Reduction of Matrix Effects with the Agilent 1200 Infinity Series Online SPE Solution

## Application Note

Environmental

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Abstract

The use of organic compounds, such as hormones, pesticides, and pharmaceuticals, leads to contamination of water resources worldwide. Therefore, monitoring these compounds is important. However, due to coeluting matrix components, a reliable quantification is critical.

With online solid-phase extraction (SPE), matrix effects are reduced because the matrix is not retained on the SPE cartridge and is flushed to waste. In this Application Note, tap, ground, river, and ultrapure water were spiked with pesticides and analyzed with a fast and automated online SPE method coupled to a triple quadrupole mass spectrometry system.

To show the reduction of matrix effects, online SPE and direct injection methods were compared. Additionally, for the evaluation of a 75-ng/L spike in river water, a standard addition calibration and external calibration were prepared. With online SPE we show that matrix effects are reduced and, therefore, an external calibration is sufficient to quantify compounds in environmental water.
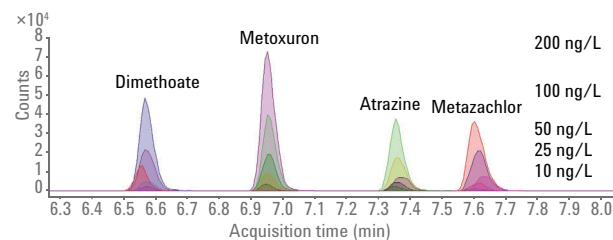
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Introduction

Quantification of pesticides in various environmental waters is important, but coeluting matrix has a negative impact on the exact quantification. Coeluting matrix can lead to ion suppression or ion enhancement, which results in under or over-examination of analytes.

The common analytical method is offline solid-phase extraction (SPE), where pesticides contained in large water volumes (> 1 L) are trapped on cartridges and afterwards analyzed and quantified with LC/MS/MS. A very fast and effective sample preparation and alternative to offline SPE is online SPE, where just small sample volumes are needed (< 1 mL). Additional advantages are little sampling handling and lower costs as well as reduction of hazardous waste compared to offline SPE[2,4].

This Application Note is aimed at the quantification of pesticides spiked into different environmental waters and measured with direct injection and online SPE. The same methods were applied for direct injection and online SPE for comparison. The performance was validated and good linearity ($R^2 > 0.991$) and great precision were obtained for online SPE and direct injection. No carryover was observed for online SPE in all water samples. Matrix effects were determined for the majority of compounds in ground water and river water by comparing spiked ultrapure water and environmental waters. In the example of aminocarb, the changes of calibration slopes and intercepts were evaluated.

To illustrate the reduction of matrix effects, river water was spiked with a suite of pesticides (75 ng/L). To determine the spiked concentration and the reduction of matrix effects with online SPE, a standard addition and an external calibration were prepared. By comparing the results of the two calibration approaches, it was observed that the reduction of matrix effects with online SPE is sufficient. Thus, there is no need for a time-consuming standard addition calibration because an external calibration is reliable to quantify compounds in environmental water.

Experimental

System configuration

The Agilent 1290 Infinity Flexible Cube is the heart of the Agilent 1200 Infinity Series Online SPE solution. The 1290 Infinity Flexible Cube hosts two 2-position/10-port valves for enabling fast method change between direct injection and online SPE. Two trapping columns are alternately in use for time saving and higher sample throughput. The piston pump and solvent selection valve can draw up to three different solvents, and are responsible for loading, cleaning and reconditioning of the cartridges[1].

With the loading process, matrix components not retained on the cartridge are flushed to waste[4]. The PLRP-S (cross-linked styrene divinylbenzene polymer) cartridges have a long life time, even with samples with high matrix content (> 200 injections). PLRP-S is a highly homogeneous material that is free from silanol groups and heavy metal ions, and is perfectly suited for the enrichment of medium and nonpolar pesticides.

Instrumentation

All experiments were carried out on an Agilent 1200 Infinity Series Online SPE system comprising:

- Agilent 1290 Infinity Flexible Cube (G4227A), equipped with:

  - Online SPE Starter Kit (G4742A), including one 2-position/10-port valve, 600 bar, capillaries, cartridge holder and cartridges

  - Online SPE Direct Injection Kit (G4744A), including one 2-position/10-port valve, 600 bar, and capillaries

- Agilent 1260 Infinity Binary Pump (G1312B) with LAN card (G1369C)

- Agilent 1260 Infinity Standard Autosampler (G1329B) with 900 µL head (G1313-60007) and Agilent 1290 Infinity Thermostat (G1330B)

- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)

