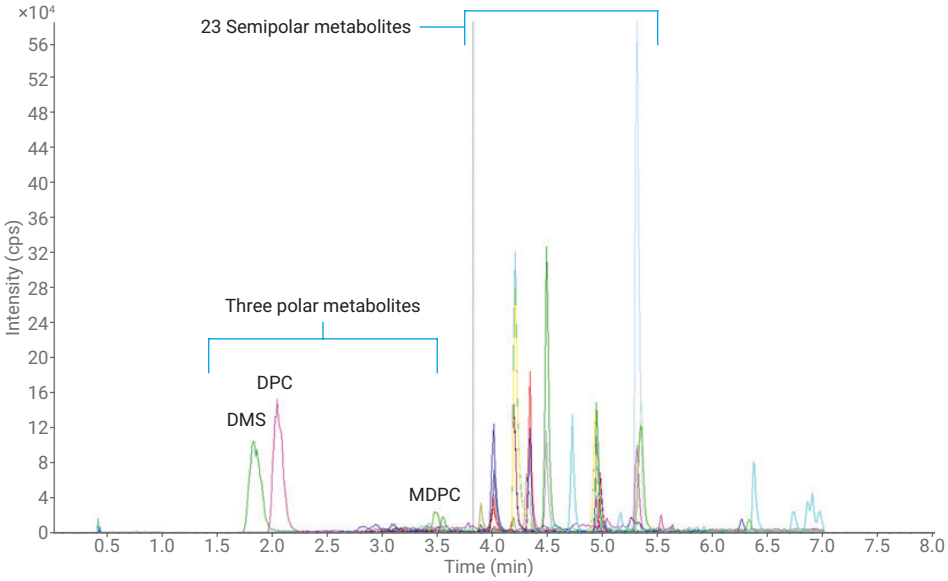
Application Note

Environmental

Rapid Quantification of Polar and Semipolar Pesticide Metabolites with Combined Online SPE and Direct Injection



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Abstract

The sensitive analysis of polar and semipolar compounds in one analytical run is challenging. Enrichment methods, such as solid phase extraction (SPE), are usually used to increase the sensitivity of an LC/MS system. However, with a conventional online SPE setup, it is not possible to analyze polar and nonpolar compounds simultaneously, because polar compounds rapidly pass through the SPE cartridge. To overcome the time-consuming need of performing two separate analytical runs, the online SPE setup was extended with an additional loop on each SPE cartridge. This enabled a direct injection for the polar compounds combined with an enrichment of the semipolar compounds on the cartridge. This Application Note develops an all-in-one online SPE triple quadrupole MS method to analyze a suite of 26 pesticide metabolites of different polarities in drinking water, with quantitation limits in the sub-ng/L range.

Introduction

For the sensitive analysis of pesticides and pesticide metabolites in water, LC/MS is the instrumentation of choice. Fully automated online SPE coupled to an LC/MS system is well suited for the analysis of all kinds of aqueous samples. This setup is known to reduce time and labor in sample preparation, and to enhance the sensitivity of the system significantly[1]. However, online SPE faces one challenge: reversed-phase chromatography is performed with nonpolar packing material (PLRP-S) to enrich a broad range of analytes. The affinity of polar compounds to a nonpolar stationary phase is weak, and these rapidly penetrate through the sorbent bed of the PLRP-S polymeric cartridge even with water as the loading solvent. This work's plan was to take advantage of the sensitivity enhancements of online SPE in combination with tandem mass spectrometry (MS/MS), and to develop a multiresidue method for the simultaneous analysis of as many compounds as possible over a wide polarity range.

To overcome the complexity of doing two separate analytical runs, one for the polar and one for the semipolar compounds, the online SPE setup in this work was modified. An additional 100-µL loop was attached in front of each SPE cartridge. After trapping 5 mL of the aqueous sample on the SPE cartridge, an additional injection volume of 100 µL was added to fill the loop attached to the SPE cartridge. After switching the loop and SPE into the analytical flowpath, the whole sample was backflushed onto the analytical column. While the semipolar analytes are enriched on the SPE cartridge resulting in a significant sensitivity enhancement, the highly polar analytes, which are not trapped on the SPE cartridge, can still be analyzed with direct injection in the same analytical run.

