

**Environmental**

# Simplifying quantitative analysis of emerging contaminants in environmental water samples with a TriPlus RSH EQuan 850 system and an Orbitrap Exploris 120 mass spectrometer

**Authors**

Jonah Toth<sup>1</sup>, Marco Pineda<sup>1</sup>,  
Viviane Yargeau<sup>1</sup>, Maciej Bromirski<sup>2</sup>

<sup>1</sup>McGill University, Montréal, Canada

<sup>2</sup>Thermo Fisher Scientific, Warsaw, Poland

**Keywords**

Emerging contaminants, PFAS, pesticides, pharmaceuticals, environmental monitoring, online sample preparation, TriPlus RSH EQuan 850 system, Orbitrap Exploris mass spectrometer

**Goal**

Assessing and demonstrating the potential for the fast and simplified analysis of emerging contaminants in environmental water samples through online solid phase extraction (SPE) on the Thermo Scientific™ TriPlus™ RSH EQuan 850 system and trace-level quantitation with the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer

**Introduction**

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is increasingly considered the leading method for analysis of emerging contaminants in environmental water samples because of its powerful qualitative and quantitative capabilities. Recent advancements in high-resolution accurate mass (HRAM) spectrometers allow researchers to perform multiresidue analyses for diverse emerging contaminants with remarkable sensitivity and precision. In particular, hybrid HRAM quadrupole Orbitrap instruments, such as the Orbitrap Exploris 120 mass spectrometer, are capable of full-scan screening followed by data-dependent MS/MS fragmentation (FS-ddMS<sup>2</sup>) to yield both holistic accurate mass measurements for non-target analysis and MS/MS fragment confirmation of target analytes with a single sample injection.

While so much focus has been placed on LC-MS/MS analysis of emerging contaminants in water samples, sample preparation prior to injection is often overlooked. More often than not, sample preparation is necessary when analyzing environmental water samples to remove matrix interferences and selectively pre-concentrate target analytes. Not only is the process often costly and time consuming, but poor sample preparation can ultimately impact the quality of data obtained through subsequent LC-MS/MS analysis.

Solid phase extraction (SPE) is a common technique employed for the clean-up of matrix interferences and preconcentration of analytes within environmental water samples. However, conventional offline SPE is labor-intensive, time-consuming, and inefficient. The process demands constant attention to apportion significant volumes of organic solvents in single-use extraction cartridges before and after loading large volumes of each water sample. Not only does conventional SPE involve significant financial and environmental costs, but the manual process introduces plenty of risk for error.

In comparison, online SPE automates the process using the TriPlus RSH EQuan 850 system and allows for increased efficiency, improved reliability, and significantly reduced labor and material inputs. Rather than using single-use cartridges, the TriPlus RSH EQuan 850 system uses a small re-usable SPE cartridge for the online loading of small volumes (typically <5 mL) of environmental water samples, followed by elution with select solvents administered by LC pumps. When integrated in succession with a robust LC-MS/MS method for analyte detections, the application of online SPE allows for increased sample throughput with ease and efficiency.

In this technical note, we present a fast and simplified workflow for the analysis of various emerging contaminants in environmental water samples through online SPE on the TriPlus RSH EQuan 850 system and trace-level quantitation with the Orbitrap Exploris 120 mass spectrometer. We present a multiresidue method validated for pesticides and discuss two other applications to analyze other emerging contaminants such as per- and polyfluoroalkyl substances (PFAS) and illicit drugs. As part of our discussion of the benefits of integrating SPE online into the workflow, we present some metrics to demonstrate the quantitative capabilities of these methods—such as precision, limits of quantitation, and matrix effects.

## Experimental

The pesticide method described herein involving online solid phase extraction and trace-level quantitation via LC-MS/MS has been described in detail for the analysis of current-use pesticides in aqueous samples.<sup>1</sup>

A method for the extraction of pesticide residues in sediment samples through accelerated solvent extraction (ASE)—also known as pressurized liquid extraction (PLE)—was also developed in compliment to the aforementioned online-SPE-LC-MS/MS method.<sup>2</sup>

Application of the methods to field samples collected from agriculture-adjacent streams near Lac Saint-Pierre in Québec, Canada has been published.<sup>3</sup>

## Sample preparation

To remove any debris or large particulates prior to online SPE, grab water samples collected from small, agriculture-adjacent streams were filtered through hydrophilic glass fiber prefilters with 1.0 µm pore size using Fisher Chemical™ Hyflo™ Supercel™ filter aid.

