



Determination of Trace Level Herbicide Metabolites with a Customized Agilent 1200 Infinity Series Online SPE System in Combination with the Agilent 6490 Triple Quadrupole MS

A comparison between online SPE and direct on column injection

Application Note

Environmental

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Abstract

This Application Note demonstrates the use of a customized Agilent 1200 Infinity Series Online SPE System for the determination of nonregulated herbicide metabolites in water. The instrumental setup was designed to enable online SPE as well as direct injection under high pressure UHPLC conditions in one system. The results for the determination of three typical nonregulated herbicide metabolites in online SPE and direct injection mode are discussed.



Agilent Technologies

Introduction

Today, plant protection is an essential part of modern agriculture. The maximum concentration of, for example, herbicides in the environment, as well as in food are strongly regulated and controlled. According to the requirements of the European Union drinking water directive 98/83/EC, the current regulation demands a limit of detection (LOD) of 25 ng/L (25 ppt) for all pesticides¹.

It has been shown that this requirement can even be exceeded by an online SPE LC/MS system with automated enrichment as well as direct injection capability^{2,3}.

If herbicide compounds are in a natural environment, they undergo decomposition by metabolism in plants and microorganisms. The occurring metabolites are often biologically active and, therefore, regulated and controlled as the originating compounds. Due to their less hazardous effect on humans, some metabolites are not regulated (nonregulated metabolites, nrM). Conversely, there are health guidelines (gesundheitliche Orientierungswerte, GOWs) for the maximum amount of nrMs in drinking water. This is especially driven by a hygienic point of view, because, if nrMs are in drinking water, it presents strong evidence showing a breakthrough of surface water into the drinking water source, for example, ground water.

This Application Note demonstrates the measurement of carboxylic acid and sulfonic acid nrMs of the herbicides metazachlor, metolachlor, and dimethachlor. The recommended maximum GOW amount is typically between 1 and 3 ppb. The comparison of enrichment by an online SPE process and direct injection is discussed. A customized instrument setup for online SPE as well as direct injection under UHPLC conditions is described.

Experimental

Instrumentation

Customized Agilent 1200 Infinity Series Online-SPE solution system

Agilent 1290 Infinity Binary Pump with internal degasser (G 4220A)

Agilent 1290 Infinity Autosampler (G4226)

Agilent 1260 Infinity Standard Autosampler (G1329B) with 900 µL head (G1313-60007)

Agilent 1290 Infinity Flexible Cube (G4227A) with two 2-position/10-port valves (G4232B)

Agilent 1290 Infinity Thermostatted Column Compartment (G1316B) with column selection valve (G4234B)

MS-detection

Agilent G6490 Triple Quadrupole LC/MS with Agilent Jet Stream Technology

Analytical columns

Two Agilent ZORBAX Eclipse Plus C18, 2.1 x 150 mm, 3.5 µm (p/n 959763-902) (one in the SPE part of the instrument, the other one directly located at the column selection valve)

Trapping columns

2 x Guard Column Hardware Kit (p/n 820999-901)

Agilent PLRP-S Cartridges, 2.1 x 12.5 mm, 15-20 µm (p/n 5982-1271)

Software

Agilent MassHunter Data Acquisition Software for triple quadrupole mass spectrometer, Version 06.00.

Agilent MassHunter Optimizer Software, Version 06.00

Agilent MassHunter Qualitative Software, Version 06.00

Agilent MassHunter Quantitative Software, Version 05.02

HPLC Method for online SPE

Agilent 1290 Infinity Binary pump

Solvent A Water, 10 mM ammonium acetate

Solvent B ACN

Flow rate 0.4 mL/min

Gradient 0 minutes – 5 % B, 3.3 minutes – 5 % B, 10 minutes – 95 % B

Stop time 12 minutes

Post time 2 minutes

Agilent 1290 Infinity Thermostatted Column Compartment

Column temperature 40 °C

Position of column selection valve To SPE system, position #4

Agilent 1290 Infinity Flexible Cube

Right valve 2-position/10-port QuickChange valve head, alternating with trapping columns for SPE

Left valve 2-position/10-port QuickChange valve head, Position for sample load on SPE trapping columns