This Application Note was produced in cooperation with SGS Institut Fresenius GmbH to demonstrate the feasibility and usability of a customized online SPE setup for the analysis of polar and nonpolar compounds. Performance parameters such as linearity and area precision for different aqueous samples are shown and discussed. A limit of quantitation (LOQ) of 3 ng/L was achieved for most analytes in aqueous samples during method validation. Compared to direct injection with an injection volume of 50 µL, a sensitivity enhancement of up to 90 times was achieved for some compounds due to the high enrichment volume of 5.4 mL. For the highly polar analytes, an injection volume of 100 µL was sufficient to achieve the required detection limits. As an additional benefit, using online SPE provides better peak shape for the polar analytes compared to direct injection without SPE. An improved peak shape might be caused by enhanced mixing of sample and mobile phase in relation to the higher dead volume.

Experimental

Instrumentation

The Agilent InfinityLab Online SPE Solution comprised the following modules:

- Agilent 1260 Infinity II Multisampler (G7167A) with 900 µL loop and metering device, standard wash
- Agilent 1260 Infinity II Quaternary Pump VL (G7111A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity Flexible Cube (G4227A) with two valve drives:
  - Agilent Online-SPE Starter Set (G4742A): one 2-position/10-port valve with two additional 100 µL loops (p/n 8002-0807)
  - Agilent Online-SPE Direct Injection Kit (G4744A): one 2-position/10-port valve
- Agilent 1290 Infinity Valve Drive (G1170A) with Online SPE High Volume Injection Kit (p/n G4745A) with 2-position/6-port valve and a 5 mL loop
- Agilent 1290 Infinity Valve Drive (G1170A) with a 2-position/6-port valve (to switch between PAL and Multisampler)
- Triple Quadrupole Mass Spectrometer
- CTC PAL HTS-xt DLW

Software

- OpenLab CDS ChemStation Edition C.01.07

Chemicals

All solvents were MS grade. Acetonitrile (ACN) and methanol (MeOH) were purchased from Promochem (Wesel, Germany). Water, ammonium formate, and formic acid were purchased from VWR (Darmstadt, Germany). Standards were purchased from different vendors, such as VWR, Sigma (St. Louis, USA), and LGC (Wesel, Germany).

Samples and methods

Samples were bottled water (France), tap water (Germany), and surface water (Germany).

Direct injections were performed by the PAL sampler with an injection volume of 50 µL.

System configuration and principle of operation

One advantage of online SPE, in comparison with conventional offline SPE, is that the entire injection volume passes into the analytical column without extract evaporation. This means that much smaller sample volumes can be used compared to those in offline SPE[1].

In general, online SPE consists of three steps (see Table 1):

1. Loading the cartridge with sample
2. Eluting the cartridge with the analytical run
3. Cleaning and conditioning the cartridge

Online SPE chromatographic method	
Column	C18 Column, 2.7 µm, 100 × 3 mm
SPE cartridge	PLRP-S Cartridges, 4.6 × 12.5 mm, 15 to 20 µm (p/n 5982-1270)
Solvents	Analytical gradient pump: 1290 Infinity Binary Pump A) H_2O + 5 mM ammonium formate B) MeOH:ACN (50:50) + 5 mM ammonium formate
Gradient	0 to 18.5 minutes: 0 %B 18.5 to 19.50 minutes: 20 %B 19.5 to 24 minutes: 75 %B 24 to 24.90 minutes: 99 %B 24.90 to 26 minutes: 99 %B 26 to 26.20 minutes: 0 %B 26.2 to 28 minutes: 0 %B
Flow rate	500 µL/min
Temperature	45 °C
Detection	MS/MS
Injection	Injection volume: 6 × 900 µL on SPE cartridge and 100 µL in extended loop Sample temperature: 8 °C Needle wash: 3 seconds H_2O:MeOH (10:90) + 0.1 % formic acid
Enrichment method	0 to 18 minutes: 2 mL/min H_2O 18 to 22 minutes: 2 mL/min ACN (switch SPE) 22 to 24 minutes: 2 mL/min H_2O

Table 1. Online SPE method setup.

