

EPRW 2012



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# Newsletter

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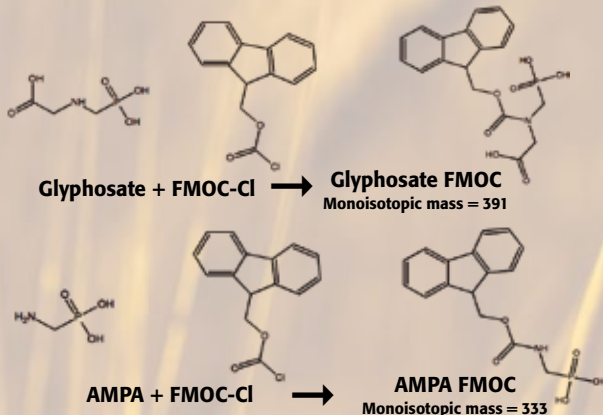
June 2012

Improved efficiency and lower LODs  
for broadband herbicides

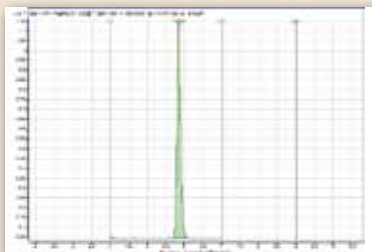
## Glyphosate / AMPA a global presence

According to recent reports, a dramatic increase in the use of Glyphosate-based broadband herbicide products brings risk to both human health and the environment. Experts agree: Monitoring and control is required. In order to be determined by HPLC-MS/MS, Glyphosate and its metabolite AMPA must be derivatized. A new automated solution dramatically improves both efficiency and sensitivity of the method.

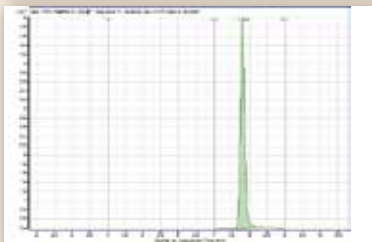
Among the most widely used broadband herbicide products world-wide, Glyphosate-based products are front-runners. Glyphosate is renowned for its non-specific impact on undesirable plants and weeds. The US-based international agriculture giant Monsanto describes Glyphosate as the most widely sold crop protection product world-wide. Glyphosate-based products in a variety of concentration levels are primarily used in agriculture, for example, to clean fields of other plants prior to sowing and it is also widely used for private gardening. The determination of Glyphosate and its main

SPE<sup>XOS</sup> on-line SPE with cartridge exchange

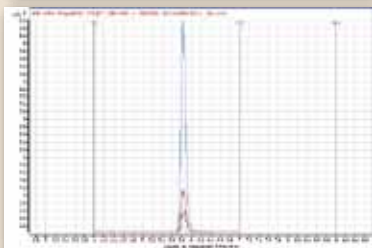
Efficiently automated derivatization of AMPA and Glyphosate with FMOCl enables accurate and sensitive determination with excellent throughput.



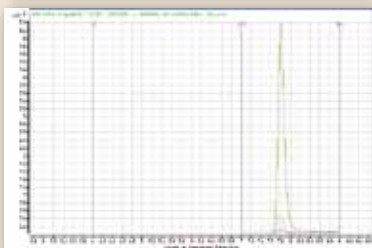
1 ng/mL Glyphosate standard after FMOCl derivatization.



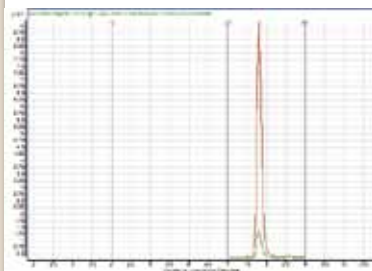
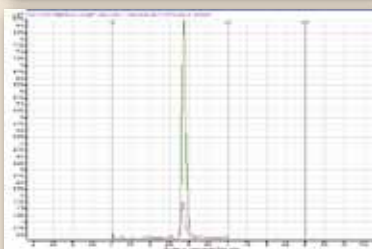
0.5 ng/mL AMPA standard after FMOCl derivatization.