Pumping rate 1.5 mL/min

Solvent A1 Ammonium acetate 10 mM in Water

Solvent B1 ACN

Time table
0 minutes – pump 200 seconds, Solvent A1
3.3 minutes – right valve change position
4.3 minutes – pump 60 seconds, Solvent B1
5.6 minutes – pump 180 seconds, Solvent A1

In this setup, the system has the capability to work under UHPLC conditions with multiple selectable 1.8- μm particle columns as well as with a part for online SPE under Standard HPLC conditions (Figure 1). For this purpose, a column selection valve is located in the thermostatted Column Compartment and the complete online SPE system part is connected to this valve as well. Depending on the purpose, the instrument can be switched easily. Use the column selection valve to select the online SPE or 1.8- μm particle column, and switch off the modules that are not needed. If the online SPE part is used, the 1290 Infinity Autosampler is switched off but is still in the flow path, which adds a delay time of approximately 0.1–0.2 minutes for the flow rate (Figure 1). A detailed description of the online SPE LC part is given in References 2 and 3.

Agilent 1260 Infinity Standard Autosampler	
Injection volume	400 μL
Needle wash In vial with MeOH	
Sample temperature	Ambient
Standard tray with 100 positions	
2-mL screw cap vials (glass, p/n 8010-0011), screw caps (p/n 8010-0061)	
Agilent 1290 Infinity Well Plate Autosampler	
Present in the system, switched off, not used	

HPLC Method for direct injection

Agilent 1290 Infinity Binary Pump	
Solvent A and B	See the method for online SPE
Flow rate	0.4 mL/min
Gradient	0 minutes – 5 % B, 3.5 minutes – 60 % B, 3.6 minutes – 95 % B
Stop time	5 minutes
Post time	2 minutes

Agilent 1290 Infinity Thermostated Column Compartment	
Column temperature	40 $^{\circ}\text{C}$
Position of column selection valve	To Column #1

Agilent 1290 Infinity Flexible Cube	
Right valve	2-position/10-port QuickChange valve head, not used
Left valve	2-position/10-port QuickChange valve head,

Agilent 1290 Infinity Well Plate Autosampler	
Injection volume	10 μL
Sample temperature	10 $^{\circ}\text{C}$
Needle wash in flush port for 10 seconds with MeOH	
Vial plate	54 \times 2 mL

2-mL screw cap vials (see above)	
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Agilent 1260 Infinity Standard Autosampler	
Present in the system, switched off, not used	

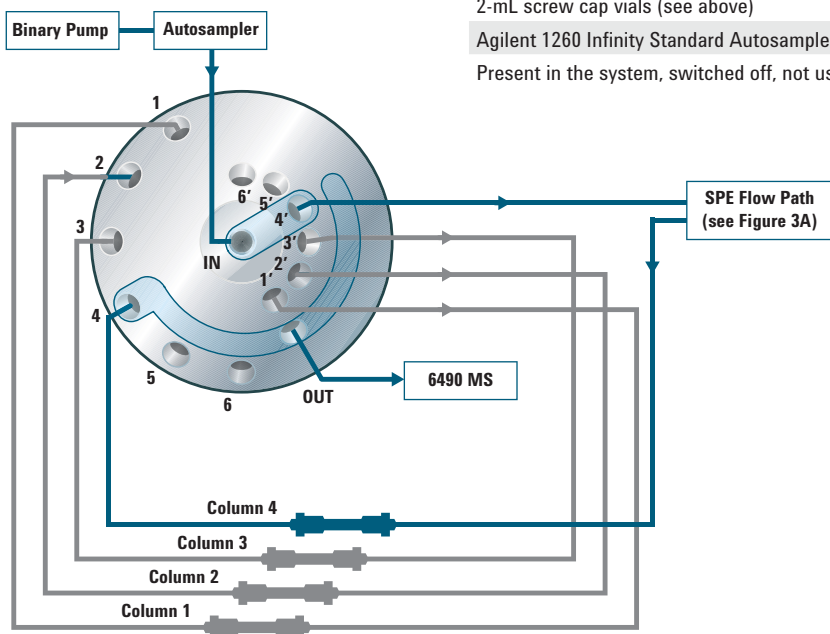


Figure 1. System set-up supporting direct analytical UHPLC-MS (1,200 bar) as well as online SPE-MS analysis.