Run time	0–18 minutes	18–28 minutes
HPLC pump	Isocratic hold and post time for analytical column	Gradient run
Online SPE	Injection and loading SPE 1 with 6 × 900 µL plus 100 µL direct injection into loop	Cleaning and conditioning SPE 2

This study based the configuration of the system on the Agilent InfinityLab Online SPE system. This system includes the 1290 Infinity Flexible Cube, which hosts two 2-position/10-port valves for direct injection and online SPE. To save time, two SPE cartridges were installed and used in an alternating fashion: while one cartridge was being eluted, the other cartridge was cleaned and prepared for the next analytical run. To avoid carryover, the Multisampler and loop were cleaned simultaneously. For loading, cleaning and reconditioning, a 1260 Infinity II Quaternary Pump was used: this is recommended when working with heavy matrix, enabling the use of four solvents within the same method.

To upgrade the instrument for high-volume injection, an additional external valve drive (G1170A) can be installed next to the 1290 Infinity II Multisampler. The external valve hosts a 2-position/6-port valve to switch the sample loop (5 mL in this example) in or out of the flowpath (Figure 1)[2]. The 5-mL loop can be filled by repetitive draw-and-eject cycles. Eventually, even more than 5 mL can be injected; during the loading process, the sample loop is connected to the SPE cartridge, which prevents sample loss. Here, 6 × 900 µL were injected into the 5-mL loop and flushed onto the online SPE cartridge. Subsequently, 100-µL were injected to fill the sample loop attached to the SPE cartridge. For this customized online SPE setup, three online SPE kits were installed, plus two 100-µL loops, which were placed in front of every SPE cartridge (Figure 1). The total injection and loading time was 18 minutes.