- Agilent 6490 Triple Quadrupole LC/MS System (G6490A) with Agilent Jet Stream technology

Software

- Agilent MassHunter Workstation Software - Data Acquisition for Triple Quadrupole Mass Spectrometer, version B.07.00

- Agilent MassHunter Optimizer Software, version B.06.00

- Agilent MassHunter Source and iFunnel Optimizer Software, version B.06.00

- Agilent MassHunter Qualitative Software, version B.06.00

- Agilent MassHunter Quantitative Software, version B.07.00

Tap water was taken from Waldbronn, Germany, surface water from the Rhein river, and groundwater from Karlsruhe, Germany. The samples and standards were stored at 5 °C, and were centrifuged for 5 minutes at 5,000 rpm prior to injection.

The water samples were spiked with different concentrations of pesticides by diluting a stock solution of 100 µg/L. A five-level calibration curve (10, 25, 50, 100, and 200 ng/L) was prepared in ultrapure, tap, ground, and river water. A standard addition calibration was prepared in river water (75 ng/L spike plus additionally 10, 25, 50, 100, and 200 ng/L).

Chemicals

All solvents were LC/MS grade. Acetonitrile was purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-μm membrane point-of-use cartridge (Millipak). Formic acid was purchased from Agilent Technologies (p/n G2453-85060).

Results and Discussion

Performance data of the online SPE method

Linearity is shown with the coefficient of determination (R^2) and was good for all compounds for online SPE and direct injection methods with $R^2 > 0.991$ (Table 1). Area precision for online SPE methods and two alternating used cartridges showed area relative standard deviations (RSDs, n = 8) of usually < 10 % in river water and even less in Milli Q water. Retention time (RT) RSDs were in the range of 0.1 to 0.2 %. Additionally, recovery of the enrichment process was determined by comparing peak areas of a 50 ng/L (n = 8) spike in ultrapure water with direct injection to the same amount of analyte obtained in online SPE mode. Generally, a recovery between 70 to 130 % was considered as acceptable[4], and all measured compounds are within this range (Table 1).

No carryover was observed for online SPE measurements, which indicated that the cleaning procedure with the 1290 Infinity Flexible Cube is sufficient to wash any remaining analytes from the cartridge. However, for direct injection, dimethoate showed carryover at 0.07 % compared to the highest standard.

Chromatographic method for direct injection and online SPE

Chromatographic conditions	
Column	Agilent ZORBAX SB-AQ, 2.1 × 150 mm, 3.5 μm (p/n 830990-914)
Column temperature	40 °C
SPE cartridge	PLRP-S Cartridges, 2.1 × 12.5 mm, 15-20 μm (p/n 5982-1271)
Flow rate	0.5 mL/min
Mobile phase	A) water + 0.1 % formic acid B) ACN + 0.1 % formic acid
Gradient	0 to 3 minutes 0 % B, 3 to 9 minutes 0 to 95 % B, 9 to 14 minutes 95 % B, 14.1 to 22 minutes 100 % A
Injection volume	200 μL
Sample temperature	5 °C

Additional method configuration for an Agilent 1290 Infinity Flexible Cube and online SPE

Agilent 1290 Infinity Flexible Cube timetable		
Time (min)	Function	Parameter
0	Pump volume	Pump 2 mL at 1 mL/min water
2.3	Right valve position change	Increase valve position
3	Pump volume	Pump 6 mL at 1.5 mL/min ACN
7.5	Pump volume	Pump 7 mL 1.5 mL/min water

Table 1. Performance data for online SPE method (retention time, recovery, area RSD in river water and R^2).

Pesticide	Retention time (min)	Recovery (%)	Area RSD % in river water (n = 8)	R^2
Aminocarb	5.34	75.0	7.3	0.996
Dimethoate	6.55	77.4	5.3	0.995
Metoxuron	6.96	98.5	7.0	0.999
Carbofuran	7.24	120.1	7.7	0.991
Atrazine	7.37	92.7	5.1	0.991
Metosulam	7.56	85.0	10.2	0.996
Metazachlor	7.67	107.5	8.3	0.995
Diazinon	8.44	86.3	16.4	0.995

Determination of matrix effects with online SPE and direct injection

As the recovery for the enrichment process was confirmed (Table 1), it is important to quantify also matrix effects when using LC/MS with electrospray ionization. Therefore, a calibration in ultrapure water and a matrix matched calibration in river and ground water were prepared and the slopes of both calibration curves were compared. It was assumed that in ultrapure water no matrix effects were present.

