Case study | 003424

Environmental

HERAtech Laboratories satisfies growing water-analysis demand and reduces costs using workflow automation

Automated sample preparation with online injection enables unattended 24/7 GC-MS/MS analysis of pesticides and PAHs in water samples

"Automation is fundamental because thirteen analysts are not enough to cope with our increase in samples. The automated approach we use allows for method consolidation with the analysis of PAHs and pesticides in a single GC run. Additionally, the possibility to perform sample extraction automatically, followed by online GC-MS/MS analysis, offers a significant reduction of labor spent and a significant reduction in solvent consumption."

—Dr. Paolo Morelli, Laboratory Manager, HERAtech S.r.l., Sasso Marconi (BO), Italy

Automation boosts sample throughput while reducing manual steps, costs, and exposure to hazardous solvents

HERAtech Laboratories, a part of the HERA Group, comprises three UNI EN ISO 17025 accredited laboratories in Central-Northern Italy that provide analytical services and support to internal and external clients. Its head laboratory near Bologna specializes in the analysis of surface water, deep water, and wastewater in compliance with legislative decree 18/2023 (water intended for human consumption) and legislative decree 152/2006 (deep waters and wastewater). Due to expansion in its share of external clients, national scope, and new legislation, demands for the lab's water sample analyses have grown substantially. In particular, many other providers have not developed the skills nor made the investments needed to address the new decree 18/2023. For context, in 2023 the lab performed more than 1.3 million analyses on more than 90 thousand samples.

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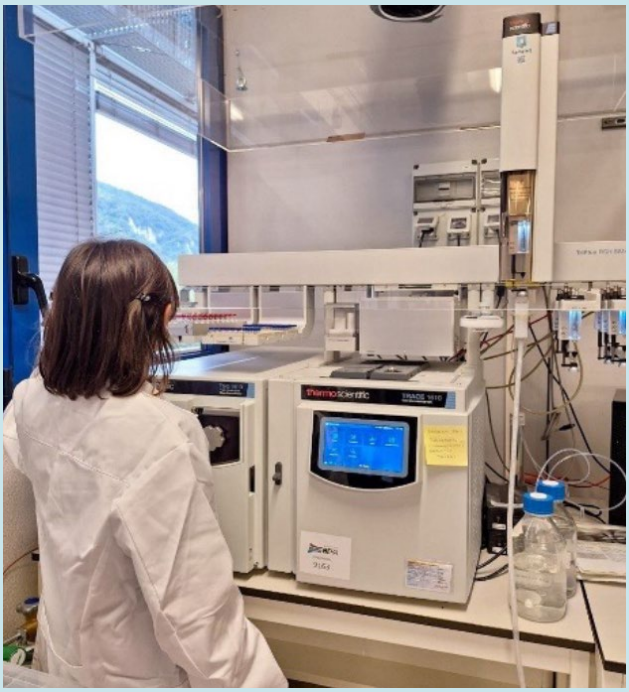
Given the increase in samples along with the need for highest-accuracy results and faster turnaround times, the lab streamlined its approach to pesticides and polycyclic aromatic hydrocarbons (PAHs) analysis in water. It went from two solid phase extractions (SPE)—one for pesticides and one for PAHs—followed by offline single quadrupole GC-MS analysis using two different methods to a single automated dispersive liquid-liquid micro extraction (DLLME) followed by online GC-MS/MS analysis of all pesticide and PAH targets in one injection. SPE followed by GC-MS analysis had required considerable time and operator skill to carry out its manual steps and was associated with higher costs for reagents, cartridges and solvents. The traditional drawbacks of LLE—manual effort and high solvent use—were overcome by using the Thermo Scientific™ TriPlus™ RSH SMART autosampler with robotic sample handling. The automated workflow allows analysis of both PAHs and pesticides in a single GC run to increase sample throughput and extends ultrasensitive Thermo Scientific™ TSQ™ 9610 Triple Quadrupole GC-MS/MS system utilization to 24/7 operation. The TriPlus RSH SMART autosampler handles all the extraction steps except for filling and placing vials in the autosampler tray, thus minimizing the chance of human error. The autosampler also allows the laboratory to substantially reduce sample and solvent volumes used per analysis, curtailing costs and staff exposure to concerning substances.

