



Analysis of Glyphosate & AMPA in water by fully automated online SPE -LC/MS/MS

Tarun Anumol Agilent Technologies Inc.



Glyphosate Application and Properties

- Thought to be the most widely used pesticide in the world
 - A non-selective herbicide, absorbed rapidly by plants
 - Sold as 'Roundup' by Monsanto in US
- Expected to have low toxicity and high efficiency
- Highly water soluble and nonvolatile
 - Very polar and ionic
- Fairly Rapid degradation in soil and is biodegradable







Aminomethylphosphonic acid (AMPA) General properties

- Chief degradation product of glyphosate
- Similarly low acute toxicity like glyphosate
- Chronic toxicity to aquatic animals has not been evaluated
- Can be degraded by bacteria in soil to phosphoric acid





Glyphosate & AMPA Presence in water

- Presence detected in several US streams and rivers¹
- Detected in European groundwater sources²
- Maximum allowable concentration in drinking water set by the European Community for several polar pesticides of 0.1 µg/L

 ¹ Battaglin et al., Glyphosate, Other Herbicides, and Transformation Products in Midwestern Streams. *JAWRA Journal of the American Water Resources Association* **2005**, *41*, (2), 323-332.
 ² B. Schmidt, B. Siegemund, H. Ehses, E. Zietz, Proceedings of XI Symp.

Pesticide Chem., Sept. 13–15, **1999**, p. 591.







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New Toxicological Characterization

World Health Organization Labels Glyphosate Probable Carcinogen

BUSINESS

Health Agency Says Widely Used Herbicide Likely Carcinogenic

Herbicide, glyphosate, is sold by Monsanto under Roundup brand

Glyphosate is a 'probably carcinogenic' pesticide. Why do cities still use it?

Cities use glyphosate to control weeds in parks and along verges. Now that the WHO says the pesticide is 'probably carcinogenic to humans', is it time to stop?



THE WALL STREET JOURNAL

Netherlands Bans Monsanto's Roundup to Protect Citizens from Carcinogenic Glyphosate

By Jennifer Lilley Global Research, May 25, 2015 Natural News 24 May 2015

Region: Europe Theme: Biotechnology and GMO, Environmen





In the Netherlands, people who have been spraying their lawns and gardens with Roundup will have to find another way to protect their land from pests. Late last year, the Dutch parliament voted to ban the sale of glyphosate-based herbicides to private parties. The ban, under which agricultural use is excluded, was initially proposed several years ago. However, it is thought that Monsanto influence prevented it from taking

place at the time.



However, it is thought that Monsanto influence prevented it from taking



Sample Volumes Reduction in sample volume, cost of transport





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Sample Volume





Agilent Infinity Flexcube OSPE system





1200 Infinity Series Online SPE product and concept



Solvent selection valve for up to three solvents

Reciprocating single-piston pump for flows up to 4 ml/min (60 bar)

up to two Quick-Change valves, according to application



Online SPE valve setup LOAD position





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Online SPE valve setup ELUTE position





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Derivatization reaction with FMOC



Adapted from: Vreeken, R.J., et al., Journal of Chromatography A, 1998. 794(1–2): p. 187-199.



Workflow





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Automated Sample Prep – Agilent 7696 Workbench





Separation Parameters Agilent 1290 LC & Flexcube

LC Column: Poroshell EC 120 C-18, 3x50 mm, 2.7 µm Flowrate: 0.35 mL/min Injection Volume: 900 µL Column Compartment Temperature: 30°C

LC Mobile Phase:

A – Water + 5mM Ammonium Acetate B – Acetonitrile

Flexcube Solvents:

A - [95/5(v/v): HPLC Water/Acetonitrile] + 0.1% Acetic Acid B - 1/1/1 (v/v/v): Acetonitrile/Methanol/Isopropanol





Gradient Profiles

Flexcube

Time (min)	Function	Volume	Flowrate
0	Pump Solvent A	4 mL	1 mL/min
4	Valve Change Position to Elution		
5	Pump Solvent B	4 mL	1 mL/min
10	Pump solvent A	2 mL	1 mL/min

Analytical

Time (min)	Mobile Phase	Flow rate (mL/min)
0	5% B	0.4
4	5% B	0.4
8	55% B	0.4
9	95% B	0.4
10	95% B	0.4
10.1	5% B	0.4

