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.

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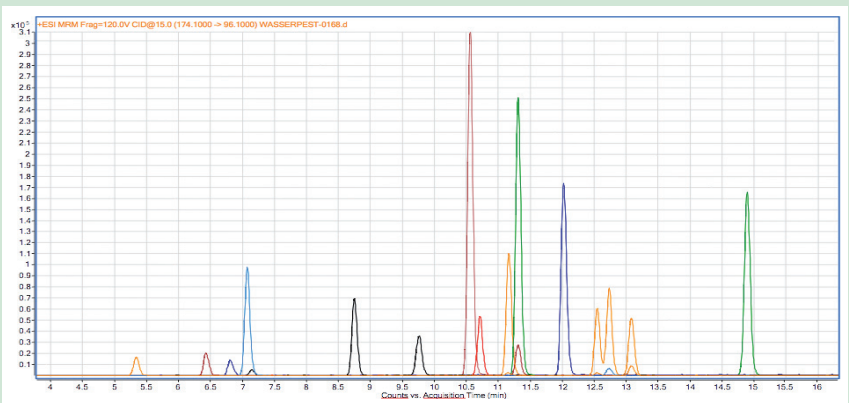
Analyzing food, water and soil for pesticide residues constitutes a significant part of the workload in laboratories that specialize in food safety and environmental analysis. Given the vast and increasing number of samples, efficiency is key and the strategy has to be automation – sensible and efficient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

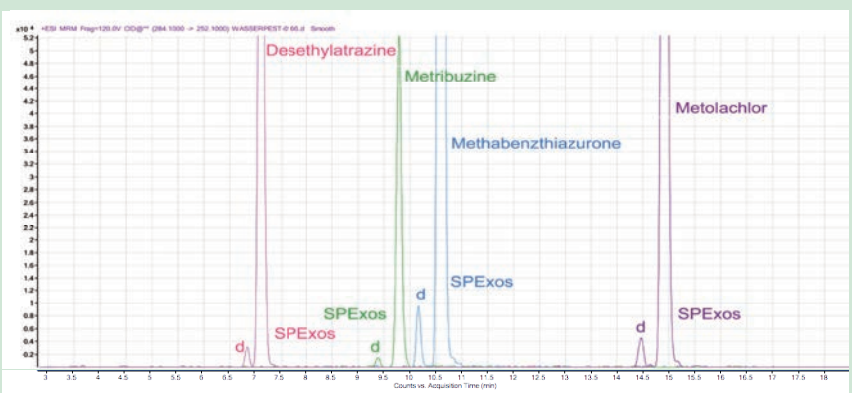
The weed killers (herbicides) most frequently used for crop protection in fruit production are based on phenyl urea or triazine compounds. Both these compound classes enter the plant through the roots and are transported to the chloroplast where they interfere with the process of photosynthesis, ultimately 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 highly sensitive HPLC-MS/MS system, but not all compounds can be determined

Pesticides are found in surface and ground water.

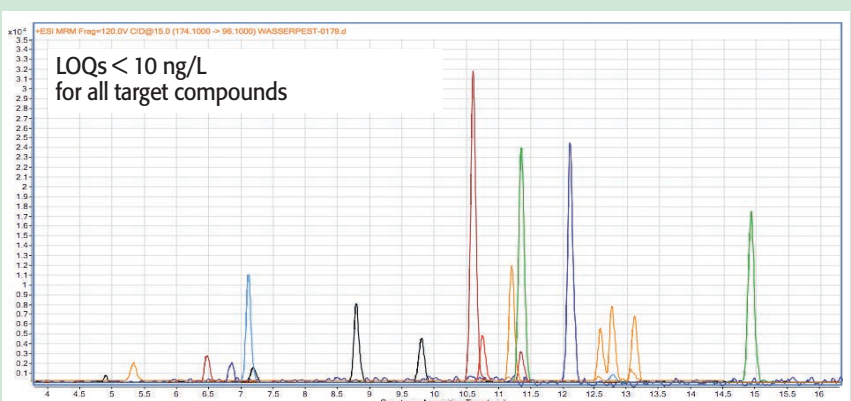
Sample preparation and LC-MS/MS analysis are performed simultaneously in parallel.



