Analysis of Glyphosate and AMPA in Drinking Water with the Agilent 1200 Infinity Series Online SPE Solution

Application Note

Environmental

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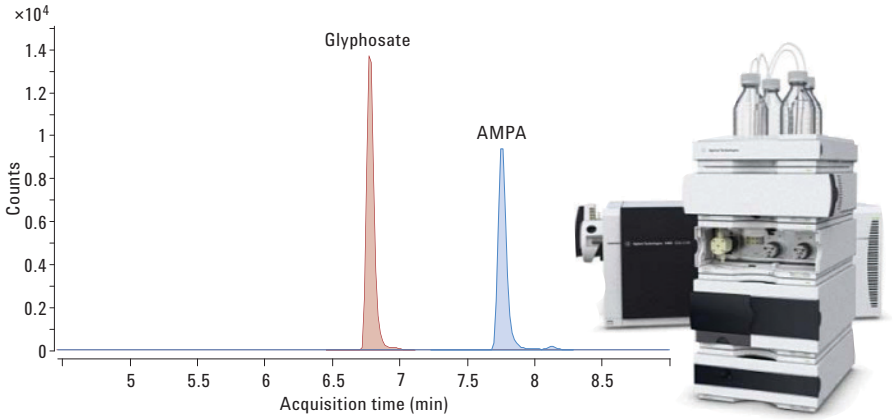
Abstract

This Application Note describes a robust and easy-to-use method for the determination of glyphosate and its degradation product AMPA using the Agilent 1200 Infinity Series Online SPE Solution.

Glyphosate and AMPA were measured in drinking water with excellent precision and linearity over a wide range of concentration.



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Introduction

Glyphosate (N-(phosphonomethyl) glycine) is the world´s biggest selling and most used herbicide for weed control[1]. It is considered to be of low toxicity to humans, but concerns for health and environment are increasing. In 2005, Richard *et al*. showed that a long exposure to glyphosate can cause endocrine effects on mammals[2].

Glyphosate has three polar functional groups (carboxyl, amino, and phosphate). It is highly soluble in water and the ability to bind to mineral components makes it persistent in the environment. For example, in 2005 in Sweden, glyphosate was found to be present in groundwater samples above the European maximum limit of 0.1 µg/L[3].

Because of the interaction of glyphosate and its metabolite AMPA ((aminomethyl) phosphonic acid) with the environment and health, both compounds were included in the annex III of the 2008/105/EC Directive as a substance subject to review for possible identification as priority substance or priority hazard substance.[4]

Hence, a fast and robust online SPE LC-MS method was developed for the analysis of glyphosate and AMPA in drinking and environmental water[6]. FMOC (fluorenylmethyloxycarbonyl chloride) was used for offline derivatization for both analytes. The derivatives were quantified with an Agilent 6460 Triple Quadrupole Mass Spectrometer coupled to a 1200 Infinity Series Online SPE Solution for online enrichment. With this method, detection limits of less than 0.01 µg/L can be achieved[6].

In this Application Note, we show excellent linearity and precision for glyphosate and AMPA analysis with the 1200 Infinity Series Online SPE Solution, which is based on a 1290 Infinity Flexible Cube for online SPE.

