



Trace Level
Measurement – LC-QQQ
Measurement of Glyphosate and AMPA



Measurement of Glyphosate and AMPA in potable and Environmental Waters using LC-QQQ and fully automated online enrichment

Glyphosate is a broad-spectrum herbicide and widely used in agriculture. The main degradation product is aminomethyl phosphonic acid AMPA which can also be formed from the breakdown of organic phosphonates in detergents.

Regulation 2008/105/EG defines quality criteria for 20 priority substances and 13 priority hazardous substances. A further set of 13 substances including glyphosate and AMPA are candidates for future action.

This flyer outlines a robust routine method for the determination of glyphosate and AMPA in potable and environmental waters at the low ng/L level using an Agilent 1200/6410 LCC-QQQ system incorporating „front end“ automated online solid phase enrichment after derivatization with fluorenylmethyloxycarbonyl chloride (FMOC).

Sensitivity and Linearity

could be shown from below 20 ng/L up to 5000 ng/L

Accuracy and Precision

were within $\pm 20\%$ of the nominal value and $< 8\%$

Recovery and Precision

was between 90% and 110%



Materials and Methods

Liquid Chromatography and online SPE: Agilent 1200 Series HPLC modules consisted of the following: Micro Vacuum Degasser, Quaternary Pump, Standard ALS with Extended Injection Volume option and Multi-Draw Kit, ALS Thermostat, 2-position/6-port valve, 6-position switching valve, Binary Pump SL and TCC.

Mass Spectrometer: Agilent 6410 QQQ system

Analytical Column: ZORBAX Eclipse Plus C-18 100 x 2.1 mm, 3.5 μm (40°C)

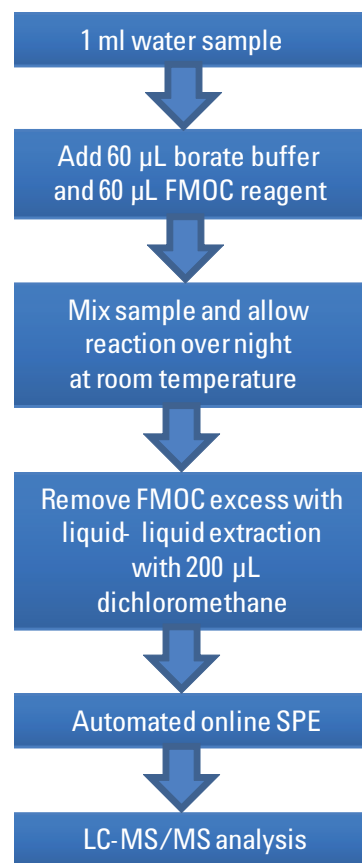
Mobile Phase: (A) 5 mM ammonium acetate (pH 9) / (B) methanol, flow rate 0.35 ml/min; linear gradient from 20 to 90% B in 6 min, 4 min isocratic at 90% B, linear gradient to 20% B in 2 min. Total run-time 20 min

Reagents: Borate buffer ($\rho(\text{Na}_2[\text{B}_4\text{O}_5(\text{OH})_4]) = 50 \text{ g/l}$; 0.25 M); FMOc solution ($\rho(\text{FMOc}) = 6 \text{ mg/ml}$)

Mass Spectrometer Settings: Electrospray ionization, positive mode, VCap -5kV, Drying Gas 11 l/min (350°C), Nebulizer 45 psi, dwell time 100 ms, unit resolution.

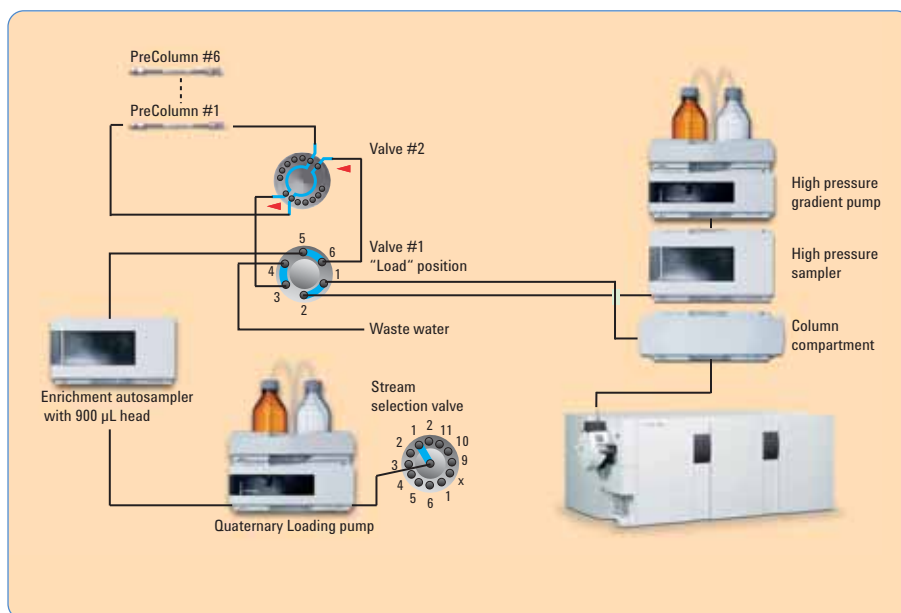
Compound	ISTD	Precursor	Product	Frag	V CE
Glyphosate	<input checked="" type="checkbox"/>	392.0	179.0	100	24
Glyphosate	<input type="checkbox"/>	392.0	88.0	100	16
$^{13}\text{C}_2^{15}\text{N}$ -Glyphosate	<input checked="" type="checkbox"/>	395.0	179.0	100	24
$^{13}\text{C}_2^{15}\text{N}$ -Glyphosate	<input checked="" type="checkbox"/>	395.0	91.0	100	16
AMPA	<input type="checkbox"/>	334.0	179.0	100	11
AMPA	<input type="checkbox"/>	334.0	112.0	100	10
Glufosinate	<input type="checkbox"/>	404.0	179.0	100	24
Glufosinate	<input type="checkbox"/>	404.0	136.0	100	20
$^{13}\text{C}_2^{15}\text{N}$ -AMPA	<input checked="" type="checkbox"/>	337.0	179.0	100	11
$^{13}\text{C}_2^{15}\text{N}$ -AMPA	<input checked="" type="checkbox"/>	337.0	114.0	100	10

MS parameters for glyphosate, AMPA, glufosinate and internal standards.