Analytical system used at HERAtech for automated determination of pesticides and PAHs in water samples

- TriPlus RSH SMART autosampler
- Thermo Scientific™ TRACE™ 1610 Series gas chromatograph with Thermo Scientific™ iConnect™ Programmable Temperature Vaporizing (PTV) injector module for large-volume injection
- Thermo Scientific™ TSQ™ 9610 triple quadrupole mass spectrometer

HERAtech



"It's necessary to evaluate every possible marginal gain on every single process in the workflow to remove any bottlenecks. The bottleneck that Thermo Fisher Scientific has helped us solve is huge for sample preparation for pesticides and in particular for PAHs by eliminating manual SPE."

—Dr. Paolo Morelli

> "The key aspect of this automated solution is an analytical system capable of carrying out automatically, in a fully unattended way, all the extraction phases of the LLE protocol using microvolumes."
>
> —Dr. Paolo Morelli

Automation simplifies workflows, eliminating manual tasks, saving analysts' time

Pesticides and PAHs are a significant source of water contamination. Their quantitation in water samples involves solvent extraction followed by gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis. Manual sample preparation procedures are time-consuming, tedious, and error prone, which creates bottlenecks and contributes to analytical variability. By using the TriPlus RSH SMART autosampler platform to automate the solvent extraction protocol, HERAtech reduces the time staff spend extracting pesticides and PAHs from water by as much as 90% (Figure 1). According to Dr. Morelli, HERAtech Laboratory Manager, "manual procedures are reduced to filling vials with sample and positioning them on the autosampler." Dr. Morrelli also noted that better traceability is an additional benefit provided by the automated workflow: "The difficulty of the manual SPE approach to manage the necessary traceability of samples through the various steps is fully overcome."

TriPlus RSH SMART autosampler configuration for automated DLLME with online sample injection

- Two tool change stations for automatic use of up to 6 syringes of different volumes to carry out the sample preparation steps
- Centrifuge
- One sample tray with up to 60 positions for 10 or 20 mL vials for extraction
- Second tray to hold 2 mL vials where the extract is transferred for injection into the GC-MS/MS
- Vortex mixer
- "Storage" station for washing solvents, calibration standards, and internal standards (IS)
- Fast Wash Module to provide longer availability of the dispersive solvent (methanol)
- Standard wash station to clean syringes with different solvents before and after addition of the IS and before sample injections

Manual steps for SPE sample preparation	Manual steps required with the automated LLE
1. Analyst measures the sample volume	
2. Analyst adds surrogates into the sample	
3. Analyst conditions the SPE cartridge	
4. Analyst quantitatively transfers the sample to the SPE cartridge	
5. Analyst washes the SPE cartridge	
6. Analyst elutes the sample from the SPE cartridge	Saved 90% manual operation for sample prep
7. Analyst performs the concentration	
8. Analyst adds Internal Standard	
9. Analyst fills the vials	1. Analyst fills the vials
10. Analyst places the vials in the sampler for GC-MS analysis	2. Analyst places the vials in the sampler for extraction and on-line GC-MS analysis

Figure 1. Comparison of manual and automated workflows. Automation provides a huge time savings by eliminating 90% of the manual steps required to extract pesticides and PAHs from water samples. The remaining manual steps are limited to filling the vials with sample and positioning them on the autosampler at the beginning of the automated sample preparation workflow.

> "We made significant investments focused on automation in terms of instruments that allow us to analyze more analytes in one run. In particular, we aimed to consolidate different methods into a single method and provide as many analytical measurements as possible with a single machine. Our goal was simultaneous analysis of 70 analytes including pesticides and PAHs, with quantification limits between 0.0005 µg/L (0.5 ng/L) and 0.02 µg/L."
>
> —Dr. Paolo Morelli

Workflow automation enables method consolidation and reduces per-sample cycle time

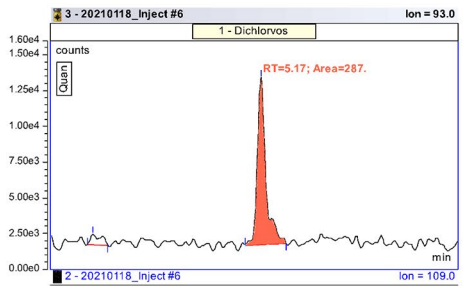
Method consolidation for pesticides and PAHs enabled by the automated workflow combined with "round-the-clock" utilization of the GC-MS/MS system dramatically reduced the time spent on each sample. Total cycle time per sample is optimized by overlapping sample preparation with the chromatographic run of the previous sample. The result is an overall increase in sample throughput and number of samples analyzed per day (Table 1).