In the online SPE system part, the 1290 Infinity Flexible Cube has two 2-position/10-port valves (Figure 2). The two trapping columns are located at the right valve and the left valve is plumbed to switch between direct on-column injection under standard HPLC conditions and injection on the trapping columns for SPE (Figure 3A). In addition to this, the Flexible Cube also contains the piston pump and the solvent selection valve for flushing the sample on the trapping columns and for the re-equilibration of those columns (Figures 3B and 3C). If the valve position for loading the sample on one of the SPE columns is selected, the piston pump inside the Flexible Cube is connected to the standard autosampler to flush the sample directly onto one trapping column (SPE 1) while the other trapping column (SPE 2) is connected to the analytical pump and is eluted in backflush mode onto the analytical column (Figure 3B). After loading the trapping column with sample, the right 2-position/10-port valve is switched and thus the positions of the trapping columns are exchanged (Figure 3C). Now, the LC pump delivers the gradient to elute the enriched analytes from the trapping column (SPE 1) onto the analytical column. Simultaneously, the trapping column (SPE 2) which had been eluted in the previous run is further cleaned and reconditioned by a purging procedure. This cleaning procedure is done by the piston pump of the Flexible Cube with the cleaning solvents selectable by the solvent selection valve.

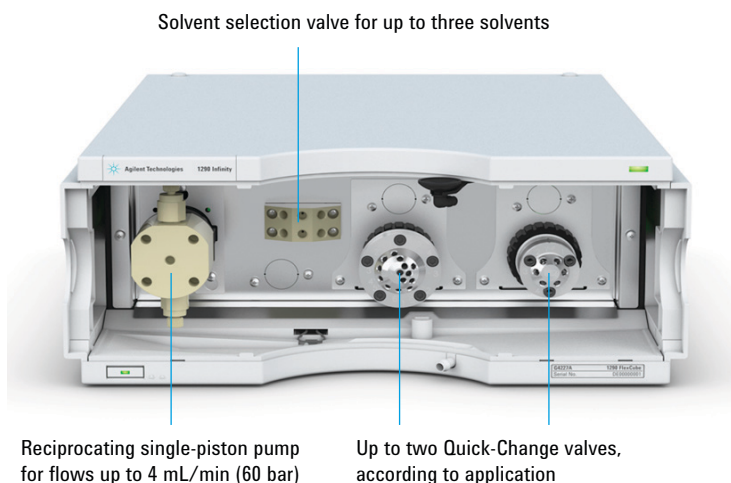


Figure 2. The Agilent 1290 Infinity Flexible Cube is an additional module to the Agilent 1290/1260 Infinity LC system, containing two Agilent 1200 Infinity Series Quick-Change valves, a piston pump, and a solvent selection valve.

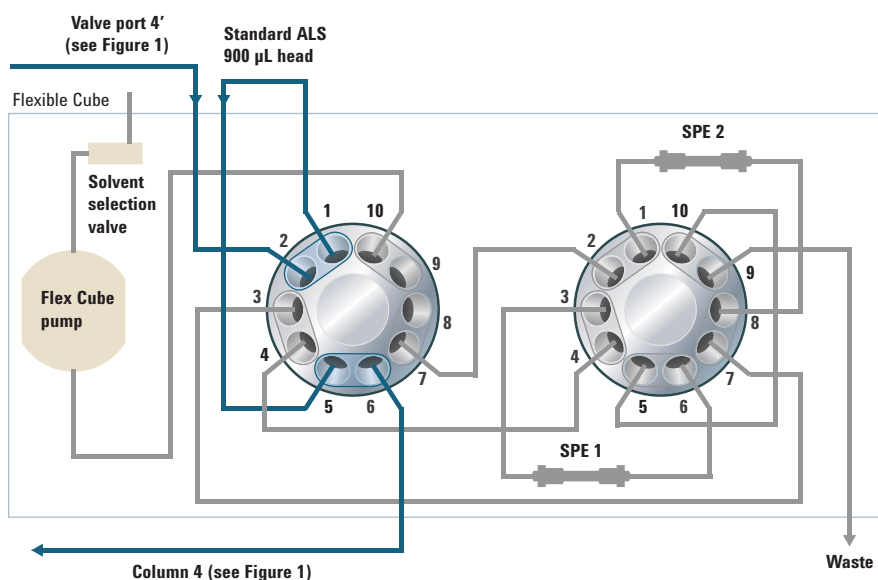


Figure 3A. Details of online SPE path plumbed for direct on column injection under standard HPLC conditions without trapping on SPE columns.