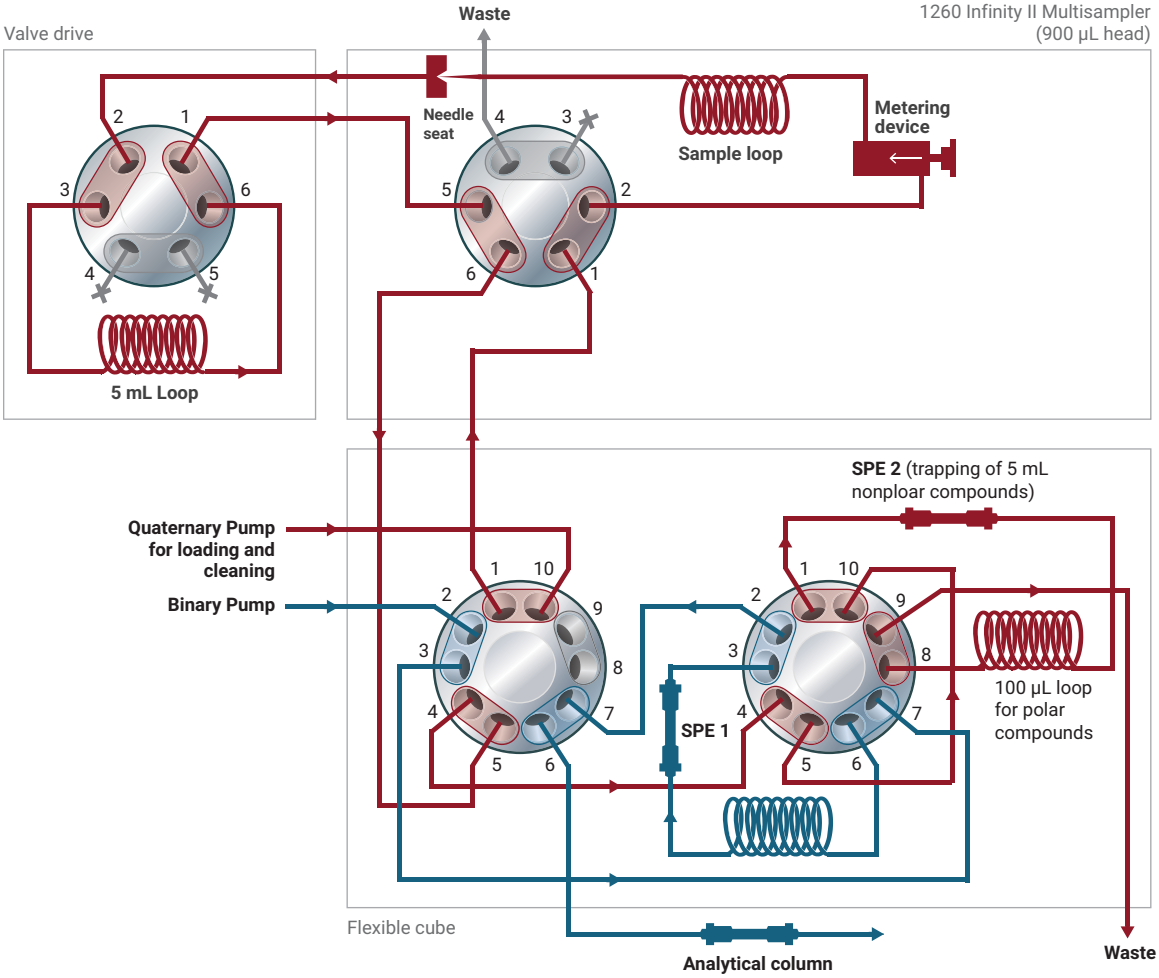

Figure 1. Online SPE setup with two alternating SPE cartridge, a 5-mL loop for high-volume injection and trapping of analytes, and additional 100-µL loops (in front of each SPE cartridge) to enable direct injection of polar compounds.

This setup enables online SPE enrichment and direct injection in one analytical run. In addition to the online SPE option with high-volume injection, the system can be used as a UHPLC instrument for direct injections, applying an additional autosampler (a PAL HTS-xt autosampler in this case). Sample batches containing online SPE including the Agilent 1260 Infinity II Multisampler, and sample batches containing the PAL HTS-xt autosampler, can easily be stacked without changing any hardware options or communication pathways with the use of an additional 2-position/6-port valve.

Results and Discussion

The challenge of analyzing numerous compounds within a wide polarity range is overcome by the described modified online SPE setup. Figure 2 shows a comparison of an online SPE chromatogram without and with the additional 100-µL direct injection (A and B, respectively). Without the added 100-µL direct injection after the SPE enrichment, the three polar metabolites included in the suite cannot be analyzed since they rapidly penetrate through the SPE cartridge.