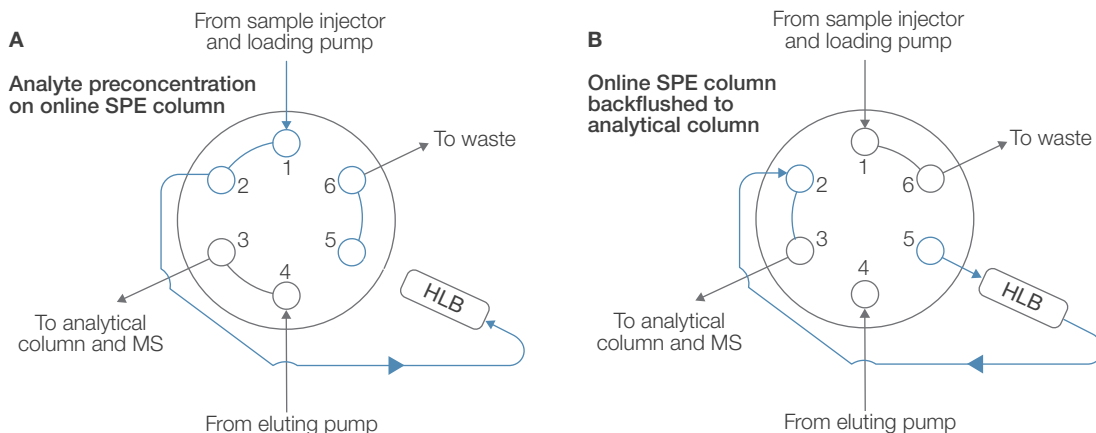
## Instrumentation

**Table 1. Workflow components**

Component	Parameter
Online SPE	- TriPlus RSH EQuan 850 system - Thermo Scientific™ stainless steel in-line filter (0.2 µm) - Waters™ Oasis™ HLB Direct Connect HP column (2.1 mm × 30 mm, 20 µm)
Liquid chromatography	- Thermo Scientific™ Vanquish™ quaternary loading pump - Thermo Scientific™ Vanquish™ binary eluting pump - Thermo Scientific™ Viper™ stainless steel capillaries
Analytical columns	- Thermo Scientific™ Hypersil GOLD™ C8 column (2.1 mm × 50 mm, 1.9 µm) - Thermo Scientific™ Accucore™ aQ polar-endcapped C18 column (2.1 mm × 150 mm, 2.6 µm)
Source	- Thermo Scientific™ EASY-IC™ heated electrospray ion source
Mass spectrometer	- Orbitrap Exploris 120 mass spectrometer
Data analysis	- Thermo Scientific™ TraceFinder™ 5.1 software

## Online SPE

A sample volume of 1 mL was injected with a loading solution of 0.1% v/v formic acid in water and methanol (98:2 v/v) at a flow rate of 2.000 mL/min. After passing through an in-line filter, a hydrophilic-lipophilic balance (HLB) column was used for sample clean-up and the pre-concentration of target analytes. The two configurations of the switching valve used to facilitate the online SPE process are shown in Figure 1.



**Figure 1. (A) In the loading position of the valve (1 → 2), the sample is injected and carried with the loading solution for preconcentration on the online SPE column; (B) In the eluting position of the valve (1 → 6), the online SPE column is backflushed by the eluting pump and analytes are eluted to the analytical columns for chemical separations.**

After a 5-minute transfer time in which the analytes were preconcentrated on the online SPE column and matrix components were washed away to reduce interferences, analytes were backflushed off the column by the eluting pump and transferred to the analytical columns for liquid chromatographic separations.

### Chemical separations

Two analytical columns were employed in sequence to optimize chemical separations between isomeric compounds within the list of target pesticides: a Thermo Scientific™ Hypersil GOLD™ C8 column (2.1 mm × 50 mm, 1.9 μm) and a Thermo Scientific™ Accucore™ aQ polar-endcapped C18 column (2.1 mm × 150 mm, 2.6 μm). Both analytical columns were warmed to 40.0 °C ± 0.5 °C.

To achieve both desorption from the online-SPE column and chemical separations on the analytical columns, a gradient of Solvent A (50 mM ammonium formate with 0.1% v/v formic acid in Milli-Q™ water) and Solvent B (0.1% v/v formic acid in methanol) was devised. When taking into account the 5-min transfer time, the total method duration was 20 min per injection.