Stop time: 12 min Post time: 1 min



Analyte transitions for LC-MS/MS analysis

Compound	Precursor ion	Product ion	Fragmentor voltage	Collision Energy
Glyphosate	392	179	100	24
	392	88	100	16
AMPA	334	112	100	10
	334	179	100	11
Glyphosate ¹³ C ₂ ¹⁵ N ₁	394	90.1	100	16
AMPA ¹³ C ₁ ¹⁵ N ₁ D ₂	338	116	100	10

Q1: Most Abundant Transition; Q2: Second-most Abundant Transition

Analysis:

ESI Positive Mode: MRM Dwell time: 20 ms Cell Accelerator Voltage: 2 eV



Source Optimization for Agilent 6460 MS/MS

Stop and	Acquisition Source Chromatogram Instrument Diagnostics
anes.tune.xml © No limit/As Pump	Source parameters
Unit Only Browse	Gas Temp: 350 °C -
source Time filtering	
S ESI 💌 🔽 Peak width 0.07 min	Gas Flow: [1] 1/min [11,0050E 1/min
ne segments	Sheath Gas Temp: [390] *C [389] *C
# Start / Scan Type Div Valve Delta Delta Stored	Sheath Gas Flow: 12 1/min 11.9896 1/min
1 0 MRM To MS 400 0 🔽	Positive Negative
	Capillary: 4000 V 3500 V 8672 nA
	Nozzle Voltage: 1000 V 500 V
	Chamber Current 1.03 µA



Linearity: Calibration Curves



Glyphosate: 0.2-500 ng/L 10 cal stds. $R^2 > 0.995$



AMPA: 0.2-500 ng/L 10 cal stds. R² > 0.999

- A

Sample Chromatogram





Limit of detection and quantification (LOD/LOQ)

Limit of Detection (LOD): S/N>3 for most abundant transition Limit of Quantification (LOQ): S/N>9 for both transitions

Analyte	S/N for 0.2 ng/L standard	Expected LOQ (ng/L)	Expected LOD (ng/L)
Glyphosate (Q1)	25.8	0.1	0.025
Glyphosate (Q2)	18.1		
AMPA (Q1)	13.1	0.2	0.045
AMPA (Q2)	10.6		



Surface & Ground Water analysis

- Samples analyzed from a ground water source in AZ and a surface water (Colorado river).
- Surface Water (n=4): Glyphosate 1.5 ng/L ; AMPA 0.5 ng/L
- Ground Water (n=4): Glyphosate ND ; AMPA ND
- Samples were spiked at two levels (20 ng/L and 100 ng/L) to determine method recoveries.



Sample Collection



Derivitization



Analysis



Method Performance

Analyte	Ground Water			
	20 ng/L spike (n=5)		100 ng/L spike (n=5)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Gylphosate	88.6	2.7	93.4	2.4
AMPA	98.9	7.7	94.1	4.5

Analyte	Surface Water			
	20 ng/L spike (n=5)		100 ng/L s	spike (n=5)
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Gylphosate	95.2	1.1	98.7	1.6
AMPA	78.4	2.4	84.5	2.5

- Recoveries for AMPA and glyphosate in both waters was 75-100%
- RSD (%) was <10% in all spikes</p>



Conclusions

- A sensitive and robust method for trace level analysis of Glyphosate & AMPA has been developed using the Agilent 1290 UHPLC coupled to the 6460 MS/MS
- The Flexcube allows automated online sample enrichment thus reducing labor time while drastically reducing required sample volume
- A second transition is added for additional validation and increased specificity
- Very low ng/L levels of quantitation are possible
- Recoveries in both surface and ground waters are good.





5991-6115EN

Automated Online SPE for LC/MS/MS Analysis of Trace Organic Contaminants in Water Using the Agilent 1290 Infinity Flexible Cube Module

Application Note

Environmental

On-line SPE Enrichment of Trace Organic Contaminants in Water and Juices

5991-4562EN

Application Note

Environment and Food



Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/talanta



Detection of Basic and Acidic Pesticides and Herbicides at Trace Levels by Online SPE LC/MS in Drinking Water

Rapid analysis of trace organic compounds in water by automated online solid-phase extraction coupled to liquid chromatography-tandem mass spectrometry

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5991-4177EN

Application Note

Environmental



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QUESTIONS ???

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