VERITAS meets increasing sample analysis demands, saves time, and minimizes costs through automation

Automated liquid-liquid extraction (LLE) for on-line GC-MS/MS and GC-FID analyses of drinking and waste waters

"The number of water samples requiring analysis for pesticides and other semi-volatile organic compounds (SVOCs) was around 150 per month until 2018, more than doubled in 2019, and is still increasing today. The growing demand to maintain high-quality water standards can only be met through methods consolidation and resource optimization. This was possible by combining the robotic automation provided by the TriPlus RSH autosampler with highly sensitive instrumentation like the TSQ 9000 GC-MS/MS."

— Gilberto Pintonello, Chromatography Lab Supervisor, VERITAS Integrated Water Service Laboratory, VERITAS, S.p.A.

Automation increases sample throughput, operating efficiency, and safety, while reducing costs

VERITAS S.p.A. is a public multi-utility company that supplies drinking water and purifies wastewater for the Veneto region of Italy. Its laboratory, VERITAS Integrated Water Service Laboratory, provides analytical support focused on wastewater, surface, and deep water intended for human consumption. One of the laboratory's goals is to monitor and guarantee the quality of water supplied to the region by VERITAS as well as by other companies. Accredited by Accredia, the laboratory analyzes samples for numerous biological and chemical quality parameters as required by regulators and plant managers.

Due to increases in population, geographical area, and ViveracquaLab Network members served, the number of sample-test reports generated and parameters analyzed by the laboratory has approximately doubled from 2016 until today. Though the laboratory analyzed 22,000 samples in 2016, the forecast for 2022 is 50,000, a huge increase. Adding to the challenge, the laboratory's clients expect increasingly faster sample turnaround with no compromise in the quality of results. Another goal is to reduce the consumption of solvents that are potentially harmful to staff and the environment, not to mention costly.

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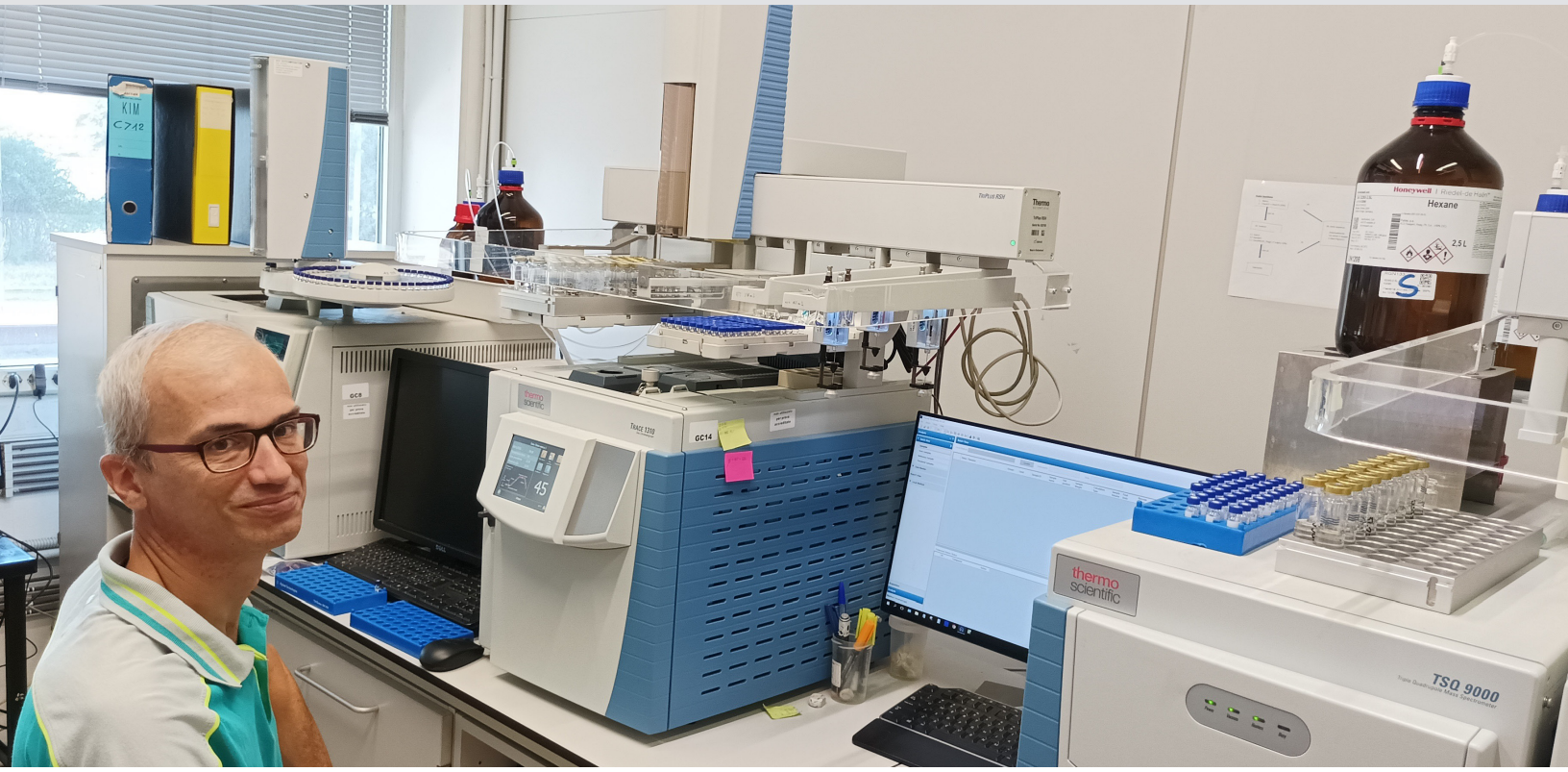
VERITAS Integrated Water Service Laboratory has exceeded these challenges with innovative technology adoption to automate workflows, increase analytical sensitivity, and consolidate the detection of a broader range of contaminants. By deploying the TriPlus RSH autosampler to automate in-vial liquid-liquid extraction (LLE) of polycyclic aromatic hydrocarbons (PAHs), pesticides, and other semi-volatile organic contaminants from drinking and wastewater prior to ultrasensitive TSQ 9000 GC-MS/MS analysis, the laboratory automated all the manual extraction steps technicians would otherwise perform, except for filling and placing vials in the autosampler tray. An additional TriPlus RSH autosampler is similarly used to automate in-vial LLE prior to GC-FID analysis of mineral oil in water. Minimizing manual procedures enables the laboratory to extract samples 24 hours per day, reducing turnaround time from sample receipt to results delivery from ten to as few as three days, while minimizing the chance of human error. The TriPlus RSH autosampler also allows the laboratory to substantially reduce required sample and solvent volumes, curtailing costs and staff exposure to concerning substances.