Experimental and Instrumentation

Agilent 1200 Infinity Online SPE Solution
Agilent 1260 Infinity BinaryPumpG1312B and LAN card G1369C
Agilent 1260 Infinity Standard Autosampler G1329B with 900 µL head (G1313-60007) and an Agilent 1290 Infinity Thermostat G1330B
Agilent 1290 Infinity Flexible Cube G4227A with two valve drives
Agilent 1200 Infinity Online SPE starter kit G4742A including one 2-position/10-port valve
Agilent 1290 Infinity Thermostatted Column Compartment G1316C
Agilent G6460A Triple Quadrupole LC/MS System with Agilent Jet Stream Technology
Analytical Column
Agilent ZORBAX Eclipse Plus C18, 2.1 × 150 mm, 3.5 µm (p/n 959763-902)
Trapping columns
2 × Guard Column Hardware Kit (p/n 820999-901) as part of G4742A
PLRP-S Cartridges, 4.6 × 12.5 mm, 15-20 µm (p/n 5982-1270) as part of G4742A
6-mL screw cap vials (glass p/n 9301-1377), screw caps (p/n 9301-1379), pre-slit septa (p/n 5188-2758)
Software
Agilent MassHunter data acquisition for triple quadruple mass spectrometer, Version 06.00
Agilent MassHunter Optimizer software, Version 06.00
Agilent MassHunter Source and iFunnel Optimizer software, Version 06.00
Agilent MassHunter qualitative software, Version 06.00
Agilent MassHunter quantitative software, Version 06.00

HPLC method

1260 Infinity BinaryPump	
Solvent A	Water, 2.5 mM ammonium acetate
Solvent B	Acetonitrile
Flow rate	0.3 mL/min
Gradient	Time (min) % B 0 5 2 5 8 70 8.5 100 13 100 Stop time: 13 minutes Post time: 5 minutes
Agilent 1290 Infinity Thermostatted Column Compartment	
Temperature	60 °C
Agilent 1290 Infinity Flexible Cube	
Valve	2-position/10-port QuickChange valve head
Solvent selection valve	Solvent A1: Water Solvent A2: Methanol
Agilent 1260 Infinity Standard Autosampler	
Injection volume	900 µL
Needle wash in vial (ACN)	
Draw and eject speed	450 µL/min
Sample Temperature	5 °C

System configuration and principle of operation

In comparison with previous configurations for online SPE analysis where seven modules have been used[6], the 1200 Infinity Series Online SPE provides a very cost-effective solution. Just five modules are needed for the same performance characteristics and application. The system is based on the 1290 Infinity Flexible Cube, which has a built-in piston pump for the loading and rinsing procedure, a solvent selection valve where up to three solvents can be used, and two valve drives for the configuration of two quick change valve heads.

The full functionality of the online-SPE system is achieved with a single HPLC pump, a standard autosampler, a thermostatted column compartment, and the 1290 Infinity Flexible Cube, which has one 2-position/10-port valve (Figures 1 and 2).

Table 1. Piston pump timetable in the Flexible Cube for the online SPE method.

Time	Function	Parameter
0	Pump volume	Pump 2.5 mL, flow: 1.5 mL/min Channel A1
2	Right valve change position	Increase valve position (switch valve)
2.1	Pump volume	Pump 5 mL, flow: 1.5 mL/min Channel A2
6.5	Pump volume	Pump 6 mL, flow: 1.5 mL/min Channel A1

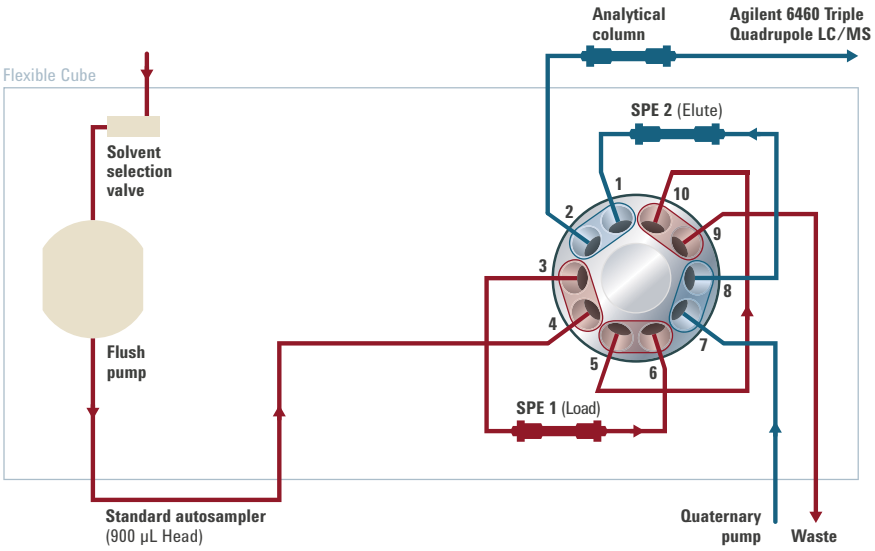

Figure 1. Valve positions for loading the sample on trapping column SPE 1 while trapping column SPE 2 is being eluted.

