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EPRW 2014
Newsletter

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Clean and sensitive Determination of Pesticides

Online SPE sample clean-up based on replaceable cartridges provides higher sensitivity and lower limits of detection – without the risk of carry-over

Analyzing food, water and soil for pesticide residues constitutes a significant part of the workload in laboratories that specialize in food safety and environmental analysis. Given the vast and increasing number of samples, efficiency is key and the strategy has to be automation – sensible and efficient automation.

The weed killers (herbicides) most frequently used for crop protection in fruit production are based on phenyl urea or triazine compounds. Both these compound classes enter the plant through the roots and are transported to the chloroplast where they interfere with the process of photosynthesis, ultimately leading to the death of the plant. It is in the nature of weed eradication through chemical agents that residues applied to the upper soil layers will reach deeper layers where the crop roots are located and will be transported into both the wider environment and the food chain. Pesticides and herbicides accumulate in ground and surface waters which are also our drinking water reservoirs. To avoid any danger to human health, governments have limited the maximum allowable concentrations for such residues in water to 0.1 μg/L with a required limit of determination ten times lower at 0.01 μg/L. Reaching this limit of determination normally requires direct introduction to a high-end highly sensitive HPLC-MS/MS system, but not all compounds can be determined this way, especially not early eluting com-
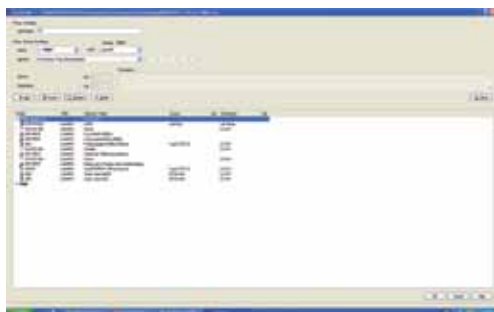
GERSTEL Seminar
THURSDAY, JULY 3,
10:50 – 11:25 a.m.

Efficiently Automated Sample Preparation workflows for the Food Safety Laboratory
Oscar G. Cabrices, Ph.D., GERSTEL, Inc.

New Concepts for GC/MS determination of pesticide residues in partially cleaned "dirty" samples
Jochen Vandenberg,
GERSTEL GmbH & Co.KG

Screenshot of the sample prep work flow as seen in MAESTRO software. Method and sequence set-up is easy and uncomplicated based simply on selecting the necessary steps from a pull-down menu or using copy-paste from existing methods and sequences.

Sample preparation and LC-MS/MS analysis are performed simultaneously in parallel. The overall analysis time per sample is only 14 minutes once the first sample has been prepared.

pounds. To circumvent these obstacles, larger volumes (up to 100 μL) are injected directly into the LC-MS/MS system – or the compounds in question are concentrated on fixed on-line SPE cartridges, a widely used procedure. However, both these alternatives are associated with certain drawbacks: The introduction of a large sample volume fre-

quently leads to peak broadening as well as to carryover effects. In addition, highly sensitive and expensive analysis instrumentation would typically be needed to reach the required limits of determination. Analyzing a series of samples using only a single fixed cartridge to concentrate analytes, on the other hand, will regularly lead to sample-to-sample carry-over and incorrect results with the need to re-analyze especially high concentration samples along with several of the following samples. Since the cartridge is typically loaded with sample from one side and eluted from the other side, the clean-up effect is also limited because the analytes don't have to traverse the entire column.

Online Solid Phase Extraction with clean-up

The goal of this project was to reach the required limits of determination based on injecting only 1 mL of water sample. In order to combine the advantages of online SPE concentration with the required clean-up, we configured our LC-MS/MS system with a separate online SPE Module (GERSTEL SPE[XOS]), which is based on replaceable cartridges. Some technical detail: SPE[XOS] cartridges contain only 50 mg of sorbent compared with 100 to 1000 mg of sorbent used in regular SPE cartridges. This means that the SPE process can be completely integrated into the HPLC process since significantly less solvent is required for analyte elution. SPE[XOS] is integrated into the system between the autosampler (GERSTEL MultiPurpose Sampler, MPS) and the LC-MS/MS system (Agilent 1260 HPLC/6460

Triple Quad MS). Sample introduction to the HPLC follows online, i.e. the SPE eluate, and thus 100 % of the analytes, is transferred directly and quantitatively into the HPLC mobile phase. In practice, the analysis requires only a very small amount of sample, in the order of 1-5 mL, and the complete process is fast enabling high throughput. System control for the complete process from sample preparation through introduction to the LC-MS/MS is conveniently controlled by mouse-click using the GERSTEL MAESTRO software. Sample preparation and analysis can be performed in parallel using the PrepAhead function to ensure that the next sample is always prepared and ready for introduction when the LC-MS/MS system is ready for the next run.