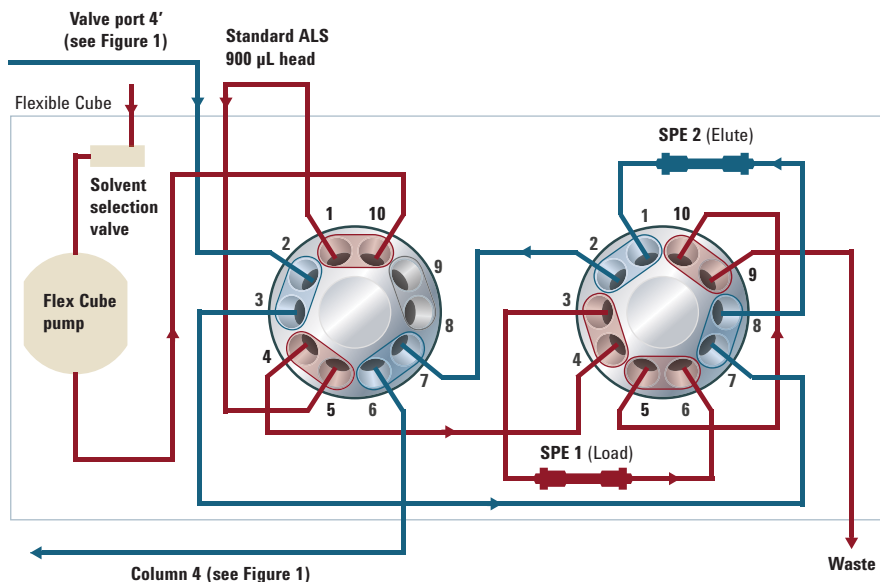


Figure 3B. SPE 1 is loaded with sample from the ALS through the Flex Cube pump.

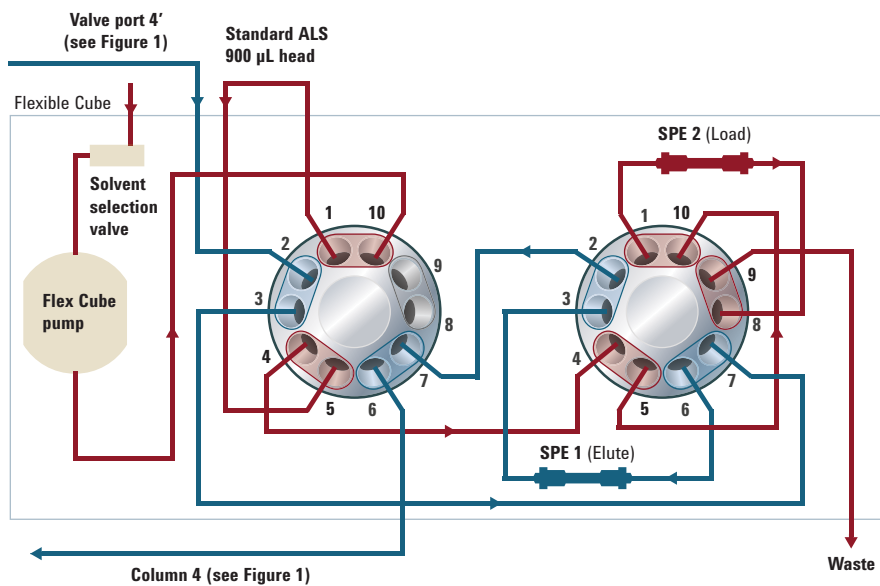


Figure 3C. While SPE 2 is re-equilibrated, SPE 1 is eluted on Column #4 (backflushed).