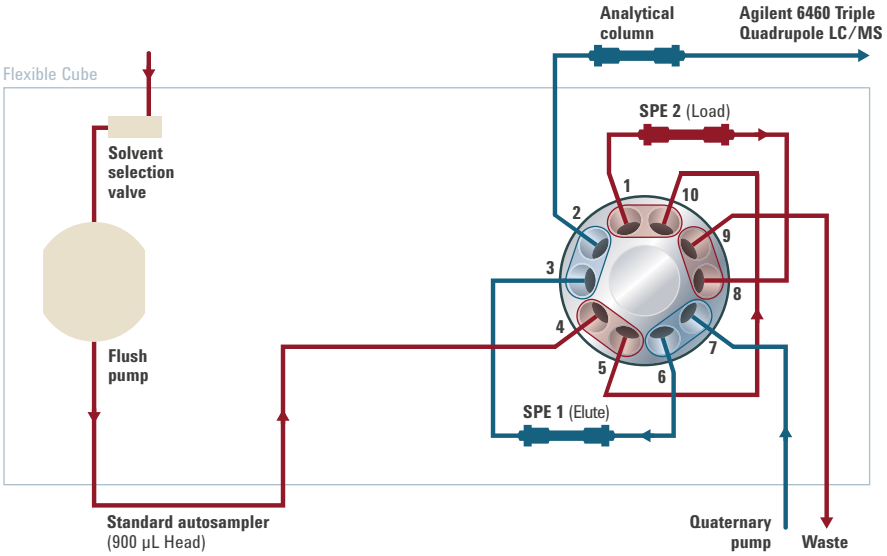

Figure 2. Valve positions for loading the sample on trapping column SPE 2 while trapping column SPE 1 is being eluted.

The 1290 Infinity Flexible Cube provides the alternate use of two trapping columns to save time and enhance sample throughput. Up to three solvents with the solvent selection valve can be used to load and clean the SPE cartridges (PLRP-S phase), which are reuseable (> 200 injections).

This system can be easily upgraded with a second 2-position/10-port valve in the 1290 Infinity Flexible Cube to run direct inject methods and online SPE methods on the same instrument without any setup modification[7].

Finally, the system was coupled to an Agilent 6460 Triple Quadrupole LC/MS System for the detection and quantification of glyphosate and AMPA in drinking water.

MS method

An Agilent 6460 Triple Quadrupole LC/MS System with negative electrospray Agilent Jet Stream technology:

Gas temperature	225 °C
Gas flow	8 L/min
Nebulizer	45 psi
Sheath gas temperature	350 °C
Sheath gas flow	11 l/min
Capillary	–4,500 Volt Nozzle: –1,000 Volt
Delta EMV	–600 Volt

Table 2 shows the MRM transitions for glyphosate and AMPA, as well as their optimum MS conditions.

Table 2. MRM transitions for glyphosate and AMPA and their internal standards.

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor voltage (V)	Collision energy (V)	Cell accelerator voltage (V)	Polarity
Glyphosate	390.2	167.8	100	6	3	negative
Glyphosate internal standard	393.2	170.8	100	6	3	negative
AMPA	332.0	109.8	100	6	3	negative
AMPA internal standard	334.0	111.8	100	6	3	negative

Chemicals

All solvents used were LC/MS grade. Acetonitrile was purchased from Rathburns, Scotland. Fresh ultrapure water was obtained from a Milli-Q Integral system. Ammonium acetate, FMOC, sodium tetraborate, di-sodium EDTA, and phosphoric acid were purchased from Sigma-Aldrich. All herbicide standards and internal standards were purchased from Qmx Laboratories.