We had anticipated that the addition of the SPE[XOS] module would provide several interesting and useful benefits. For example, since the cartridges are exchangeable we expected carry-over to be eliminated. Further we expected the clean-up effect to be superior since analytes had to travel the entire length of the sorbent bed. Further, different - even specific – clean-up steps were conceivable since we could freely select sorbent materials. Finally, focusing the analytes on the analytical column after they had been transferred quantitatively from the SPE column would lead to sharp peaks and improved separation and sensitivity – thus the theory and the high expectations.

## A glimpse at the technical details of the analysis

The practical analysis was performed as follows: The one and only manual sample preparation step was to load water samples into vials and place them in the proper positions on the MPS autosampler. All further steps were performed automatically, as specified in the software.

SamplePrep Solution for Glyphosat and AMPA

In cooperation with TeLA GmbH, a food safety laboratory and GERSTEL partner located in Bremerhaven, Germany, GERSTEL has developed an LC-MS/MS-based solution for the determination of glyphosate and its main metabolite(s). The Glyphosate / AMPA solution from GERSTEL encompasses an LC-MS/MS system from Agilent Technologies, the GERSTEL MultiPurpose

Sampler (MPS) used for automated sample preparation, as well as the GERSTEL SPE[XOS], a Solid Phase Extraction (SPE) module based on smaller cartridges requiring only smaller sample volumes and less solvent while reaching the required limits of detection. The MPS-SPE[XOS]-LC-MS/MS solution leads to a significant increase in efficiency and throughput for the determination of glyphosate and AMPA in various food, water and soil samples.
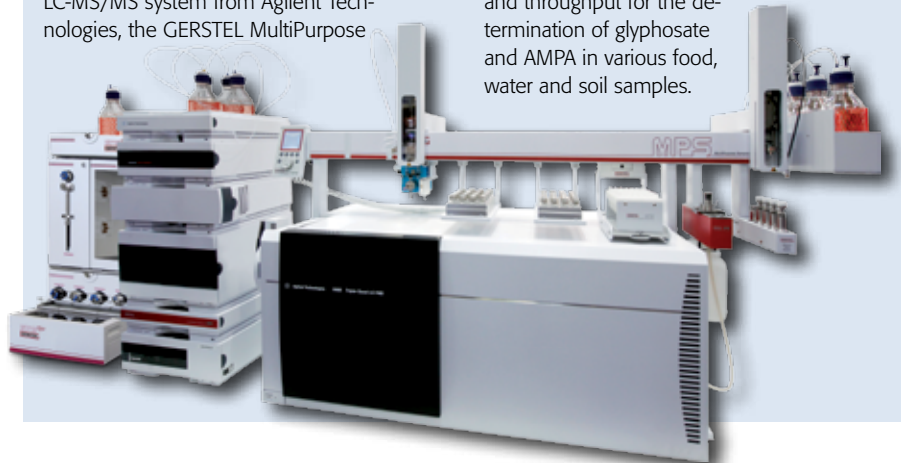
[LOAD]
Load the SPE[XOS] cartridge

[SPE PREP]
Condition with 4 mL of methanol

[SPE PREP]
Condition with 4 mL of water

[ADD]
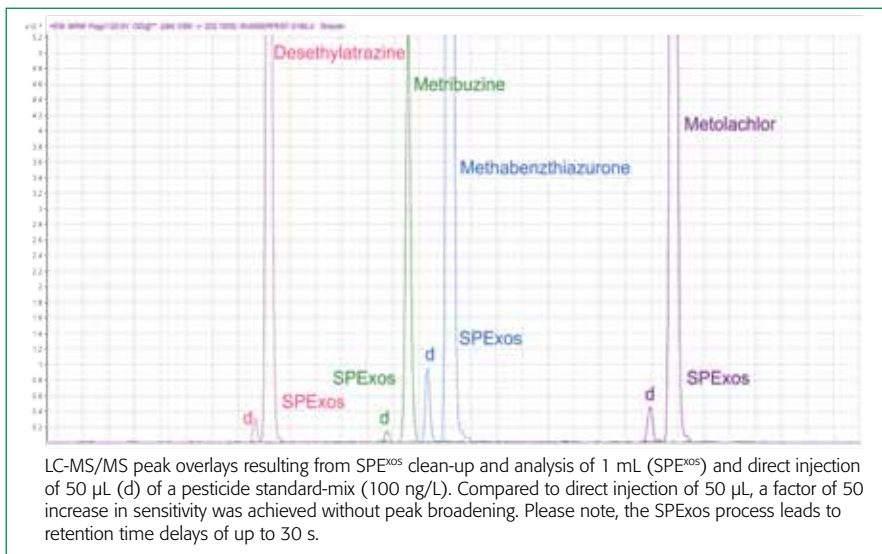Load 1 mL of sample into the MPS injection valve loop.