The MRM triple quadrupole MS method was developed by means of the MassHunter optimizer software and flow injection of individual pesticide standard solutions (10 mg/L) into the mass spectrometer. The optimization was done to find the optimum collision energies for the fragmentation to the quantifier and qualifier ions (Table 1). The optimum parameters for the Agilent Jet Stream were found using the source optimizer software.

Chemicals

All solvents and buffers were LC/MS grade. Acetonitrile and water was purchased from Fisher Scientific, Germany.

All pesticide standards were purchased from Dr. Ehrenstorfer GmbH, Germany at a concentration of 100 mg/L in acetonitrile.

Triple quadrupole MS method

Agilent Jet Stream thermal gradient focusing technology	
Gas temperature	140 °C
Gas flow	18 L/min
Nebulizer	40 psi
Sheath gas temperature	380 °C
Sheath gas flow	12 L/min
Capillary	4,000 V
Nozzle	300 V
Polarity	positive

Table 1. Optimized MRM conditions for each individual compound for optimum collision energies for the fragmentation to the quantifier and qualifier ions.

Compound	Precursor ion	Product ion	CE	CAV	Dwell
Metolachlor sulfonic acid	330	298	15	1	25
	330	202	32	6	25
Metolachlor acid	280	248	11	3	25
	280	73	20	3	25
Metazachlor sulfonic acid	324	134	29	2	25
	324	69	8	7	25
Metazachlor acid	274	134	23	2	25
	274	69	10	3	25
Dimethachlor sulfonic acid	302	270	14	2	25
	302	132	34	3	25
Dimethachlor acid	252	220	10	3	25
	252	105	35	3	25

Results and Discussion

For the comparison of direct injection under UHPLC conditions versus online SPE, calibrations for all herbicide metabolites were done in direct injection as well as SPE mode. For the direct injection, nine calibration points from 1,000 ng/L down to 3.906 ng/L (dilution factor 1:2) were acquired. The level showing a signal-to-noise (S/N) ratio of 10 was used as the limit-of-quantification (LOQ) and the LOD was calculated downwards from this point to the S/N ratio of 3. For example, metolachlor acid showed a LOQ of 3.906 ng/L for direct injection (Figure 4). For the calibration of metolachlor sulfonic acid, eight points of the calibration were used and the LOQ was found at 7.8125 ng/L (Figure 5).

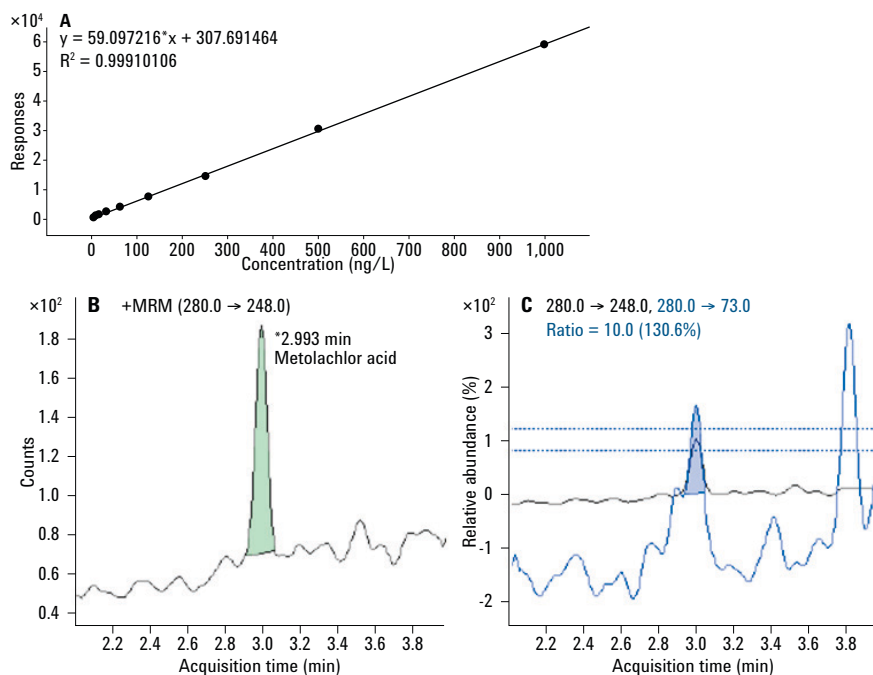


Figure 4. Calibration of metolachlor acid for direct injection (A), quantifier transition (B), and quantifier-qualifier-ratio (C).