Samples and sample pre-treatment

Glyphosate and AMPA were spiked in different concentrations with their internal standards in drinking water (0, 100, 200, 500, and 1,000 ng/L). Each calibration concentration was measured four times. One quality control standard (QC) was also measured four times at a concentration of 100 ng/L.

Glyphosate and AMPA are both highly polar compounds, therefore, an offline derivatization step with FMOC (fluorenylmethyloxycarbonyl chloride) is needed to achieve chromatographic separation on a reversed phase column.

Further required reagents were EDTA solution (0.1 M), tetraborate buffer (30 g/L), acetonitrile, FMOC reagent (20 mM in ACN) and phosphoric acid (Figure 3)[6].

Internal standards were added to compensate for recovery variations in different waters due to matrix effects.

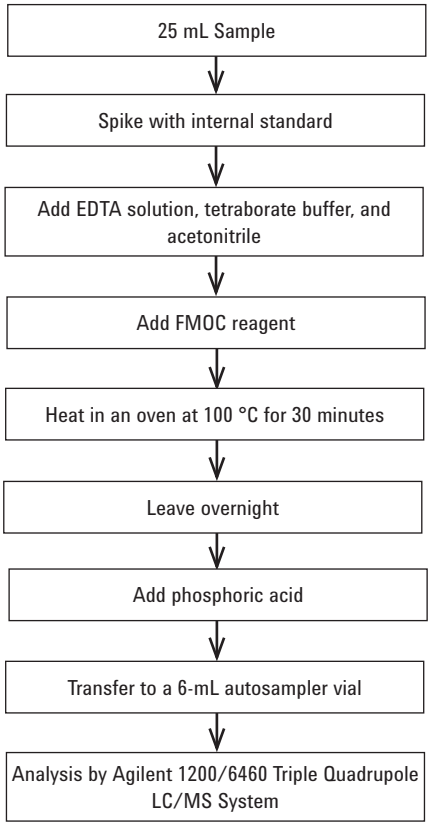

Figure 3. The offline derivatization of water with FMOC reagent.

Results and Discussion

After the offline derivatization step, glyphosate and AMPA could be separated on a reversed phase column with retention times (RT) at 6.7 and 7.7 minutes (Figure 4). The online SPE made it possible to inject the derivatives directly onto the SPE cartridges and to flush the FMOC reagent in the waste during the loading process to protect the analytical column from borate buffer and derivatization reagent.

Linearity

Glyphosate and AMPA show excellent linearity in the measured range of 100 to 1,000 ng/L, with R^2 of 0.9997 for both analytes (Figure 5).

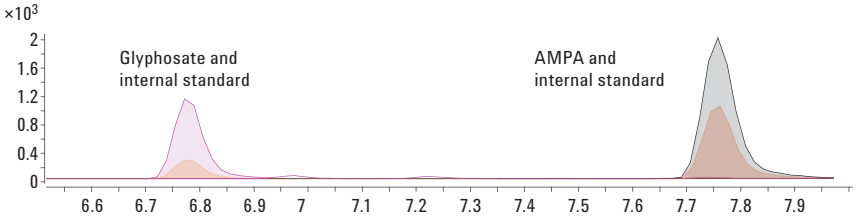

Figure 4. Chromatogram of glyphosate and AMPA and their internal standards at 100 ng/L.