Dr. Maria Chiara Gaeta, HERAtech Analyst explained: "Now we have a single analysis for PAHs and pesticides. Combining the two analyses in one was the turning point because the analysis of PAHs separately required a huge amount of manual work. Since we were working in compliance with EPA Method 8270, the addition of surrogate and internal standard required lengthy manual preparation. It would have been unfeasible—it took the whole day when we had a tenth of the samples that we run now. For pesticides analysis, working without addition of ISTD and surrogate went a bit faster, but the system we used before was only running either pesticides or PAHs, so it was necessary to alternate the analyses."

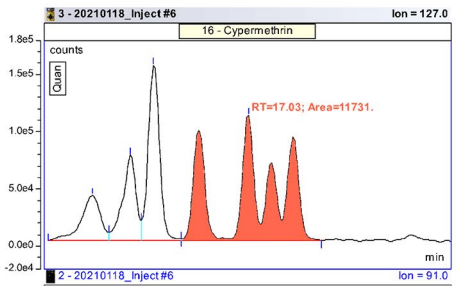
"To give you an estimation of the productivity, the system takes about 70 minutes to prepare a batch of 20 samples with the calibration standards and the QC samples as per the EPA method. For each sample, this is followed by 22 minutes of GC runtime, plus 5 additional minutes of GC cooling. The sequential operations, including centrifugation and transfer of the extract, are performed during the GC run of the previous sample, optimizing the overall cycle time," added Dr. Morelli. "The key benefit is the unattended operation of the system overnight. Since we implemented the automated workflow, the number of samples has at least doubled. There has been a decided expansion and acquisition of new contracts for our laboratory."

Analytical performance addresses reference methods

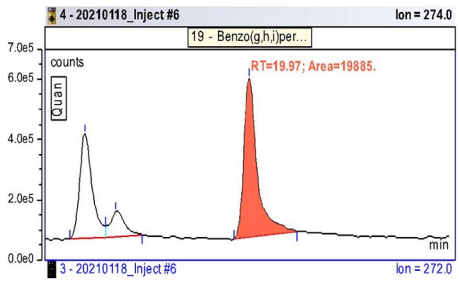
Automation reduces variability and increases repeatability, while large-volume injection on the TSQ 9610 Triple Quadrupole GC-MS/MS system delivers the sensitivity required by the Legislative Decree 18/2023 (Figure 2). The analytical performance obtained permits the use of the same instrumentation to carry out both drinking water and wastewater methods. "For our operations, it is important to use the same instrumentation and method for both simple and more complex matrices. The high sensitivity provided by the system, and the very good signal-to-noise ratio, allow us to validate a single analytical method for both clean water and wastewater, thanks to the ability to make the appropriate dilutions while still obtaining the required limits of detection," explained Dr. Morelli. "The linearity and repeatability of data obtained during the validation phase were very good. Automation of sample preparation provided clear improvement in terms of the precision of the results, compared to the same parameter measured with the manual SPE technique, for which the RSD exceeded, even doubled, the values obtained with the automated system." Improved data quality can reduce the time spent manually reprocessing data (Table 1).



1 ng/L water, Dichlorvos



1 ng/L water, Cypermethrin



0.6 ng/L water, Benzo (*ghi*) perylene

Figure 2. DLLME with large-volume injection onto the GC-MS/MS system provides sub-ppt sensitivity for drinking water analyses.

> "Only two solvents are necessary to perform automated DLLME, and the much lower volumes offer significant cost savings. The sample volume required is much lower, reducing logistic costs of transportation and storage."
>
> —Dr. Paolo Morelli

Automated DLLME minimizes amounts of solvent and sample needed per analysis

Automated sample handling and extraction not only increases sample throughput, it also reduces solvent consumption and cost, sample volume requirements, and chemical exposure risks to staff. The TriPlus RSH SMART autosampler can more precisely handle small volumes than most human analysts. As shown in Figure 3, automated DLLME uses small amounts of solvent—just hundreds of microliters—dispersed in a reduced volume of water sample to create an emulsion. Due to its high surface area, the emulsion provides enhanced extraction efficiency. Additionally, the limited volume of extraction solvent allows enrichment of approximately 15 times, leading to ppt and sub-ppt sensitivity, removing the need to re-concentrate the extract. Minimizing manual steps and solvent volumes also minimizes staff exposure to hazardous chemicals.