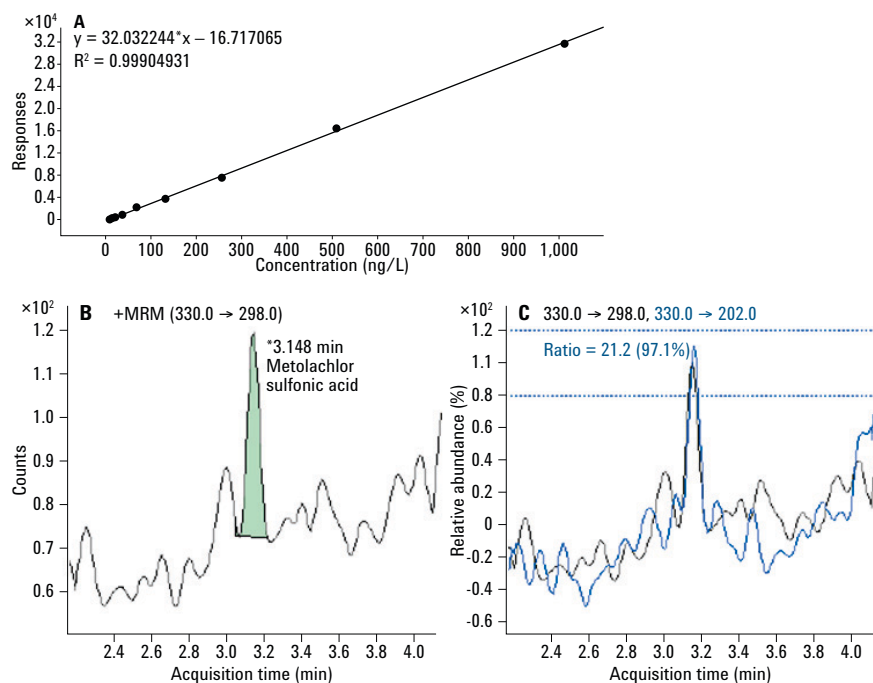


Figure 5. Calibration of metolachlor sulfonic acid for direct injection (A), quantifier transition (B), and quantifier-qualifier-ratio (C).

For online SPE, calibrations were done at a level of 1/10 of the amount used for direct injection. The calibrations started at 100 ng/L over nine levels down to 0.3906 ng/L (dilution factor 1:2). For example, in the SPE mode, metolachlor acid showed an LOQ of 0.3906 ng/L (Figure 6). For the calibration of metolachlor sulfonic acid, nine points of the calibration were used and the LOQ was found at 0.3906 ng/L (Figure 7).

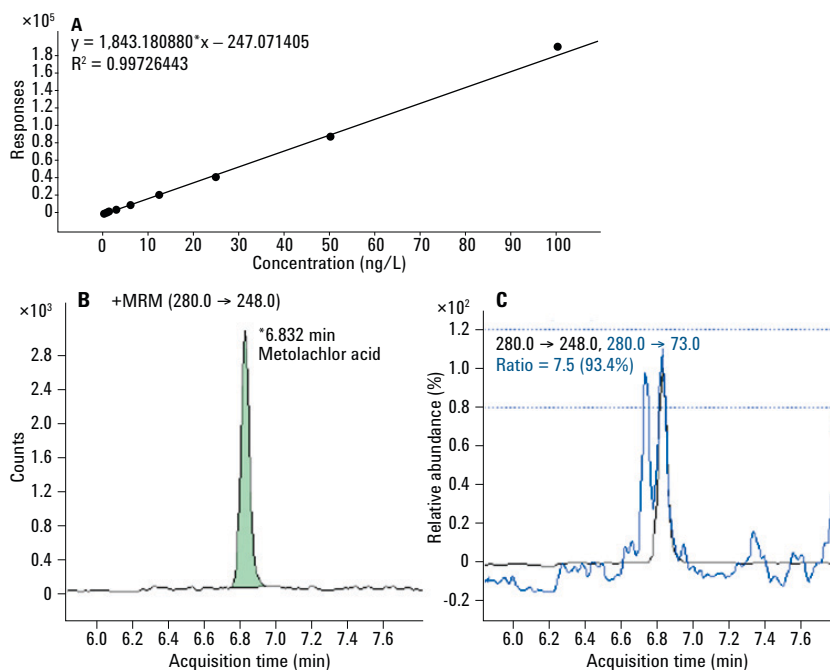


Figure 6. Calibration of metolachlor acid for online SPE (A), quantifier transition (B), and quantifier-qualifier-ratio (C).