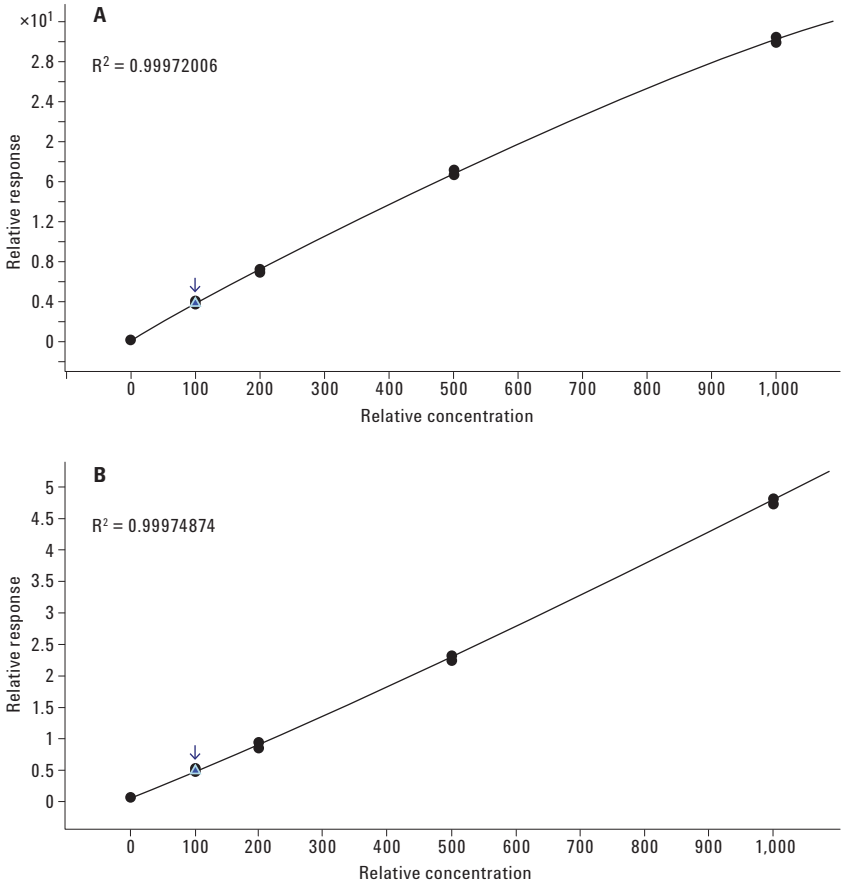

Figure 5. Calibration curve of glyphosate and AMPA 0–1,000 ng/L (five levels, quality control standards at 100 ng/L, n = 4).

Precision

Precision data were determined for all spiked concentration levels (100–1,000 ng/L, n = 4). Area RSD were < 5 % for all concentrations and both analytes. R^2 for glyphosate and AMPA showed excellent linearity with 0.9997. Excellent retention time (RT) precision with RSD of 0.111 % was observed for both analytes. Table 3 summarizes these results.

Table 3. Area RSD for four concentration levels (n = 4), linearity coefficient, and retention time RSD (n = 20) for glyphosate and AMPA.

	100 ng/L	200 ng/L	500 ng/L	1,000 ng/L	0–1,000 ng/L	RT RSD (%) (100–1,000 ng/L) n = 20
	Area RSD (%)	Area RSD (%)	Area RSD (%)	Area RSD (%)	R^2	
Glyphosate	2.18	2.27	1.49	1.76	0.999	0.111
AMPA	3.29	2.48	4.45	4.07	0.999	0.111

Conclusion

The Agilent 1200 Infinity Series Online SPE Solution based on the Agilent 1290 Flexible Cube offers a robust and fast analysis of glyphosate and its metabolite AMPA in drinking and environmental water. The performance data show excellent linearity and great precision for a wide concentration range. The described method, with fully automated online SPE, achieves detection limits of less than 0.01 µg/L with an injection volume of 900 µL[6].

The 1200 Infinity Series Online SPE Solution provides a cost-effective way for online enrichment. It does not only provide the flexibility to run online SPE methods, but also offers an opportunity to easily upgrade the 1290 Infinity Flexible Cube to direct inject methods. Furthermore, every Agilent HPLC can be upgraded to an online SPE system with the 1290 Infinity Flexible Cube and its flexible solutions.

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Published in the USA, October 1, 2013
5991-3208EN

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