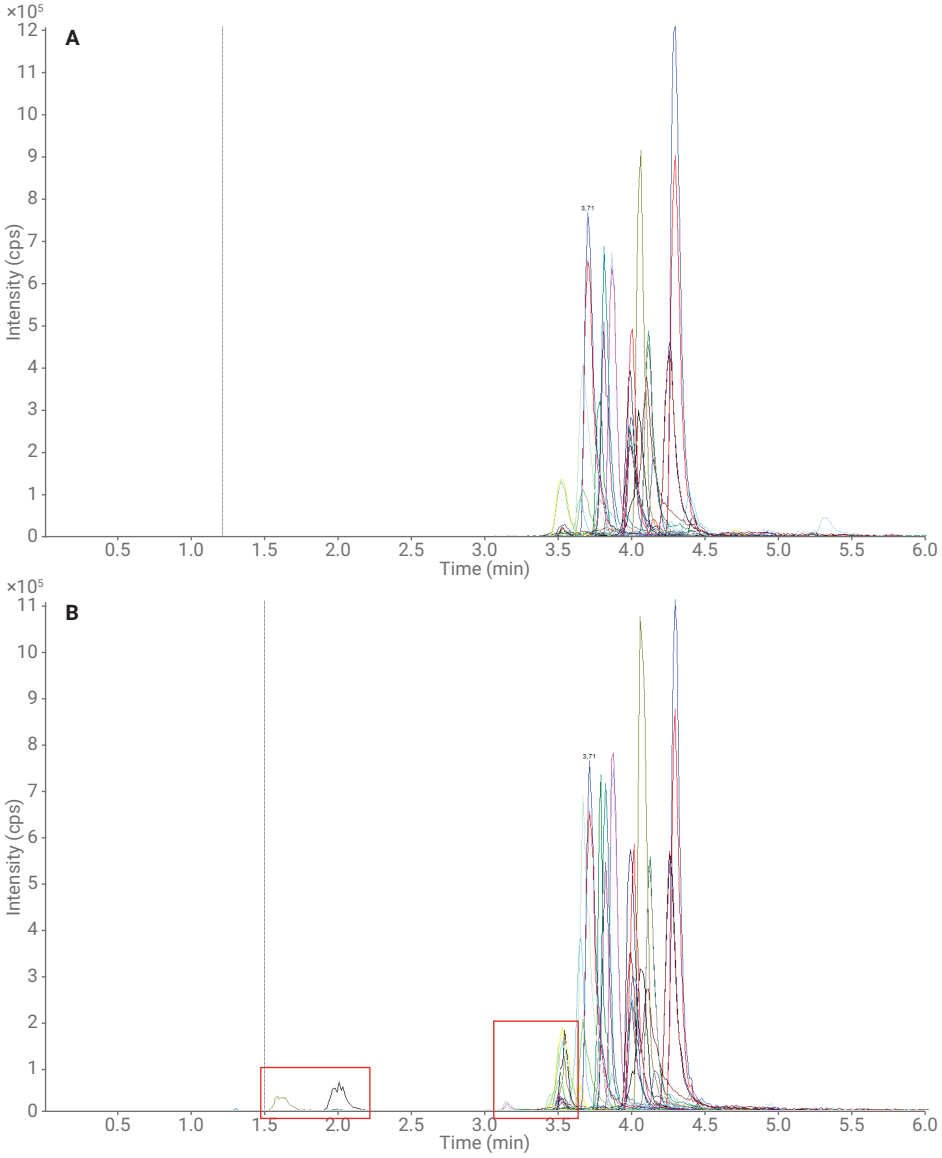

Figure 2. Comparison of A) online SPE analysis and B) online SPE with direct injection.

A comparison of the direct injection with a PAL autosampler, and the online SPE coupled to direct injection, shows the enhanced sensitivity and peak shape of the polar compounds DMS, DPC, and MDPC. In Figure 3, even poor peak shape/broadening and response were observed for the direct analysis of polar compounds, whereas online SPE coupled to direct injection leads to improved peak shape of the early eluting compounds.

While backflushing the enriched analytes from the cartridge onto the analytical column, the additional sample volume of the 100-µL loop is also pushed through the cartridge before reaching the column. This procedure leads to an increased column length and an additional focusing effect for the polar metabolites DMS, DPC, and MDPC, resulting in improved peak shapes. MDPC responds in a unique way, appearing as a split peak in the online SPE chromatogram but not in direct injection. A certain amount of MDPC is trapped on the SPE cartridge, but a higher sensitivity can be achieved when coupled to direct injection (by a factor of five). The additional column length results in a slightly longer retention of the sample from the extra loop.