> “Automation is key to extending analysis overnight and over the weekend, resulting in a significant increase in sample throughput. The procedure is simplified and automated, and therefore has a low probability of manual errors and the advantage of high repeatability”
>
> — Gilberto Pintonello



Analytical systems used by VERITAS Integrated Water Service Laboratory for semi-volatile organic compounds (GC-MS/MS) and mineral oil (GC-FID) in water samples with automated liquid-liquid extraction (LLE): Thermo Scientific™ TRACE™ 1300 Series Gas Chromatograph with Thermo Scientific™ iConnect™ Programmable Temperature Vaporizing (PTV) Injector Module for large-volume injection, Thermo Scientific™ TSQ 9000 triple quadrupole GC-MS/MS, with the Thermo Scientific™ TriPlus™ Robotic Sample Handling (RSH) autosampler.

"An important trend is to reduce solvent use. With an automated workflow we have less solvent consumption, less waste, and less exposure. Using manual procedures, we exceeded 500 liters of dichloromethane per year. With the automated approach, not only was it possible to convert to the less hazardous n-pentane, but its consumption is only about one liter per month."

— Gilberto Pintonello

Automation reduces manual tasks, increasing sample throughout and reducing variability

Semi-volatile organic compounds (SVOCs) are a significant source of water contamination. Quantitation of SVOCs such as chlorobenzenes, pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and phenols in water samples involves solvent extraction followed by gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis. Manual sample preparation procedures like LLE are time-consuming and tedious, representing a bottleneck for most analytical laboratories. Quantitation typically involves preparation of IS at different concentration levels for calibration, which adds more manual steps. Other drawbacks of manual methods include their expense; high solvent use, exposure, waste, and contribution to analytical variability.