[SPE PREP]
Transfer the sample from the loop to the SPE[XOS] cartridge using 1.5 mL of water

[SPE PREP]
Valve switch: The flow from the binary pump is switched to the SPE[XOS] cartridge

[INJECT]
Start signal for the Agilent MassHunter Software and the LC-MS/MS system.

LC-MS/MS peak overlays resulting from SPE^{xos} clean-up and analysis of 1 mL (SPE^{xos}) and direct injection of 50 μL (d) of a pesticide standard-mix (100 ng/L). Compared to direct injection of 50 μL, a factor of 50 increase in sensitivity was achieved without peak broadening. Please note, the SPExos process leads to retention time delays of up to 30 s.

retention time was shifted with a delay of 30 seconds. Carry over effects were not observed when comparing injections of a standard [c = 100 ng/L] through the SPE^{XOS} system with subsequent blank injections. Apart from the increase in sensitivity, the additional clean-up step resulted in a significantly cleaner solution, which should have a positive long-term effect on system stability. Not least, a limit of determination of < 10 ng/L was reached for all compounds. The calibration resulted in good linearity throughout and the overall analysis time per sample was approximately 14 minutes.

Authors

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LC/MS method parameters

Mobile phase:

Flow: 0.35 mL/min; A - Formic acid 5 mmol/L; B - Acetonitrile; 0 min: 5 % B – 10 min: 50 % B – 22 min: 100 % B – 22.1 min: 5 % B - End: 28min.

Column Oven Temperature:	60 °C
Column material:	C18
MSD Source:	Agilent Jetstream, ESI positive
Gas Temperature:	300 °C
Gas Flow:	9 L/min
Nebulizer:	45 psi
Sheath Gas Temperature:	270 °C
Sheath Gas Flow:	12 L/min
Capillary:	5500V
Nozzle:	300 V

Results and discussion

A method is only useful if it proves itself in practice. The idea of using online SPE for analyte concentration while also using it as clean-up step proved highly useful and SPE^{XOS} reliably replaced SPE cartridges between samples. In samples of only 1 mL volume, the following analytes were determined: metolachlor, metazachlor, diurone, terbuthylazine, metoxurone, methabenzthiazurone, chloridazone, atrazine, metribuzine, chlorotolurone, isoproturone, metamitron, desethylatrazine and desisopropylatrazine.

If you are considering automating your sample preparation, or if you have already decided to automate, please take a moment to visit the GERSTEL booth to discuss the possibilities with our highly experienced specialists. You will find us on booth 22a.

Compared to a direct injection of 50 μL, we achieved a factor 50 increase in sensitivity – without peak broadening. Only the

Intelligently automated sample preparation

Whoever wants to increase laboratory efficiency and productivity should likely consider automating at least key parts of the work-flow. The greatest potential for efficiency improvements is typically found in the sample preparation processes.

GERSTEL is a leading company in the field of automated sample preparation and sample introduction for GC/MS and LC/MS. Most of our solutions are based on the GERSTEL MultiPurpose Sampler (MPS) a highly flexible and efficient autosampler and sample preparation robot. Simple or complex processes can be automated The highly modular system enables easy and flexible adaptation as laboratory requirements change and new tasks must be performed. Extraction and clean-up techniques are among the most widely used sample preparation steps.
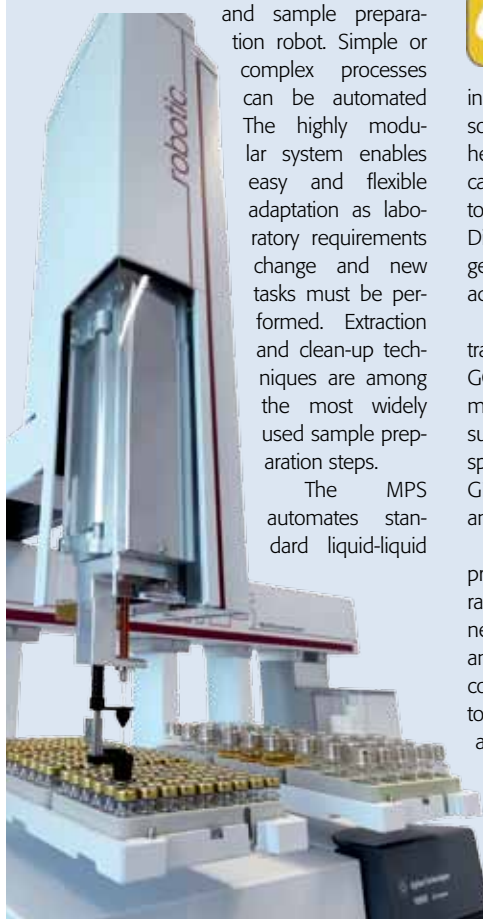
The MPS automates standard liquid-liquid extraction as well as Membrane Assisted Solvent Extraction (MASE) and addition of internal standard(s) and finally clean- up including a range of SPE techniques. Among these are standard SPE, dispersive SPE and online SPE with replaceable cartridges (GERSTEL SPE^{XOS}), directly coupled to the LC/MS analysis.