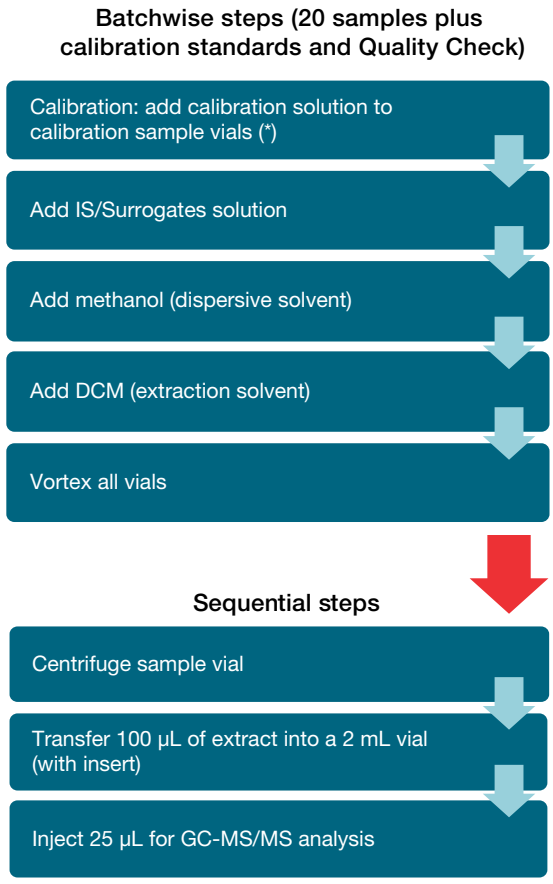


Figure 3. Automated DLLME workflow for pesticides and PAHs analysis in water. The process starts by adding a small amount of dispersive solvent (methanol) and a few hundred microliters of dichloromethane (DCM) extraction solvent to a 5–10 mL sample. This is followed by vortexing and then centrifugation for 2 minutes to separate the solvent from the aqueous phase. After centrifugation, the DCM deposited on the bottom is recovered and transferred to vials for injection onto the GC-MS/MS system. *Calibration standards are automatically prepared, spiking the proper amount to 5 mL of water contained in 9.5 mL high-recovery vials.

Using automated DLLME, the laboratory was able to reduce the sample used per analysis from 200 mL and 500 mL each for pesticides and PAHs, respectively, to 5 mL for the consolidated method (Table 1). Said Dr. Gaeta, "An added value of the automated system is the reduction of sample volumes. We went from two 1.5 L bottles for pesticides and PAHs to about 40 mL, with several benefits in terms of cost and time savings for collecting samples, transport, storage, and disposal."

Usability speeds deployment

The solution was the lab's first instrument-software purchase from Thermo Fisher. Though staff had to learn to use a new system, usability features facilitated its adoption. "It was a new software for me, but I like it. In particular, I like that there is the separate part of Studio (Thermo Scientific™ Chromeleon™ CDS Customizable Studio Ribbon) for data reprocessing so I can review the data during acquisition," said Dr. Gaeta. Despite the many features that Chromeleon CDS has, it is very intuitive, and it was quick to learn. One very useful thing is the reporting is like Excel™ so you can add formulas. If you are able to manage Excel, you can work with it and create custom templates."

The TRACE 1610 GC is likewise easy to use. "I used the how-to videos that are on the TRACE 1610 GC. From the beginning, they were very useful to quickly learn the basic operations like changing the liner, replacing the ferrule, or installing the column with the right length. Everything is very simple," explained Dr. Gaeta. Dr. Paolo Morelli added, "the injectors of the Thermo Scientific GC system are easy to maintain thanks to the modular design."

"It was not easy for me either to decide to invest in a different vendor, but the liquid-liquid automation offered by Thermo Fisher was quite unique, and we had no doubts since it responded to our needs. From this experience, we have seen that buying a turnkey system, completely validated, is the path we will take again in the future. There are obviously pros and cons because a turnkey system is not as flexible and does not easily allow changes to the method such as adding other analytes. However, the positive experience of not having had to invest time and resources to optimize the method compensates for the fact that the system is dedicated."