Matrix effects were observed for both environmental (river and ground water) and for most compounds, which is illustrated by comparing matrix matched standards to a calibration in ultrapure water. The analysis of pesticides in ground and river water was performed with direct injection and online SPE (same sample and same method) to compare and determine the matrix effects. Figure 1 and Figure 2 illustrate the example of aminocarb. An external calibration curve (blue) was prepared in ultrapure water and compared to a matrix matched calibration in ground (green curve) and river water (red curve).

There are two main matrix effects; a variable signal change proportional to the analyte amount, or a constant signal change independent from the analyte amount. A variable signal change is also called rational effect, and arises when the size of the signal derived from the analyte is affected by nonanalyte constitutes of the test solution. The size of the matrix effect is usually proportional to the signal and changes the slope of the calibration function, but not its intercept[5]. On the example of aminocarb, the slope for direct injection changes for both waters, up to 22 %, and indicates a rational effect. For online SPE, the slope for both waters changes comparatively less, at 15 %.

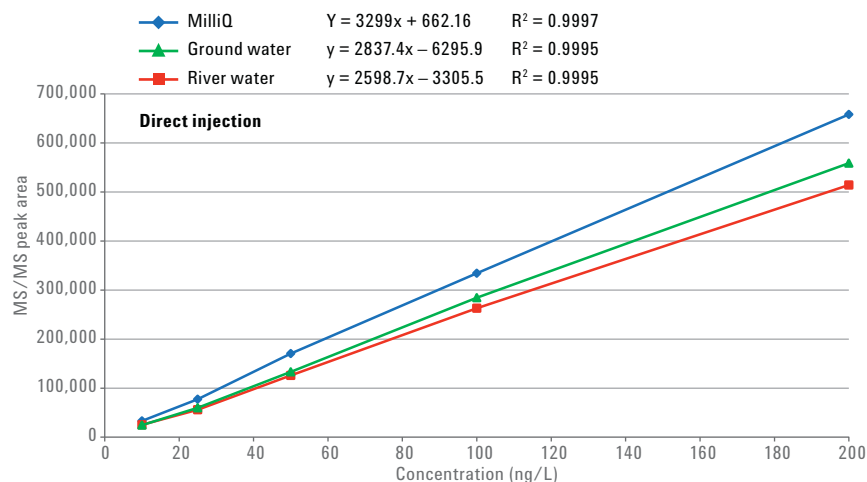|		
◆ MilliQ	Y = 3299x + 662.16	R^2 = 0.9997
▲ Ground water	y = 2837.4x − 6295.9	R^2 = 0.9995
■ River water	y = 2598.7x − 3305.5	R^2 = 0.9995

Figure 1. Matrix effect for aminocarb in river water (red, 27 % ion suppression) and ground water (green, 22 % ion suppression) calibrated in ultrapure water (blue) and measured with direct injection.

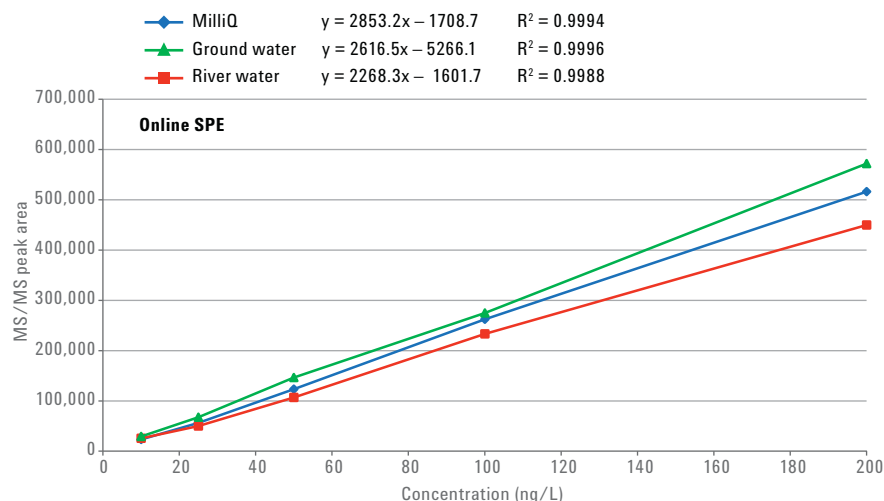|		
◆ MilliQ	y = 2853.2x − 1708.7	R^2 = 0.9994
▲ Ground water	y = 2616.5x − 5266.1	R^2 = 0.9996
■ River water	y = 2268.3x − 1601.7	R^2 = 0.9988

Figure 2. Matrix effect for aminocarb in river water (red, 16 % ion suppression) and ground water (green, 7 % ion enhancement) calibrated in ultrapure water (blue) and measured with online SPE.

