg Acc %	103	103	101	102	104	102	
	Avg Area	2.99E+05	1.99E+05	4.07E+05	4.32E+05	1.76E+05	1.53E+05	2.71E+05
~200	Avg Acc %	104	101	100	101	104	103	
	Avg Area	6.31E+05	4.19E+05	8.13E+05	8.63E+05	3.50E+05	3.10E+05	2.89E+05
~500	Avg Acc %	100	98.7	98.9	99.8	102	105	
	Avg Area	1.74E+06	1.18E+06	2.03E+06	2.15E+06	8.58E+05	7.90E+05	3.33E+05
~1000	Avg Acc %	98.9	100	100	99.8	97.8	96.5	
	Avg Area	3.49E+06	2.45E+06	4.12E+06	4.30E+06	1.65E+06	1.45E+06	3.41E+05
	R	1.000	0.999	0.998	0.998	0.999	0.998	
	R2	0.999	0.999	0.997	0.996	0.997	0.996	

Spiked Tap Water Results (N=9)

Compound Name	Spiked µg/L	Average µg/L	%Average Recovery	Std Dev µg/L	%RSD
Glyphosate (a)	100.0	101.2	101.2	6.4	6.3
Glyphosate (b)	100.0	101.6	101.6	6.4	6.3
AMPA (a)	200.0	163.8	81.9	14.5	8.9
AMPA (b)	200.0	156.6	78.3	13.9	8.9
Glufosinate (a)	91.4	70.4	77.0	7.3	10.3
Glufosinate (b)	91.4	72.7	79.5	7.4	10.2

Spiked Surface Water Results (N=10)

Compound Name	Spiked µg/L	Average µg/L	%Average Recovery	Std Dev µg/L	%RSD
Glyphosate (a)	100.0	97.9	97.9	2.7	2.7
Glyphosate (b)	100.0	100.6	100.6	3.5	3.5
AMPA (a)	200.0	151.4	75.7	15.6	10.3
AMPA (b)	200.0	147.4	73.7	16.9	11.5
Glufosinate (a)	91.4	57.2	62.5	8.3	14.6
Glufosinate (b)	91.4	56.8	62.1	8.4	14.8

Spiked Groundwater Results (N=9)

Compound Name	Spiked µg/L	Average µg/L	%Average Recovery	Std Dev µg/L	%RSD
Glyphosate (a)	100.0	93.7	93.7	4.1	4.3
Glyphosate (b)	100.0	94.3	94.3	2.7	2.9
AMPA (a)	200.0	142.1	71.1	14.5	10.2
AMPA (b)	200.0	140.8	70.4	14.8	10.5
Glufosinate (a)	91.4	61.1	66.8	6.2	10.2
Glufosinate (b)	91.4	60.4	66.1	6.5	10.7

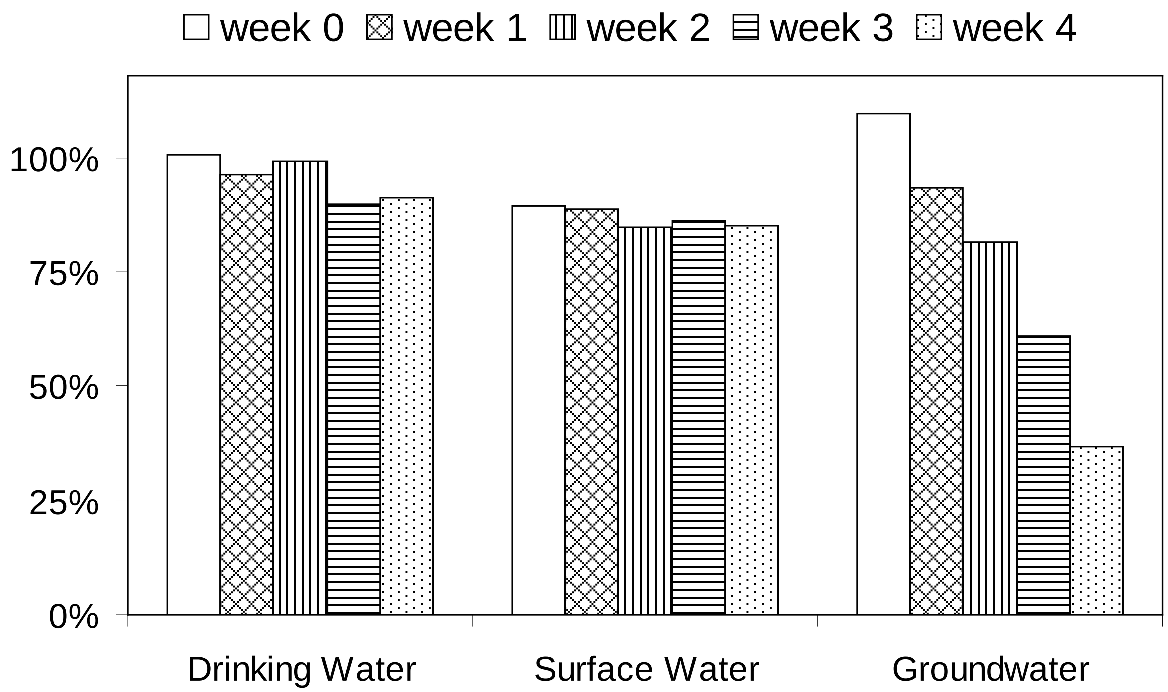
MDL, MQL, Inter-day Accuracy for Spiked Tap Water Samples (N=33)