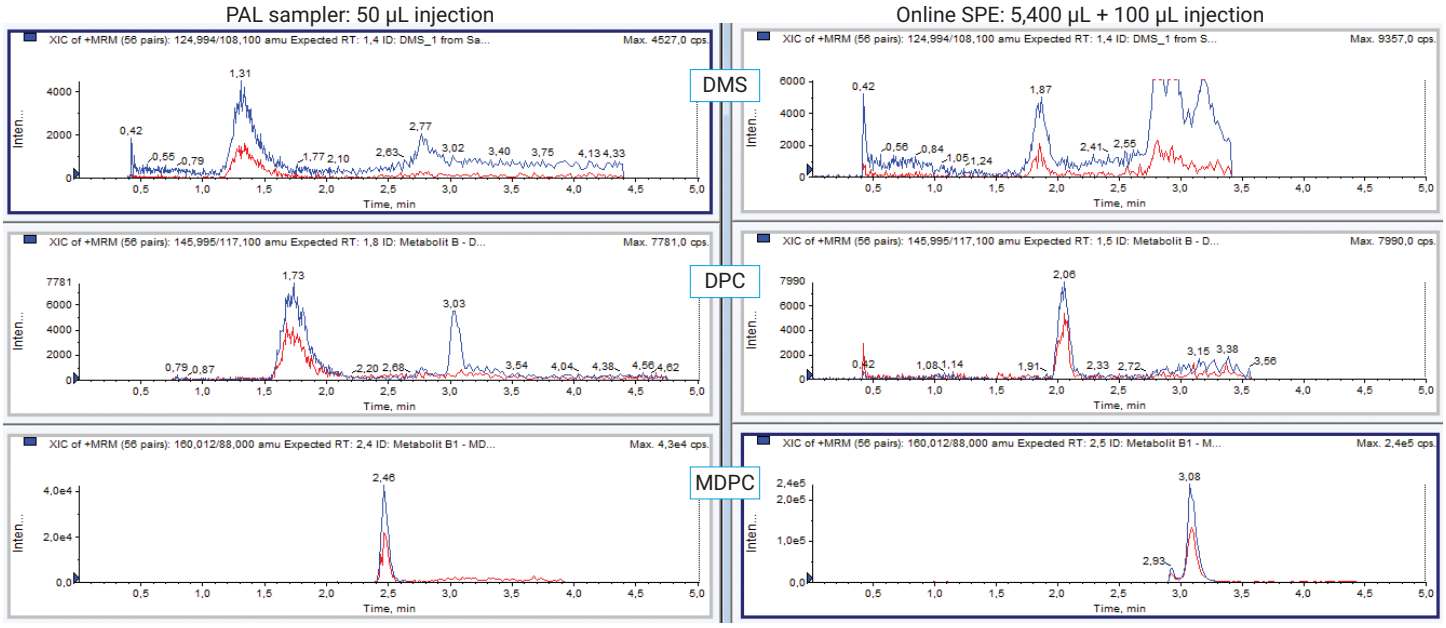

Figure 3. Direct injection coupled to online SPE enables the analysis of DMS and DPC with the rest of the semipolar pesticide metabolites.

Table 2 demonstrates the overall enrichment efficiency of the high-volume injection. All 26 compounds are listed with LOQs for the direct injection of 50 µL and online SPE, with a total volume of 5,500 µL. The enrichment efficiency is calculated by the ratio of the injection volume to the peak area. DMS and DPC are not enriched, and the increases in sensitivity are negligible. Different enrichment efficiencies can be explained by distinct bonding effects between analytes and the SPE cartridge. With online SPE, a sensitivity improvement of up to 90 times was achieved with an average enrichment factor of 35. An LOQ of 3 ng/L was achieved for most analytes in water samples during method validation.

Table 2. List of pesticide metabolites with LOQ of PAL (50 µL direct injection) and online SPE (5,400 µL enrichment and 100 µL direct injection), as well as the enrichment efficiency of online SPE compared to PAL.