Overlay of Glyphosate standard chromatograms at 0.1; 0.5; 1; and 5 ng/mL after derivatization and cleanup.



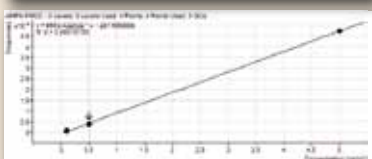
Overlay of AMPA standard chromatograms at 0.1; 0.5; 1; and 5 ng/mL after derivatization and cleanup.



Overlay chromatograms of Glyphosate (top) and AMPA in wheat matrix at 0.01 and 0.1 mg/kg respectively after derivatization and cleanup.



Glyphosate-FMOCl calibration curve (peak areas).



AMPA-FMOCl calibration curve (peak areas).

metabolite, aminomethylphosphonic acid (AMPA), mainly relies on HPLC and multi-dimensional mass selective detection (MS/MS). The method presented in this work was developed based on a trace analysis method for Glyphosate and AMPA in water, which is based on direct injection and LC-MS/MS and which follows an official method [1]. The focus in this work was on automating and further developing the existing method while improving the overall process efficiency, sensitivity and accuracy of analysis using standard LC-MS/MS systems. We especially aimed to improve the analytical accuracy at low concentration through an enrichment step.

### HPLC-MS/MS Method Development Step By Step

Both the active compound Glyphosate and its most important metabolite, aminomethylphosphonic acid (AMPA) are so polar that they are not easily determined using HPLC. However, the derivatization products formed with 9-Fluorenylmethylchloroformate (FMOCl), as specified in ISO/DIS 21458 [2], can be quite nicely determined using LC-MS/MS. This part of the sample preparation process, adding both a derivatization reagent and a standard solution, we performed using the GERSTEL MultiPurpose Sampler (MPS). In order to clean up the sample and to improve the sensitivity of the analysis, solid phase extraction (SPE) was performed using a special extraction module, the GERSTEL SPE<sup>XOS</sup>. SPE<sup>XOS</sup> performs on-line solid phase extraction, transferring the eluate 100% to the HPLC system for maximum recovery and sensitivity. A new cartridge is inserted for each sample, eliminating sample-to-sample carry-over. The SPE<sup>XOS</sup> unit was connected to an Agilent Technologies

1290 UHPLC system. Unlike standard SPE systems, SPE<sup>XOS</sup> is based on smaller SPE cartridges with only 50 mg of adsorbent compared with 100 to 1000 mg in standard cartridges. SPE<sup>XOS</sup> cartridges withstand up to 200 Bar pressure, enabling them to be inserted directly into the HPLC mobile phase and enabling injection, cleanup and analysis of larger sample volumes. In contrast, the elution can be performed with significantly smaller eluent volume, leading to a higher concentration factor. As mentioned, the eluate is typically transferred quantitatively to the HPLC system for maximum sensitivity. The total process from extraction through cleanup to elution is performed automatically, and is easily set up and controlled by mouse-click as part of the sample preparation method. MAESTRO software enables stand-alone control or integrated operation with the LC-MS/MS sequence table for simple and error-free operation. Using PrepAhead, the next sample is prepared during the on-going LC-MS/MS analysis enabling just-in-time injection as soon as the system is ready. This ensures best possible system utilization and return on investment; the analysis system never has to wait for the next sample to get ready. For the detection of Glyphosate and AMPA, we used a 6460 Triple Quadrupole MS from Agilent Technologies. The FMOCl-derivatization products of Glyphosate and AMPA were detected in negative ionization mode (ESI).