Compound Name	MDL µg/L	MQL µg/L	Between Run %Accuracy	%RSD
Glyphosate (a)	1.51	4.53	102	4.05
Glyphosate (b)	1.52	4.56	102	4.28
AMPA (a)	3.85	11.5	80.2	13.1
AMPA (b)	3.91	11.7	77.7	14.1
Glufosinate (a)	1.85	5.55	70.4	19.4
Glufosinate (b)	1.68	5.04	70.6	20.7

MDL: method detection limit

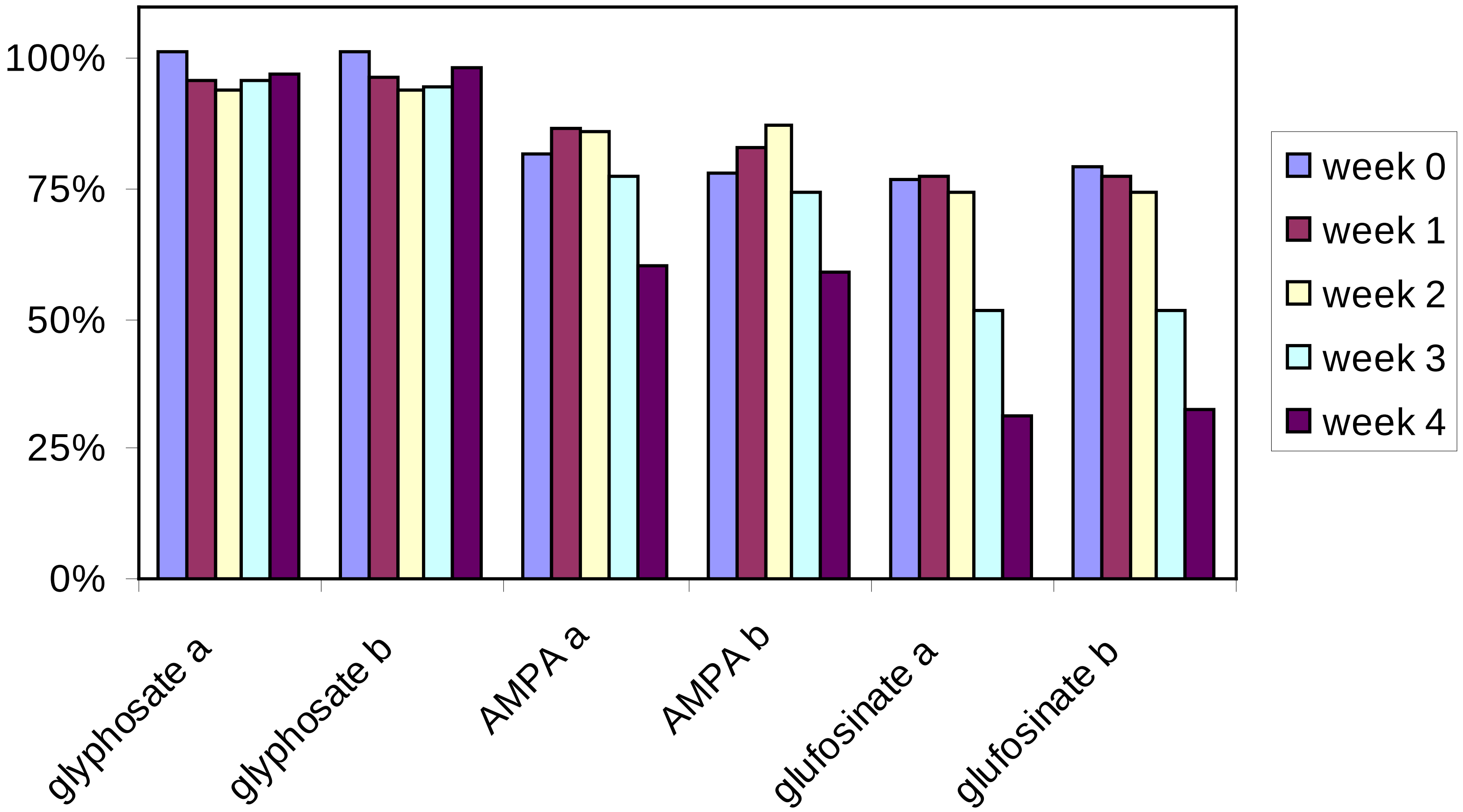
MQL: method quantification limit

Storage Study — ^{13}C,^{15}N-glyphosate