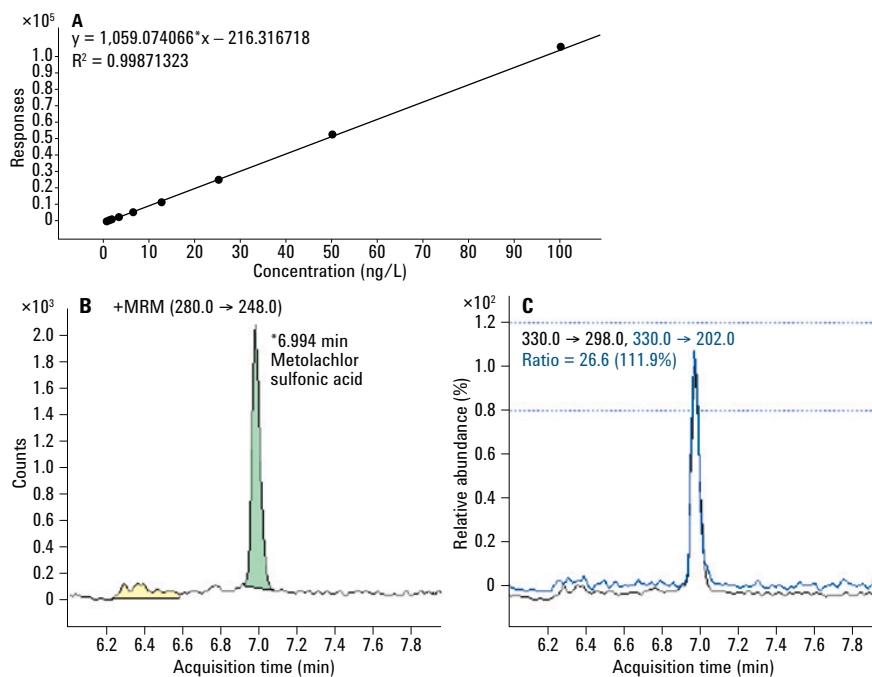


Figure 7. Calibration of metolachlor sulfonic acid for online SPE (A), quantifier transition (B), and quantifier-qualifier-ratio (C).

The measured LOQs, calculated LODs, and linearity coefficients are outlined in Table 2.

For the determination of the recovery of the SPE process, the results of both methods were compared. A mixture of standards was injected directly (10 μL at 1 ng/mL) and on the SPE column (100 μL

at 0.1 ng/mL). The online SPE process took 5 minutes to flush the sample on the SPE column. The gradient time was the same for both experiments. For the more nonpolar compounds, a nearly complete recovery can be seen when the direct injection is compared to the SPE method (Figure 8). Due to the stronger focusing effect on the SPE column,

the peaks are more narrow and higher but have the same area as the direct injection. The more polar compounds suffer from a certain loss and, therefore, need optimization of the trapping process including sample volume and washing volumes after sample introduction.

Table 2. Comparison of LODs, LOQs, and calibration coefficient of all used herbicide metabolites in direct injection mode versus online SPE mode.