### Mass spectrometry

The Orbitrap Exploris 120 mass spectrometer was operated in polarity switching mode using capillary voltages of +3,500 V and -2,500 V, respectively. Using a heated electrospray ion source, MS analysis began with a full scan at an Orbitrap resolution of 60,000 full width at half maximum (FWMH) and radio frequency (RF) lens of 60% followed by data-dependent MS<sup>2</sup> (dd-MS<sup>2</sup>) scans at 15,000 FWHM resolution using dynamic exclusion and targeted mass filters. In total, three dd-MS<sup>2</sup> scans in positive ionization mode were performed using stepped collision energies of 20%, 40%, and 70% and two dd-MS<sup>2</sup> scans in negative ionization

mode were performed using stepped collision energies of 15%, 35%, and 60%.

### Data analysis

Compound identification was performed using TraceFinder 5.1 software based on a HRAM database compiled in-house for the target list of pesticides. Quantitation of analytes was automated based on the measurement of precursor and product ions with a mass tolerance of ±5 ppm and retention time window of 0.5 min. The matching of ion fragment ratios and isotopic patterns provided further confirmation of target analytes.

### Results and discussion

Application of Thermo Scientific™ instrumentation with these methods results in a number of benefits for researchers when analyzing emerging contaminants in water samples, including greater time efficiencies, reduced consumable requirements, and improved reliability of sample analysis and analyte quantitation.

### Significant time savings

After samples are loaded into the trays and a sequence is programmed for analysis, the TriPlus RSH EQuan 850 system can automate the online SPE process and all associated sampling handling. Samples can be analyzed consecutively without delay—and without any input from the researcher, aside from occasional monitoring.

By comparison, performing manual SPE is labor-intensive and requires constant oversight throughout the process, from conditioning the cartridges and loading the samples to washing the cartridges and eluting the target analytes. Even after the manual SPE process is completed, extracts are often dried and reconstituted prior to LC-MS/MS analysis, requiring even more time and effort.

Whereas the manual SPE process can take hours per sample, the TriPlus RSH EQUan 850 system allows researchers to reduce analysis times to minutes. Figure 2 visualizes the potential time savings for the analysis of a single sample via online SPE. With a total run time of just 20 minutes, the online SPE method allows for the analysis of up to 72 samples within a 24-hour period, whereas performing manual SPE for a dozen samples can easily consume a full day.

The automated nature of online SPE also mitigates the risk of introducing unnecessary error into the process, including the risk of the sorbent drying if not constantly monitored and the variable flow rates per cartridge that are commonly associated with the manual SPE process.

### Reduced consumable requirements

In addition to significantly reducing the time and efforts required by a researcher, online SPE also represents a “greener” process with reduced consumable requirements. This directly translates into cost savings for laboratories performing routine analysis.

Among the advantages of the use of an online SPE method, and perhaps the most notable, is the cost of single-use cartridges

during the manual SPE, representing a large proportion of the cost, source of variability on the process efficiency, and source of waste generation, particularly when processing a significant quantity of samples. In comparison, a single online SPE column is capable of processing hundreds of extractions with washing, conditioning, and equilibration of the sorbent integrated directly into the method resulting in conservative savings of up to eight times compared to single-use SPE cartridges.

Figure 3 shows other potential benefits of using an online SPE column with the TriPlus RSH EQUan 850 system versus single use SPE cartridges.

In addition, online SPE reduces the volumes of solvents needed during the extraction stage. Considering the loading pump flow rate of 2.000 mL/min and eluting pump flow rate of 0.250 mL/min, the method presented herein requires a total of a 14 mL of solvent per sample. In contrast, a manual SPE procedure can consume upwards of 50 mL of solvent throughout the various steps of the process representing six times savings on solvents compared to that used on a manual SPE.