Standard mixture of the target analytes (100 ng/L) following extraction and cleanup in the SPE^{XOS} unit.



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 SPE^{XOS} process leads to retention time delays of up to 30 s.



Standard mixture of the target analytes (10 ng/L) following extraction and cleanup in the SPE^{XOS} unit. A Limit of Quantitation (LOQ) below 10 ng/L was achieved for each analyte.

this way, especially not early eluting compounds. 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 frequently leads to peak broadening. 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 on-line SPE concentration with the required

Automated sample prep workflow

[LOAD]
Load the SPE^{XOS} cartridge

[SPE PREP]
Condition with 4 mL of methanol

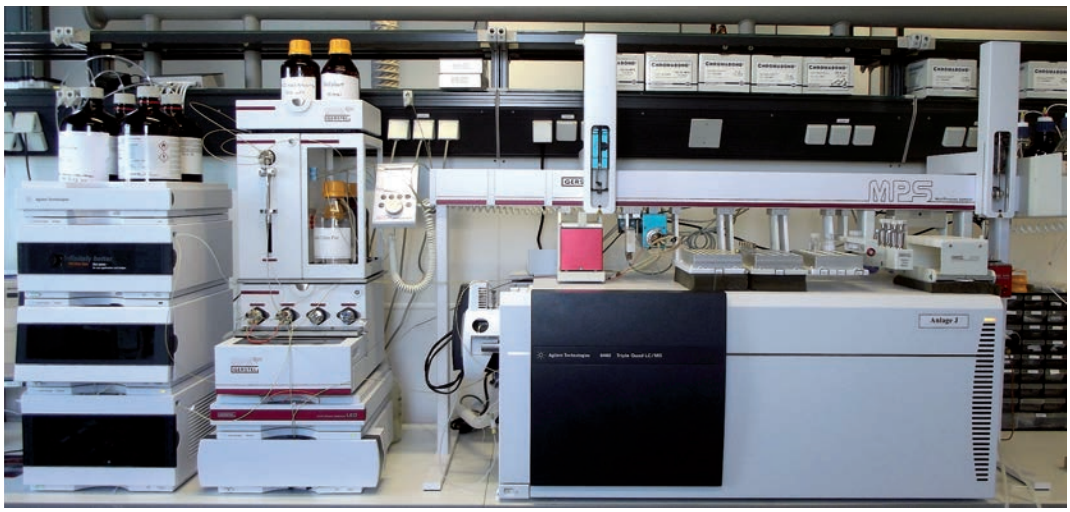
[SPE PREP]
Condition with 4 mL of water

[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.



The SPE^{XOS}-HPLC-QqQ-MS system used for the determination of phenylurea and triazine herbicides.

Action	MPS	Method / Value	Source	Vial	Destination	Vial
PREP Vials 1-5		No Overlap				
CARTRIDGE	Left MPS	LOAD	Left Rack		Left Clamp	
SWITCH INJ	Left MPS	Active			LC Vlv1	
SPE PREP	Left MPS	Cond MeOH 4000µl				
SPE PREP	Left MPS	Cond_AquaUltraPure 4000µl				
ADD	Left MPS	Sample Introduction 1000µl-10ml/Vial	Tray2,VT32-10		LC Vlv1	
SWITCH INJ	Left MPS	Standby			LC Vlv1	
SPE PREP	Left MPS	Rinse with 1500µl AquaUltraPure				
SWITCH INJ	Left MPS	Active			LC Vlv1	
SPE PREP	Left MPS	Valve Switch and Elution with LC Pump				
INJECT	Left MPS	WATER_PEST-SPExos-0µl.mth	Tray2,VT32-10		LC Vlv1	
ADD	Left MPS	Wash valve MeOH	SFS2Wsh1		LC Vlv1	
ADD	Left MPS	Wash valve H2O	SFS2Wsh2		LC Vlv1	
END						

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.

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.

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: 28 min.

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:	5500 V
Nozzle:	300 V

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 instrument method.

Results and discussion

A method is only useful if it can prove itself in practiceal use. The idea of using online SPE for analyte concentration and as a 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, methabenz-thiazurone, chloridazone, atrazine, metribuzine, chlorotolurone, isoproturone, metamitron, desethylatrazine and desisopropylatrazine.

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