A constant signal change is called translational effect and is produced by coeluting substances present in the matrix but not by the analyte (background interference). It affects the intercept of a calibration function, but not its slope. Here, aminocarb shows a translational effect and appears also for online SPE and direct injection.

For aminocarb, both types of matrix effects led to overall ion suppression in river water of 27 % for direct injection and just 16 % for online SPE. For ground water, direct injection showed 22 % ion suppression and online SPE 7 % ion enhancement. In summary, both types of matrix effects can have the same effect on the observed signal, and it is not unusual for both effects to be present simultaneously[3,5]. The other compounds, which are not shown here, also indicate the same effects with more or less intensity. For most compounds, less matrix effects were detected with online SPE measurements compared to direct injections. Aminocarb is an interesting example that shows different matrix effects with direct injection and online SPE. While direct injection leads to ion suppression, online SPE showed negligible ion enhancement. This shows that with the enrichment process parts of the matrix, such as salts and minerals, are flushed out and matrix components cannot interfere with the analyte.

Standard addition calibration and external calibration

When matrix effects are sample dependent, it is recommended to use the standard addition calibration (SC)[3]. For the following experiments, river water was spiked with 75 ng/L pesticide standard. Additionally, for standard addition calibration five aliquots of increasing standard concentration were added (10, 25, 50, 100, and 200 ng/L). For comparison, an external calibration (EC) was prepared in drinking water, as an EC is less time and labor consuming, especially when many samples have to be measured. Finally, the results of EC and SC calibration were compared and the spiked samples were evaluated. The measured concentration of EC and SC is shown in Figure 3 as well as a ± 20 % tolerance of 75 ng/L spike.

Figure 3 shows the results for external calibration and standard addition calibration for online SPE. In summary, the external and standard addition calibration achieved good results and precision for the determination of 75 ng/L spiked in river water. The results were comparable for both calibration methods, which indicate that with online SPE an external calibration is perfectly suited for the quantification of organic compounds in environmental water and the standard addition approach is not mandatory. Additionally, the standard addition method can correct rational effects only[5].

The online SPE enrichment and matrix removal process can still be optimized. Different flow rates for the loading procedure can have a positive impact on the sensitivity and different loading volumes can be chosen as well as loading solvents. Anumol and Snyder[4], for example, showed a better removal of matrix when adding 5 % of acetonitrile and 0.1 % acetic acid in the SPE loading water.
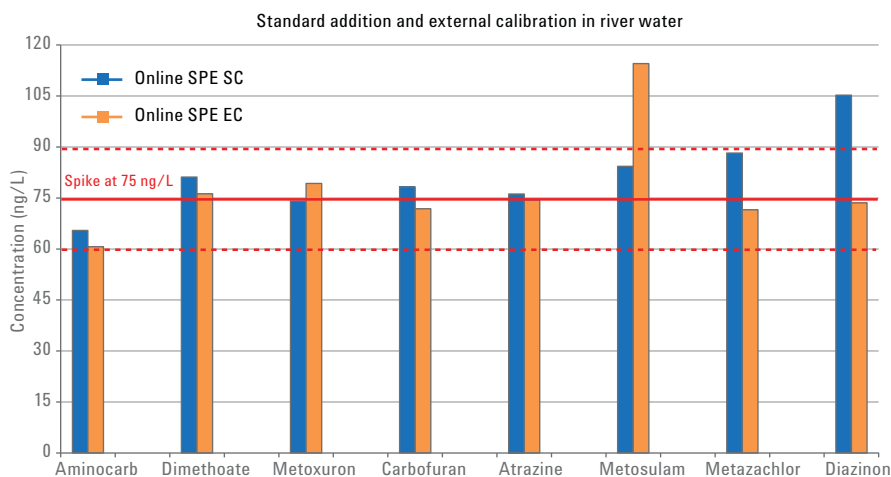

Figure 3. Comparison of the standard addition calibration to an external calibration in drinking water. A 75 ng/L amount of pesticides were spiked in river water.

Conclusion

This Application Note demonstrates the reduction of matrix through the enrichment process of the online SPE. During the loading procedure, different solvents can be selected to wash matrix to the waste. With online SPE, standard addition calibration is not necessary, and an external calibration is sufficient to quantify organic compounds in environmental water, which saves time and labor. No carryover was determined, which indicates a successful cleaning procedure of the cartridges with the Agilent 1290 Infinity Flexible Cube. The recovery of the online SPE analysis was evaluated and showed good results as well as linearity, area and retention time precision.

References

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