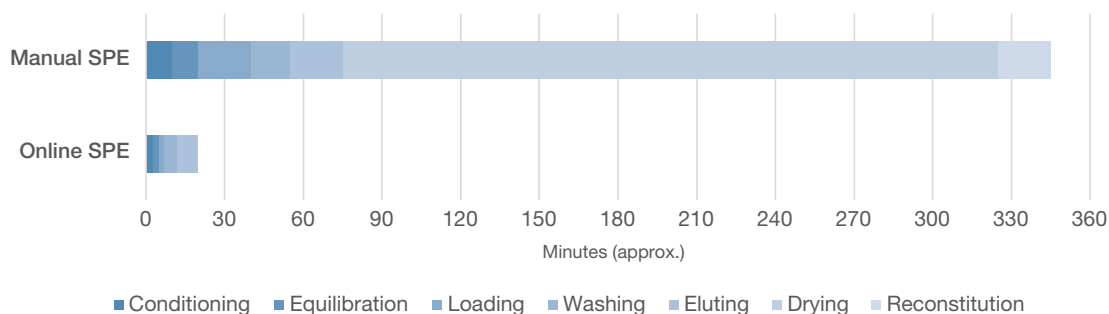


Figure 2. Estimation of the time required to process one sample via a manual SPE process versus online SPE with the TriPlus RSH EQUan 850 system

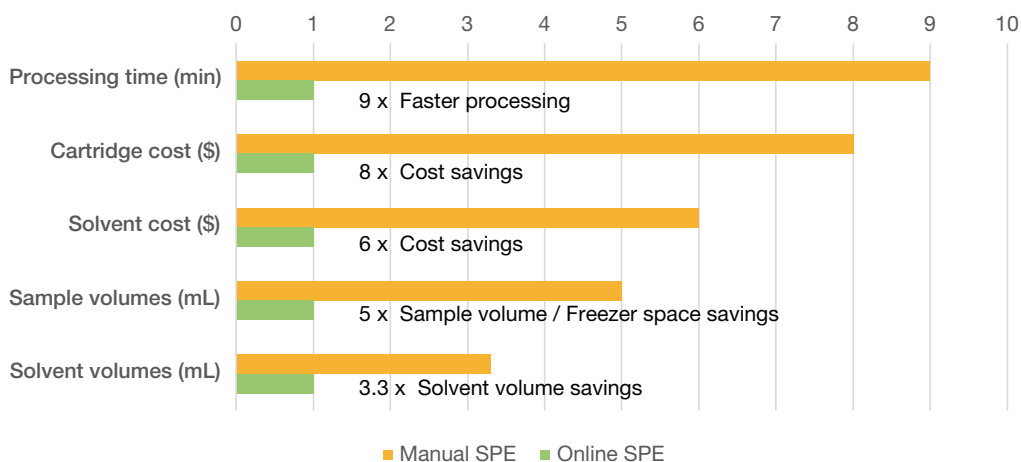


Figure 3. Efficiencies of online SPE column with the TriPlus RSH EQUan 850 system (scaled to value of 1) versus processing of manual SPE cartridges



## Reduction of matrix effects

Matrix effects, whether resulting in signal suppression or enhancement, represent a major challenge when performing trace analysis of emerging contaminants in complex sample matrices. Interference from the matrix has the potential to impact the accuracy and reproducibility of the quantitative analysis. The use of online SPE with the TriPlus RSH EQuan 850 system helps to remove matrix interferences and reduce co-extractives to effectively minimize matrix effects.

To quantify matrix effects, the ratio was calculated between the slopes of calibration curves prepared in environmental surface waters versus Milli-Q water. A positive value indicates signal enhancement, and a negative value indicates signal suppression, with values between -20% and +20% generally considered to reflect minimal matrix effects.

In the multiresidue study of current-use pesticide residues in water samples collected from agriculture-adjacent streams,<sup>1</sup> the vast majority of pesticides were found to experience minimal matrix effects, as shown in Figure 4. The significant reduction of matrix effects in these environmental samples is attributed to the efficiency of the online SPE process, including the selectivity of the sorbent to adsorb target compounds and wash away interfering co-extractives.

## Reproducibility of method

The application of online SPE with the automation of the TriPlus RSH EQuan 850 system contributes to strong reproducibility, as evidenced by the percent relative standard deviation (% RSD) calculated between seven intra-day injections at 800 ng/L. For the method reported by Toth et al.,<sup>2</sup> the % RSD values for the target pesticides ranged from 1.8% to 8.4%. This strong intra-day reproducibility can be attributed to the automation of the online SPE method for consistency between runs and across time.

The capability of the Orbitrap Exploris 120 mass spectrometer to perform positive and negative switching modes on a single analytical chromatographic run reduced in half the processing time, consumables, sample volume, and analytical effort needed for the analysis of these pesticides. The precision of the quantitative analysis relies on the HRAM Orbitrap 113 scans on average across each of the pesticide peaks obtained during the quantitative analysis for the precursor ions at 60,000 resolution full width at half maximum (FWMH) with and average scan speed for the ddMS2 spectra collected at 15,000 FWHM across each peak for identification purposes was about 11 scans per second. Figure 5 shows the average number of points detected across the peaks, as well as the scan speed, showing the capacity of the system to generate a minimum of 10 points across each peak, even operating under UHPLC conditions where peak width is about 10–20 seconds.