Name	Area	Concentration
1	12345	100
2	23456	200
3	34567	300
4	45678	400
5	56789	500
6	67890	600
7	78901	700
8	89012	800
9	90123	900
10	01234	1000

### Solid phase extraction and on-line sample introduction

Listed below are the individual sample preparation steps that are performed synchronizing SPE<sup>XOS</sup> with the MPS under GERSTEL MAESTRO software integrated with the ChemStation or MassHunter software from Agilent Technologies:

- Load the SPE<sup>XOS</sup> cartridge
- Condition the cartridge with 1 mL of methanol
- Rinse the cartridge with 2 mL of water
- Introduce 1000 µL of sample
- Wash the cartridge adsorbent bed with 1100 µL of water
- Switch SPE<sup>XOS</sup> from MPS mode, i.e. sample preparation- and extraction mode, to injection mode for transfer of the eluate to the HPLC system
- Elution and derivatization of the next sample

All steps were fully automated. The sample preparation was completed in 25 minutes. The HPLC-MS/MS analysis cycle time was around 20 minutes.

### Conclusion and Outlook

Using the newly developed MPS-SPE<sup>XOS</sup>-LC-MS/MS method, we were able to successfully determine Glyphosate and its most important metabolite, Aminomethylphosphonic Acid (AMPA) in various spiked water samples. The determination of a dilution series resulted in highly satisfactory linearity of 0.999 and a very low Limit of quantitation of 10 ng/L (LoQ). Following cleanup, no matrix effects were observed for any of the different samples, and recovery and reproducibility were found to be excellent. The method is highly sensitive, reproducible and rugged. In our experience, it is also well suited for both selective and sensitive determination of Glyphosate and AMPA in water as well as in foods and environmental matrices. As analysts, we were pleased to see that the above-described MPS-SPE<sup>XOS</sup>-LC-MS/MS system enabled us to combine analyte concentration with sample clean-up and on-line sample introduction. Last, but not least, smaller SPE cartridges are used in the SPE<sup>XOS</sup> system enabling us to reduce the amount of solvent used significantly compared with standard SPE systems. The MPS offers further bandwidth in terms of available sample preparation techniques such as filtration, centrifugation, and evaporation, which can be selected by mouse-click and used whenever the need arises for any particular sample type. All steps are automated and synchronized with the LC-MS/MS analysis for best possible productivity and throughput. In short, the analyst can deliver more and better results while reducing his or her workload.

### Literature

- [1] "LC-MS/MS trace analysis of water without the use of concentration steps. Determination of Glyphosate and AMPA in water using direct introduction of Fmoc-derivatization products". Poster presentation found under [www.lw-online.de](http://www.lw-online.de), Zweckverband Landeswasserversorgung, Stuttgart, Germany and Aqua Service, Schwerin, Germany.
- [2] International Organization for Standardization (ISO) Technical Committee ISO/TC 147, Water quality, Subcommittee SC2, Physical, chemical and biochemical methods: Determination of Glyphosate and AMPA using high performance liquid chromatography (HPLC) with fluorescence detection.

### Authors

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## Automated Liner EXchange ALEX

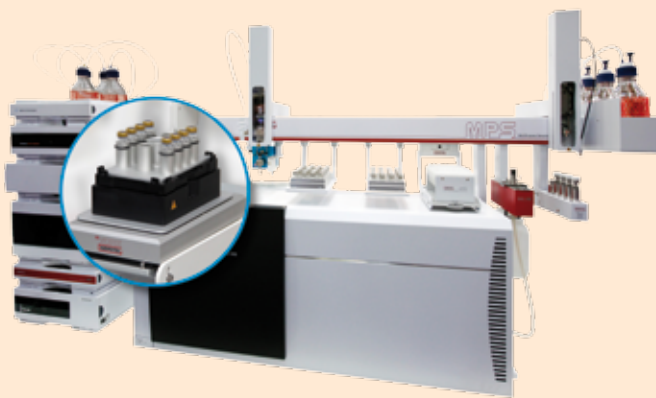
GERSTEL's Automated Liner EXchange (ALEX) enables routine GC analysis of samples containing matrix or other solid residue. When deposited in the GC inlet, matrix residue can cause adsorption and loss of active analytes. One such example is pesticides extracted using the QuEChERS method. ALEX replaces the GERSTEL CIS, PTV-type, inlet liner at user-defined intervals, eliminating the need for time-consuming clean-up steps during sample preparation. ALEX is an add-on module for the GERSTEL MultiPurpose Sampler MPS. Software control is fully integrated with the GC/MS software. Just one method and one sequence table operate the complete system.