—Dr. Paolo Morelli

Planning for the future

In the future, the lab intends to automate other time-consuming and costly manual processes. "The bottleneck of operator worktime can be improved. Another operation is the preparation of the calibration standards and ISTD addition to samples, which is absolutely influential on the quality of the data. I would like to automate all these manual processes," said Dr. Morelli. "For the future, we are also thinking about a possible use of the robotic autosampler as a stand-alone bench station to automate the extraction of multiresidue pesticides—about 500 target compounds—prior to analysis by LC-MS and GC-MS to further increase our laboratory productivity."

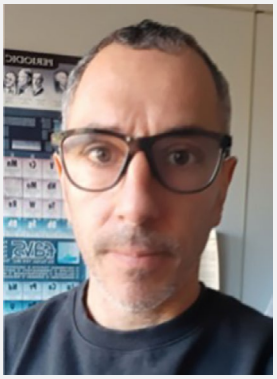
Conclusion

HERAtech Laboratories has met rising demand for sample analysis and better serves its customers with faster response times through deployment of the TriPlus RSH SMART autosampler to automate DLLME for online GC-MS/MS analysis of pesticides and PAHs in drinking and wastewater. The TriPlus RSH SMART autosampler performs all the extraction steps except for filling and placing vials in the autosampler tray, minimizing the chance of human error. Environmentally friendly, the autosampler also allows the laboratory to reduce sample and solvent volumes used per analysis, reducing costs, and staff exposure to hazards. The automated workflow increases sample throughput and lab efficiency via method consolidation for the analysis of both PAHs and pesticides in a single GC run and 24/7 GC-MS/MS system utilization. Automation combined with highly sensitive and repeatable GC-MS/MS analysis facilitates compliance with stringent legislative requirements for drinking water analyses, while allowing analysis of more complex matrices using the same unattended workflow.

Table 1. Summary of efficiencies and savings provided by the automated workflow

	Previous SPE + GCMS	Current automated DLLME + LV GC-MS/MS	Impact
Solvent consumption	Pesticides: 9 mL methanol + 1 mL DCM + 1 mL ethyl acetate PAHs: 9 mL methanol + 3 mL DCM + 3 mL hexane + 0.5 mL ACN	0.4 DCM 0.8 methanol	Cost savings
Sample volume	Pesticides = 200 mL PAHs = 500 mL	5 mL for all targets	Smaller volumes and weights reduce picking and transport costs
Analyst time	Batch 20 samples: Pesticides 4 h PAHs 12 h	30 min for all targets	Optimization of labor-time
Samples/day	24 sample /day	48 sample / day	Increased productivity
Manual processing residues	Many…	Only few…	Increased productivity

About Dr. Paolo Morelli



Dr. Paolo Morelli is Laboratory Manager, Water Service at HERAtech Laboratories' facility in Sasso Marconi, Italy. He received his master's degree in industrial chemistry in 2001 and then began his career in the analytical testing laboratory working on several types of analytical instruments (GCMS, ICP, FTIR, CI, etc.). For the last few years, he has been responsible for the Analytical Laboratory at HERAtech in Sasso Marconi, Italy, which is ISO17025 accredited for the analysis of wastewater, surface, and deep water intended for human consumption.

About Dr. Maria Chiara Gaeta

Dr. Maria Chiara Gaeta is an analyst at HERAtech Laboratories' facility in Sasso Marconi, Italy. She graduated with a degree in chemistry from the University of Bologna in 2019. Since then, she has been a lab technician at HERAtech, with experience in many different analytical techniques. Her current focus is on GC and GC-MS analysis for hydrocarbons, PAHs, and pesticides in water samples.

About HERAtech Laboratories

HERAtech Laboratories, a part of the HERA Group, comprises three UNI EN ISO 17025 accredited laboratories in Northern Italy that employ 84 technical staff to provide analytical services and support to internal and external clients. The laboratories' services include on-site sampling and analysis; chemical, physical, microbiological and radiochemical analyses; electron microscopy analysis for asbestos; interpretation of analytical results and consultancy for specific analytical problems; research and monitoring of chemical and microbiological risk in workplaces; and characterization and classification of waste.

The Hera Group's business activities range from water, energy, and environment to public lighting, telecommunications, and more. The company's mission is to be the leading Italian multiutility for its customers, workers, and shareholders, focused on the fair and inclusive growth of the local economy while building a sustainable future with the communities in which it operates.

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