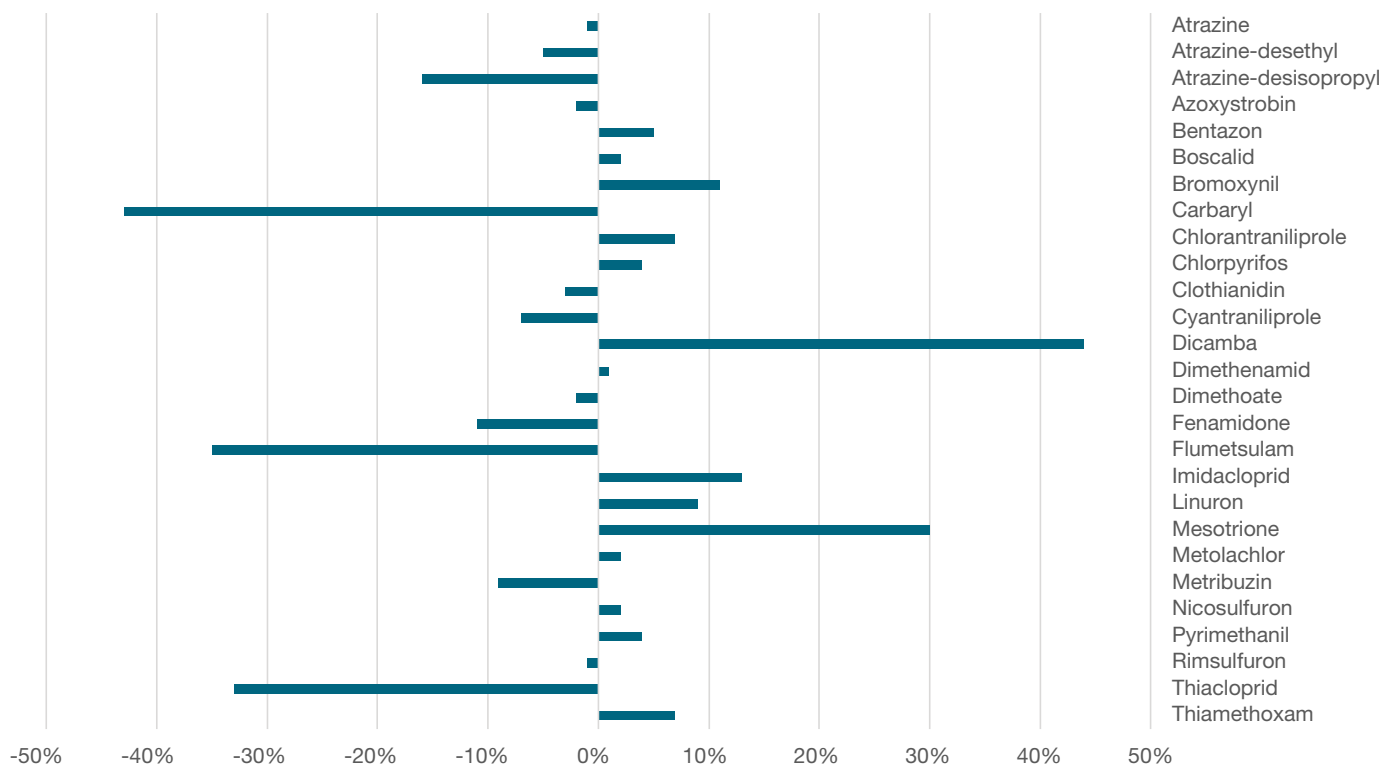


Figure 4. Calculated matrix effects for various pesticides in environmental water samples collected from agriculture-adjacent streams