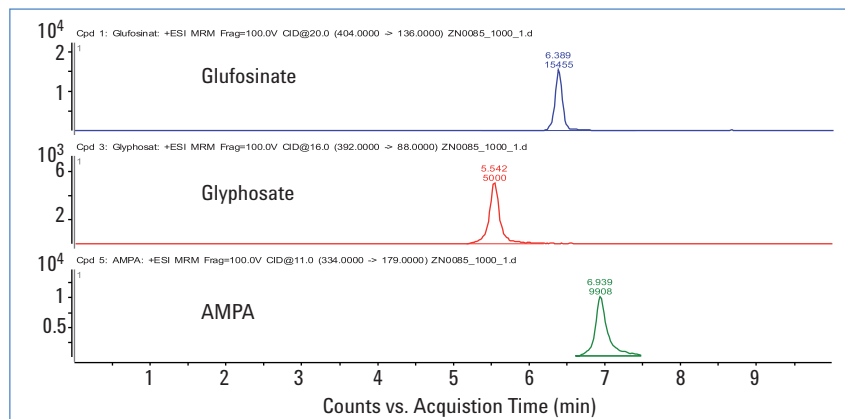


Automated online enrichment

900 μL of the derivatized sample is loaded onto the SPE cartridge from a standard 2 mL vial in the Enrichment autosampler using a 1 mL/min loading flow rate from the Quaternary Loading pump. The SPE cartridge is then desorbed in 'backflush' mode using the gradient from the High pressure gradient pump and trapped analytes are separated on the 2.1 x 100 mm ZORBAX Eclipse Plus C18 column, 3.5 μm .



Performance Examples



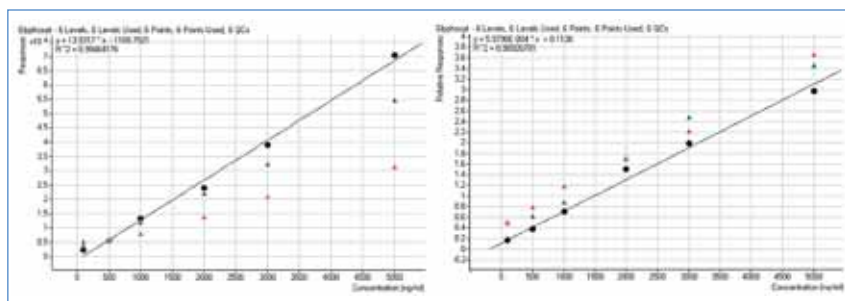
Online SPE – LC-MS/MS chromatograms for the quantifier ions of derivatised glufosinate, glyphosate, and AMPA of a groundwater sample spiked to a concentration of 1 µg/L.

Derivatization with FMOC at pH >9 produces derivatives that can be separated by reversed phase chromatography and detected by positive ion electrospray.

Online SPE with 900 µL of sample allows for LOQs below 20 ng/L and eliminates any detrimental effects of borate buffer and excess FMOC reagent reaching the analytical column.

Liquid-liquid extraction of the derivatized sample with dichloromethane reduced the background noise and therefore increased the S/N ratio of the method. The addition of isotopically labelled internal standards compensates for any recovery variation due to matrix effects.

Derivatization has been optimized in terms of pH, excess amount of FMOC, reaction time, and different water sources (surface water, hard and soft groundwater, saline waters).



Comparison of Calibrations for ultrapure water (black), surface water (blue) and groundwater (red) without (A) and with (B) internal standard correction.

Sampling point	AMPA in ng/L	Glyphosate in ng/L	Glufosinate in ng/L
Elbe, Sampling point 1	780	220	n.d.
Elbe, Sampling point 2	460	900	n.d.
Lausitzer Neiße, Sampling point 1	2300	1000	n.d.
Lausitzer Neiße, Sampling point 2	750	510	n.d.
Lausitzer Neiße, Sampling point 3	820	470	n.d.
Weißer Elster, Sampling point 1	1300	530	n.d.
Weißer Elster, Sampling point 2	1600	380	n.d.

Real samples – Concentrations of AMPA and glyphosate in river waters in southeast Germany.

Conclusion

The application of online SPE to the analysis of glyphosate and AMPA after derivatization with FMOC increases the sensitivity compared to direct injection and the overall method robustness by protecting the analytical column from borate buffer and derivatizing agent which might otherwise cause column degradation.

By using an injection volume of 900 µL LOQs below 20 ng/L have been shown for all compounds.

The use of internal standards compensates for any recovery variations in natural waters due to matrix effects.

The method has been successfully applied to the routine analysis of surface and groundwater samples. Glyphosate concentrations of up to 1 µg/L and AMPA concentrations of up to 2.3 µg/L have been detected. Glufosinate has not been detected in the analyzed water samples.

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Printed in the Germany April 1, 2012
5990-8136EN