Compound	LOQ (ng/L) of 50 µL direct injection	LOQ (ng/L) of SPE + direct injection	Enrichment efficiency
Metazachlor metab. BH 479-11	50	0.01	43
Metolachlor metab. CGA 354743 (Metolachlor ESA)	20	0.25	32
Metolachlor metab. CGA 50267	20	0.05	84
Metalaxyl metab. CGA 62826	20	0.1	46
Dimethenamid OA (M23)	20	0.5	30
Tolylfluanid metab. DMS (N,N-Dimethylsulfamid)*	20	20	1
Tolylsulfonyldiamid metab. DMST	20	5	10
Flufenacet ESA (M2)	20	0.5	25
Chloridazon metab. B (DPC)*	20	20	1
Chloridazon metab. B1 (MDPC)*	20	20	5
Metolachlor metab. NOA 413173	20	2.5	21
Propachlor OA	20	0.5	90
Propachlor ESA	20	0.5	32
Dimethachlor metab. SYN 528702	20	1	20
Trifloxystrobin metab. CGA 321113	20	0.05	67
Desmethylchlorotoluron	20	0.05	39
Flumioxazin metab. APF	50	2.5	5
Azoxystrobin metab. R 234886	20	0.05	38
Thiamethoxam metab. CGA 353968	20	0.5	83
Thiamethoxam metab. CGA 355190	20	0.1	21
Metolachlor-morpholinon	20	0.5	47
2-Hydroxyterbutylazin	20	0.01	52
Propazine-2-hydroxy	20	0.05	49
2,6-Dichlorbenzamid	20	1	15
Picoxystrobin M8	20	0.05	17
Irgarol M1	20	0.05	41
Average	**22.31**	**2.92**	**35**

* Polar metabolites

The linearity of the method was studied by analyzing standard solutions in duplicate (using each SPE cartridge once) at 12 concentration levels ranging from 0.01 to 500 ng/L. Satisfactory linearity was assumed when the correlation coefficient (R^2) was higher than 0.99.

Figure 4 shows the calibration of the polar metabolite DPC with good linearity in the range of 5 to 500 ng/L with an R^2 of 0.9949. In Figure 5, metolachlor ESA, a less polar compound, shows a very good R^2 of 0.99966 in the range from 0.01 to 500 ng/L.

Method accuracy and precision (expressed as relative standard deviation (RSD)) were evaluated for the ^{13}C-labeled internal standard of DPC, spiked at a concentration of 600 ng/L in ultrapure water. Area RSDs were less than 9 % for 30 replicates, and retention time (RT) RSDs were <1.3 %. A difference was observed between the two alternating SPE-loop setups due to the additional volume of the bridging capillary required for the online SPE setup using a 2-position/10-port valve.

In general, it is possible to upgrade the system with a larger loop for the loop-SPE combination, if a 100-µL direct injection is insufficient (maximum 900 µL).

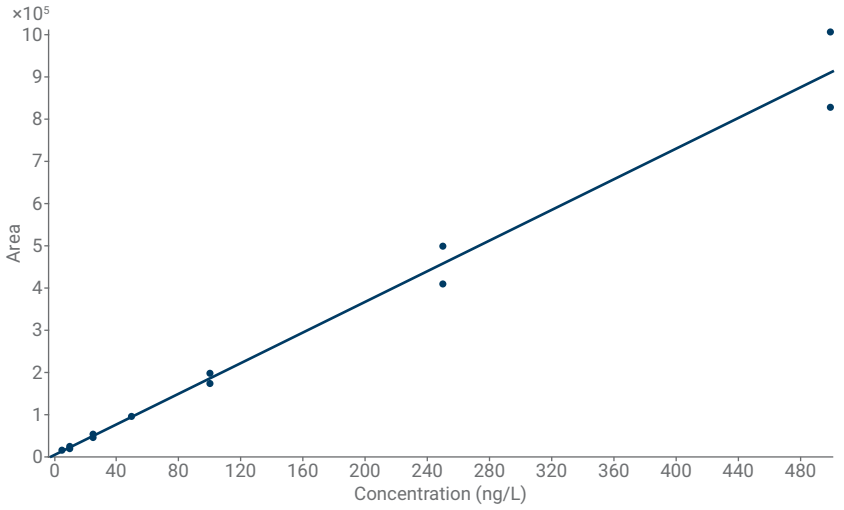

Figure 4. Calibration curve of DPC with online SPE coupled to direct injection (5–500 ng/L), R^2 = 0.99499, with two replicates for each standard concentration.