The MPS is the perfect platform for automating a host of techniques including vortexing (^{m}VORX), ultrasonic bath, centrifugation, microwave heating and filtration. Equally, liquids can be added to vials and weighed to generate highly accurate calibration standards. Dilution series and calibration standards can be generated automatically or internal standards added.

When it comes to extraction, analyte concentration and introduction of volatile compounds for GC/MS determination, the MPS is the ideal automation platform performing standard techniques such as SPME, Headspace, Dynamic Headspace (DHS), Stir Bar Sorptive Extraction (SBSE, GERSTEL Twister) as well as Thermal Desorption and Pyrolysis.

With the MPS, you are assured the highest productivity and flexibility. The MPS works with a range of different sample containers as per the needs of the customer for many different tasks and applications. Sample trays can be heated or cooled, samples can be stored without exposure to daylight and a large number of sample stored and processed in the smallest space.

As needed, the MPS can even unscrew and reseal your sample vials.

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.
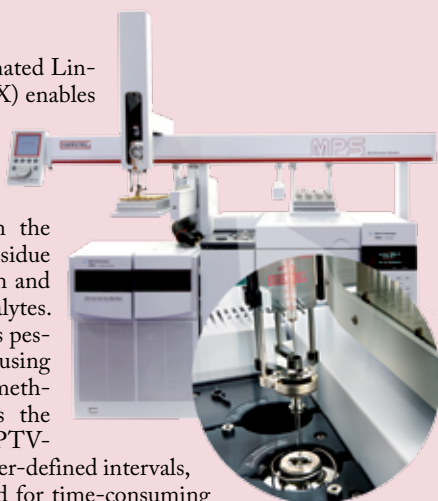
Vortex- and Shaker option for the MPS

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.
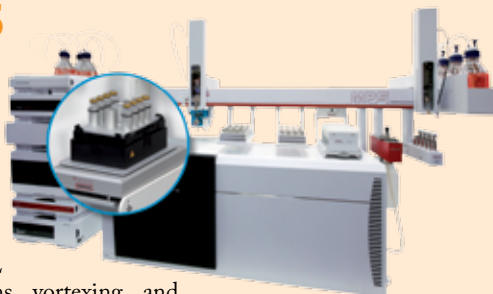
Multi-Position Evaporation Station (mVAP)

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 and solvent exchange to a GC- or HPLC compatible solvent 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 and just one sequence table for 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.

Online SPE system SPEXOS

GERSTEL SPEXOS performs online SPE with automated cartridge exchange. SPEXOS uses small cartridges, which are inserted directly into the HPLC mobile phase for elution resulting in quantitative transfer of analytes to the HPLC column. Much smaller sample sizes can be used to reach required detection limits and much less solvent is needed, for example, for analyte elution, increasing the concentration factor and reducing both cost and environmental impact. In combination with the GERSTEL MPS and MAESTRO software, liquid sample preparation, clean-up and LC-MS/MS analysis can be performed in one integrated system operated with one integrated sequence table. The Prep-Ahead functionality with multi-sample overlap ensures maximum efficiency and throughput for the complete system, including the LC-MS/MS.

Suggested reading:

Development of an Automated Sample Preparation and Analysis Workflow for Mycotoxin Residues in Different Food Matrices
www.gerstel.de/pdf/p-gc-an-2013-10.pdf

Automated Derivatization, SPE Cleanup and LC/MS/MS Determination of Glyphosate and Other Polar Pesticides
www.gerstel.de/pdf/p-gc-an-2013-09.pdf

Automated Sample Preparation and Analysis Workflows for Pesticide Residue Screenings in Food Samples using DPX-QuEChERS with LC/MS/MS
www.gerstel.de/pdf/p-gc-an-2013-08.pdf

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

GERSTEL Automated Liner Exchange (ALEX) and its Benefits in GC Pesticide Analysis
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-gc-an-2010-04.pdf

Improving MS Detection of Malachite Green in Fish Products using Automated SPE coupled with an LC/Ion Trap MS system
www.gerstel.de/pdf/p-gc-an-2007-05.pdf

Multi-Residue Method for the Determination of Five Groups of Pesticides in Non-Fatty Food Samples by Dual Stir Bar Sorptive Extraction (Dual SBSE) and Thermal Desorption GC-MS
www.gerstel.de/pdf/p-gc-an-2005-03.pdf