Compound	LOD direct injection (ng/L)	LOD SPE (ng/L)	LOQ direct injection (ng/L)	LOQ SPE (ng/L)	Linearity coefficient direct injection	Linearity coefficient SPE
Dimethachlor acid	2.343	0.09	7.8125	0.3	0.9987	0.9993
Dimethachlor sulfonic acid	2.343	0.15	7.8125	0.5	0.9993	0.9982
Matazachlor acid	4.687	0.57	15.625	1.9	0.9998	0.9965
Metazachlor sulfonic acid	1.171	0.15	3.906	0.5	0.9990	0.9987
Metolachlor acid	1.171	0.03	3.906	0.1	0.9991	0.9972
Metolachlor sulfonic acid	2.343	0.09	7.8125	0.3	0.9990	0.9987

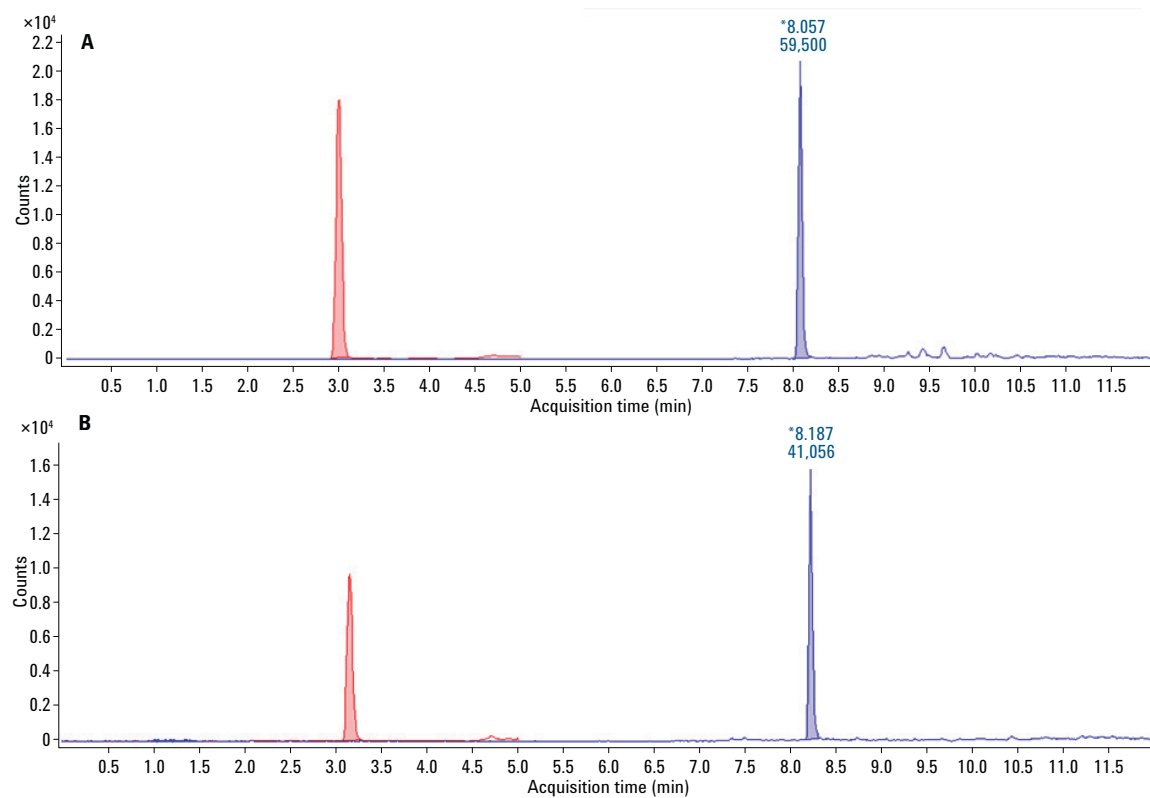


Figure 8. Injection of 10 μL standard mixture at 1 ng/mL for direct injection (red) and 100 μL of a standard mixture at 0.1 ng/mL by Online-SPE (blue) – 10 pg on column each. A) Metolachlor acid, B) Metolachlor sulfonic acid.

Conclusion

This Application Note shows a customized system which is able to work under both UHPLC conditions and online SPE conditions. The system was used for the determination of nonregulated herbicide metabolites in drinking water by SPE. LOQs far below 1 ng/L (1 ppt) were achieved. The comparison to the direct injection revealed that the SPE process offers increased sensitivity by a factor of 10.

References

1. European Union Drinking Water Directive 98/83/EC http://ec.europa.eu/environment/water/water-drink/index_en.html.
2. Agilent Application Note, Publication number 5991-1738EN.
3. Agilent Application Note, Publication number 5991-2140EN.

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Published in the USA, September 1, 2013
5991-2776EN