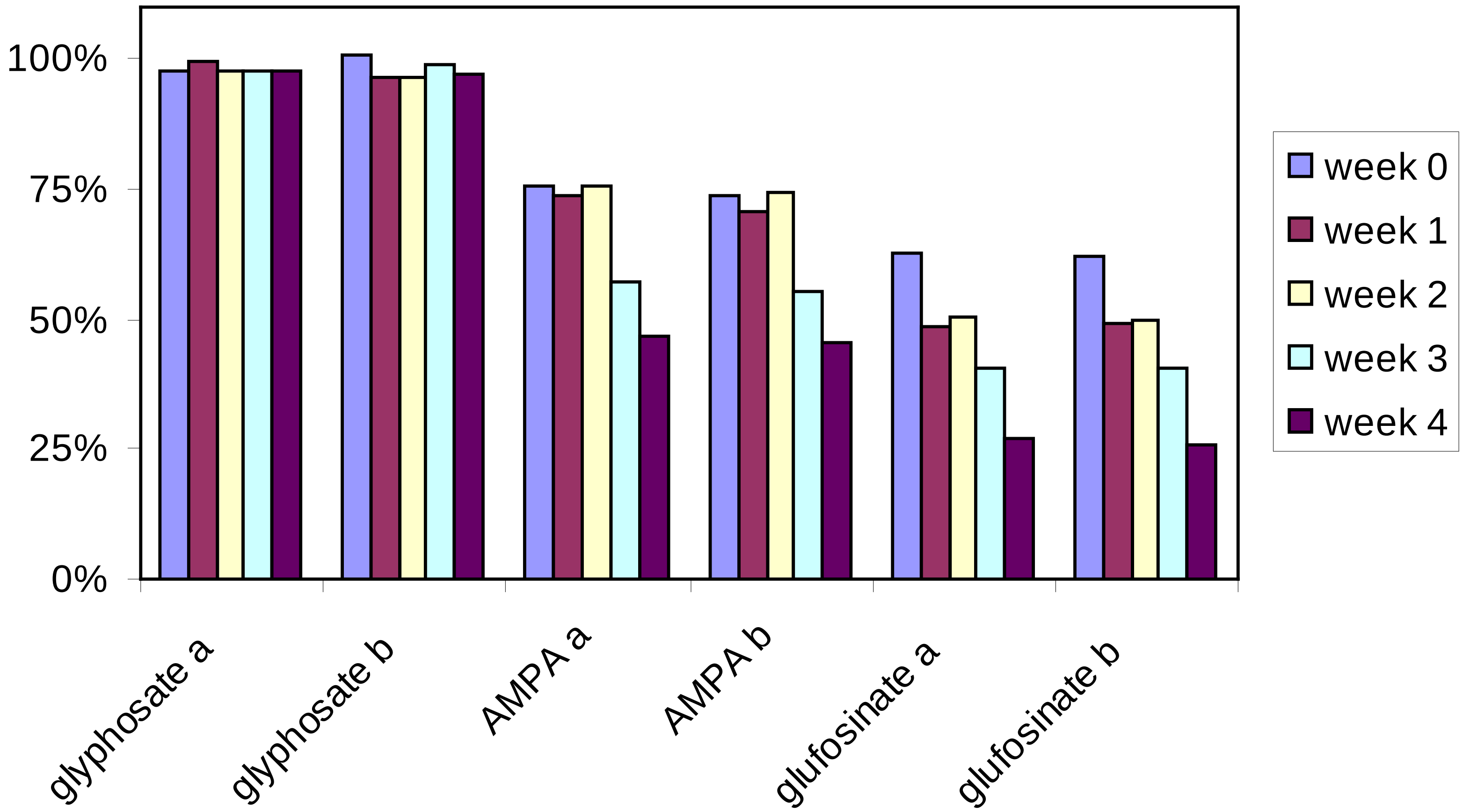


^{13}C,^{15}N-glyphosate decreased significantly with time in groundwater. Similar phenomena were also observed for glyphosate by Ibanez et al. and Freuze et al. due to slow complexation with cations. References: M. Ibáñez, O. J. Pozo, J. V. Sancho, F. J. López, F. Hernández, J. Chromatogr. A 1134 (2006) 51 & I. Freuze, A. Jadas-Hecart, A. Royer, P. Y. Communal, J. Chromatogr. A 1175 (2007) 197