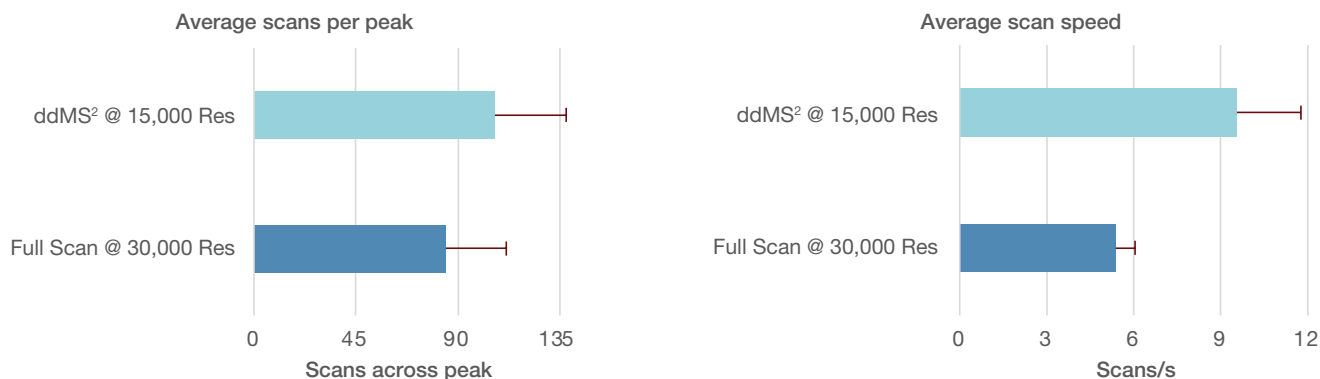


Figure 5. Average points detected across the peak with simultaneous positive and negative mode switching on the Orbitrap Exploris 120 mass spectrometer, for a panel of 31 pesticides in real environmental water samples collected from agriculture-adjacent streams

Table 2. Method performance for various emerging contaminants. The Illicit drugs panel is based on the 2023 SCORE successful inter-laboratory blind test. This international annual wastewater-based epidemiology initiative surveys and monitors six compounds with an inter-laboratory proficiency study (ILS) plus in this case ketamine as an additional marker. The PFAS panel consisted of seven compounds. Reported concentrations are free acid concentrations. Determination of the limits of detection and quantification for the method was performed in accordance with a published procedure from the United States Environmental Protection Agency. Full details are provided in our manuscript.

	Pesticides	Illicit drugs	PFAS
Detections	<p>Total pesticide concentration in stream water sample = 7,977 ng/L</p>	<p>Total illicit drug concentration in wastewater sample = 2,147 ng/L</p>	<p>Total PFAS concentration in wastewater sample = 2,054 ng/L</p>
Example calibration curve	<p>Atrazine, R<sup>2</sup> = 0.9986</p>	<p>Ketamine, R<sup>2</sup> = 0.9991</p>	<p>PFOA, R<sup>2</sup> = 0.9995</p>
LOD range	10–28 ng/L	3.93–6.49 ng/L	1.01–1.49 ng/L
LOQ range	30–86 ng/L	13.10–21.62 ng/L	3.37–4.97 ng/L

## Application to other emerging contaminants

While a method involving online SPE with the TriPlus RSH EQuan 850 system and trace-level quantitation with the Orbitrap Exploris 120 mass spectrometer was developed for pesticides,<sup>2</sup> this analytical approach is the latest result of other methods developed on diverse water source samples for the monitoring of other chemicals, including emerging contaminants such as per- and polyfluoroalkyl substances (PFAS) and illicit drugs in wastewater-based epidemiology projects.

In all cases, the methods were able to detect the emerging contaminants—whether pesticides in stream water or illicit drugs and PFAS in tap water and wastewater. They show strong linearity across the calibration curves and low limits of detection (LODs) and limits of quantitation (LOQs). The percentage values indicate the share of each compound detected in an average sample (whether stream water or wastewater) as a proportion of the total contaminant concentration. Representative data on the methods performance for various contaminants is presented in Table 2.

## Conclusion

The potential for the fast and simplified analysis of emerging contaminants in environmental water samples has been demonstrated through online solid phase extraction (SPE) on the TriPlus RSH EQuan 850 system and trace-level quantitation with the Orbitrap Exploris 120 mass spectrometer. Key performance criteria of the workflow include:

- Significant savings of time, labor, and consumables for researchers through application of online SPE processes versus manual SPE
- Exceptional sample throughput while reducing matrix effects from complex environmental sample matrices and ensuring strong reproducibility
- Remarkable flexibility to effectively analyze pesticides, illicit drugs, and PFAS with the potential to develop other analytical methods in the evolving environmental contaminants landscape. Other emerging contaminants of concern, such as endocrine-disrupting chemicals (EDCs), can also be investigated using this approach.

## References

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