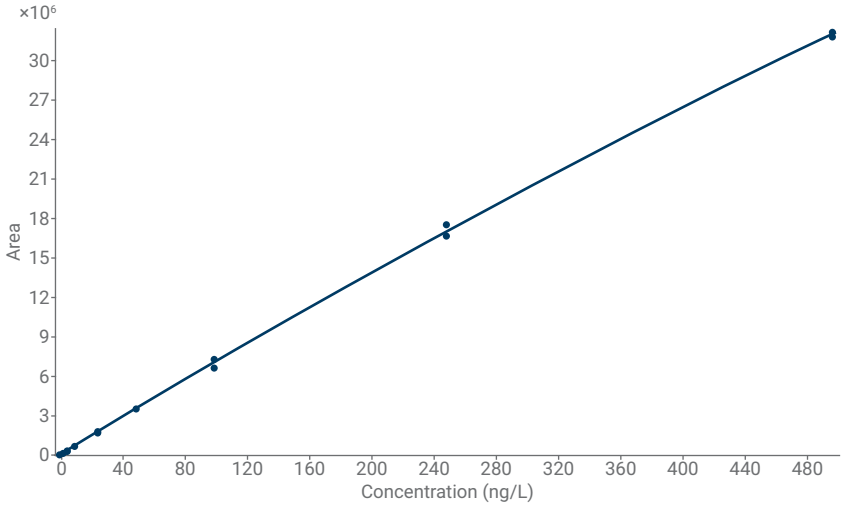

Figure 5. Calibration curve of metolachlor ESA with online SPE coupled to direct injection (0.01–500 ng/L); R^2 = 0.99966.

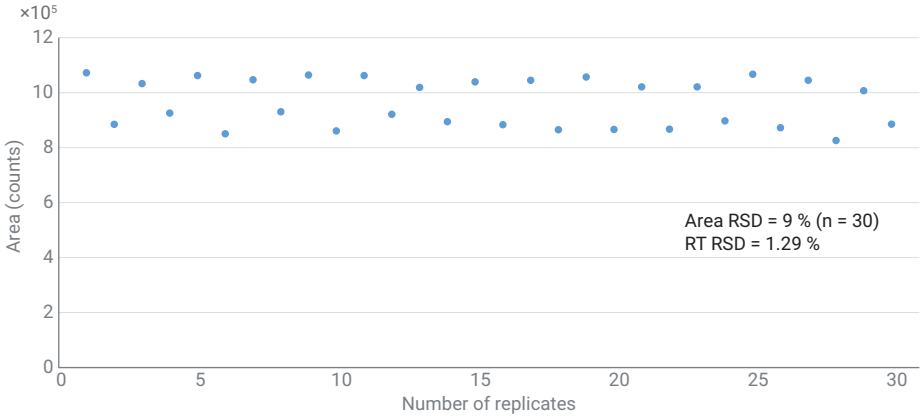

Figure 6. Internal standard of DPC shows good RSD values for area and retention time.

Conclusions

This Application Note describes the development and validation of a rapid and sensitive multiresidue online SPE LC/MS/MS method for the analysis of 26 pesticide metabolites. Special attention was paid to the combination of polar and semipolar pesticide metabolites in one analytical run. A direct injection of aqueous samples (100 µL), combined with a high-volume injection for online SPE, has been shown to be an attractive approach to avoid time-consuming sample preparation while achieving high sensitivity. Online SPE coupled to a direct injection setup further improves the retention and peak shape of early eluting polar analytes, which are notorious for having poor peak shapes when injected directly. This combined method enables the analysis of many analytes using an overall run time of just 28 minutes for automated sample enrichment and separation.

References

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