Storage Study – Drinking Water



Storage Study – Surface Water



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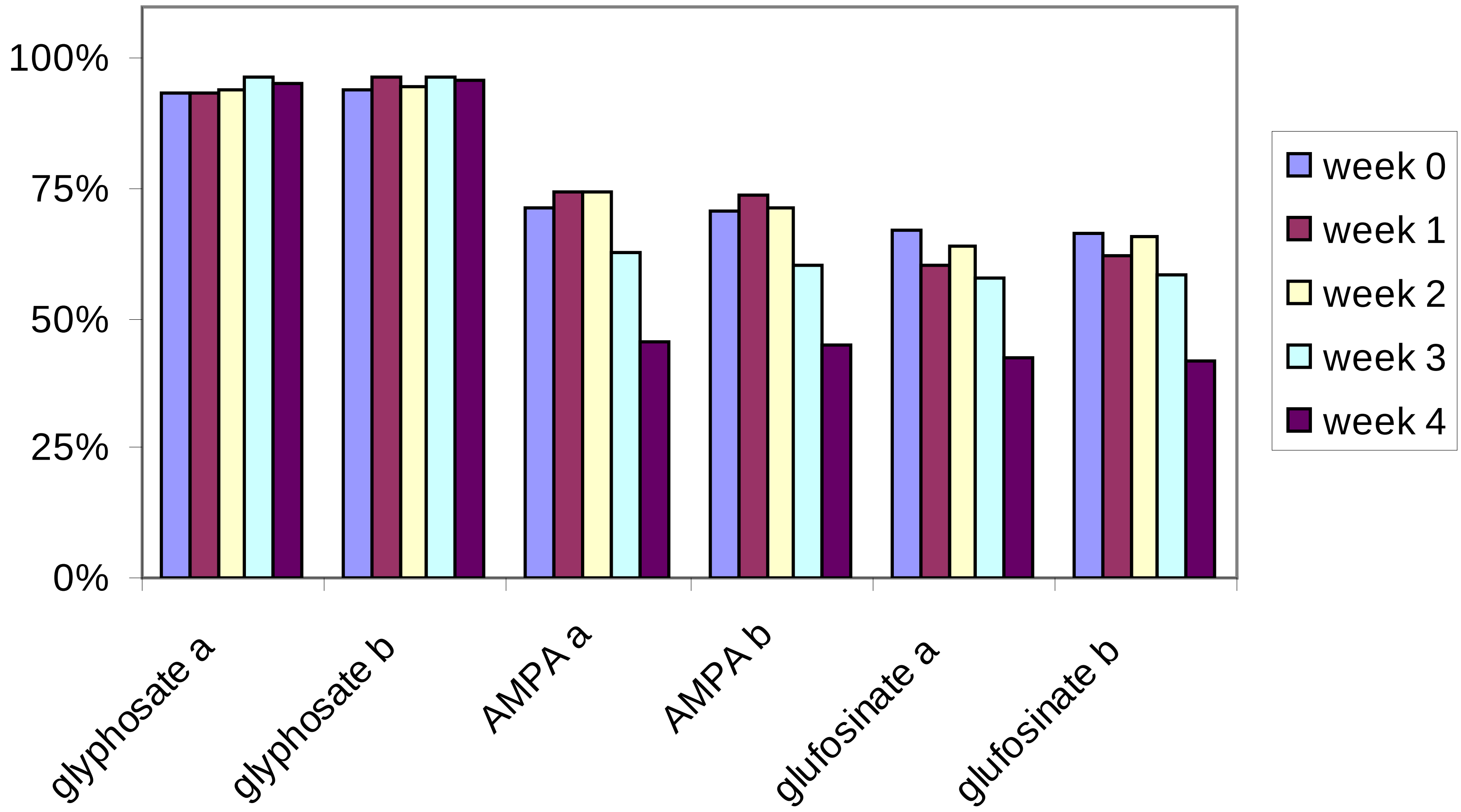
Storage Study — Groundwater



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Inter-laboratory Study Results for Glyphosate

Two Environmental Resource Associates (ERA) PT samples:

August 2010 (WS-169):	reported: 715 µg/L grand mean target: 692 µg/L (RSD 5.18%) accuracy: 103% data points: 7
April 2011 (WS-177):	reported: 380 µg/L grand mean target: 383 µg/L (RSD 6.23%) accuracy: 99.2% data points:10

PT: proficiency testing

Conclusion

- 12-minute LC/MS-MS method for glyphosate, AMPA and glufosinate in environmental water
- direct injection with no sample concentration and derivatization steps
- quick, easy and reliable approach to satisfy the needs in North American for:
    1. emergency response
    2. drinking water quality monitoring
    3. regulation enforcement
- expected trade-offs:
    1. matrix effects
    2. higher detection limits (µg/L instead of ng/L)
    3. narrower linearity range (10^2 instead of 10^3)

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