For more information, please visit the GERSTEL booth.

## Vortex / Shaker option for the MPS

The novel Vortex and shaker option (mVORX) for the GERSTEL MultiPurpose Sampler (MPS) is now available. The GERSTEL mVORX performs vortexing and agitation at up to 3,000 rpm, speeding up sample preparation steps such as liquid-liquid extraction, dissolution, and homogenization. The mVORX performs efficient simultaneous vortex mixing of up to eight samples depending on the vial size. The mVORX orbital motion is provided by a precise linear, direct drive motor for finely-tuned operation. Mixing movements are restricted to the horizontal plane, allowing even the most sensitive samples to be mixed efficiently without over-agitation or wetting of the vial cap. Automated operation under MAESTRO software control enables reliable and flexible sample processing, the PrepAhead functionality ensures best possible productivity and throughput.



## Multi-Position Evaporation Station (mVAP)

A six-position evaporation station (mVAP) is available for the GERSTEL MultiPurpose Sampler (MPS). Samples are concentrated at user-defined temperature and vacuum, enabling significantly improved limits of detection. Solvent exchange to a GC- or HPLC compatible solvent can be performed for improved chromatography. mVAP can be used in combination with SPE, Dispersive SPE (DPX) or liquid/liquid extraction to evaporate solvent from extracts combined with injection to GC/MS or LC/MS. Every step is controlled by mouse-click using the MAESTRO PrepBuilder. Just one method and one sequence table is needed for the entire process including GC/MS or LC/MS analysis.



## LC/MS Sample Preparation

Integrated sample prep and LC/MS analysis with Agilent LC MassHunter®, AB SCIEX Analyst®, and ThermoScientific® XCalibur® software is performed using one integrated sequence table in combination with GERSTEL MAESTRO software. Addition of standards, derivatization reagents, or diluents; heating; cooling; mixing; centrifugation; SPE or dispersive SPE is performed followed by sample introduction. Sample Prep is performed in parallel with the ongoing LC-MS/MS analysis using MAESTRO PrepAhead for optimized throughput. Priority samples can be inserted into the running sequence without slowing or halting the workflow. MAESTRO operates independently or integrated with the LC/MS sequence table.

### Suggested reading:

Automated QuEChERS Extraction for the Determination of Pesticide Residues in Foods using Gas Chromatography/Mass Spectrometry.

[www.gerstel.de/pdf/p-gc-an-2011-05.pdf](http://www.gerstel.de/pdf/p-gc-an-2011-05.pdf)

GERSTEL Automated Liner Exchange (ALEX) and its Benefits in GC Pesticide Analysis

[www.gerstel.de/pdf/p-gc-an-2010-07.pdf](http://www.gerstel.de/pdf/p-gc-an-2010-07.pdf)

Automated QuEChERS Extraction for the Confirmation of Pesticide Residues in Foods using LC/MS/MS

[www.gerstel.de/pdf/p-lc-an-2010-04.pdf](http://www.gerstel.de/pdf/p-lc-an-2010-04.pdf)

Automated Multi-Residue Pesticide Analysis in Fruits and Vegetables by Disposable Pipette Extraction (DPX) and Gas Chromatography/Mass Spectrometry

[www.gerstel.de/pdf/p-gc-an-2009-01.pdf](http://www.gerstel.de/pdf/p-gc-an-2009-01.pdf)