VERITAS Integrated Water Service Laboratory minimizes the manual steps involved in analysis of SVOCs by fully automating in-vial LLE extraction and internal standard (IS) addition (Figure 1) with the TriPlus RSH autosampler platform. According to Gilberto Pintonello, Chromatography Lab Supervisor, VERITAS Integrated Water Service Laboratory, "Our workflow is fully unattended and the total cycle time per sample is optimized by overlapping the sample preparation steps with the chromatographic run of the previous sample. Also, it can extract samples 24 hours per day, whereas before, extractions could only be performed during working hours. The result is an overall increase in sample throughput and an ability to respond to customers in two or three days." Pietro Leinardi, Chemical Laboratory Technician, VERITAS Integrated Water Service Laboratory, added, "I significantly reduce the time spent on sample preparation and to monitor the instrument. I used to spend about 20 minutes per sample, and now I spend about 20 minutes for 60 samples, and about 1 hour a day—a half an hour in the morning and half an hour in the evening in front of the instrument. I'm saving a lot of time for more value-added activities in the laboratory."

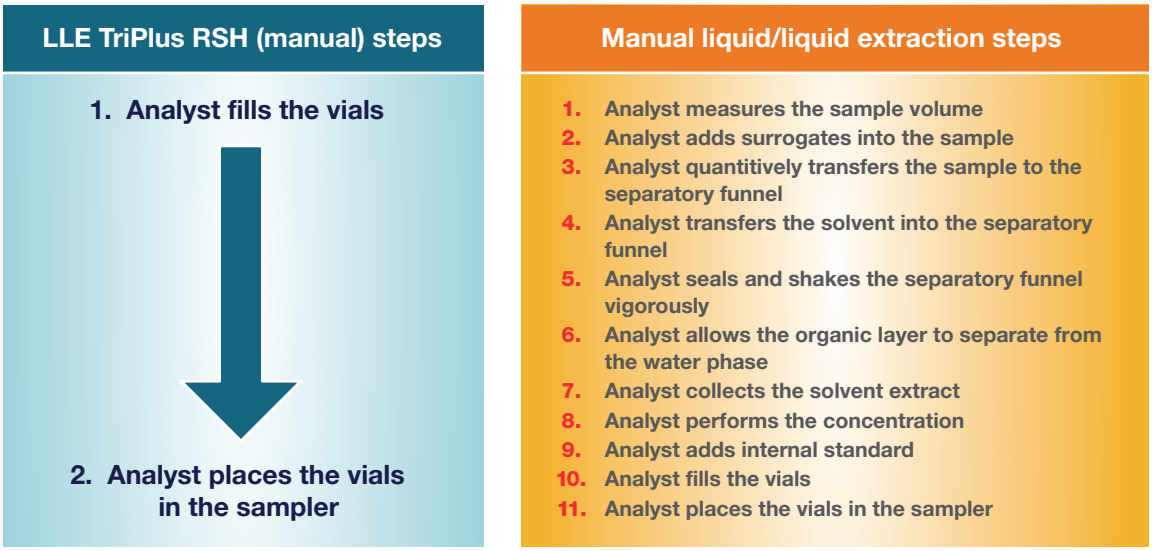


Figure 1. Automated LLE workflow. All the operator must do is fill the 20 mL vials with 10 mL sample and place the vials in the autosampler tray. The TriPlus RSH autosampler adds solvents and internal standards (IS), performs vortexing, and injects samples into the PTV injector for GC-MS/MS analysis. The automated LLE is overlapped with GC separation of the previous sample to optimize cycle time and increase sample throughput.

To carry out the workflow, the TriPlus RSH autosampler automatically chooses from a number of different syringes according to its programming: a dedicated 1 mL syringe for the solvent addition (1.8 mL), 50 µL syringe for internal standard addition (20 µL), and a 100 µL syringe for GC injection (50 µL). Pintonello explained, "The use of dedicated syringes, combined with efficient syringe washing, eliminates any risk of cross-contamination." Due to precise control, fully unattended sample preparation also reduces sample-to-sample variability that is typically encountered with manual pipetting or washing steps. Many laboratories, including VERITAS, chose Thermo Fisher Scientific because of its collaboration with SampleQ (part of Interscience, NL) for offering to customers validated solutions and rapid implementation.

Automation minimizes amounts of solvent and sample needed for each analysis

Automated sample handling and extraction not only increase sample throughput; they reduce solvent consumption and cost, sample amount requirements, and staff exposure to chemicals. In fact, the autosampler can more precisely handle smaller volumes and vortexing (Figure 2) results in more efficient extraction. "Manual operations and the solvent volumes used in the traditional procedure could be a source of health problems. While manual LLE required up to 200 mL of dichloromethane solvent per sample, the automated procedure uses only 2 mL of the less hazardous n-pentane per sample, allowing analysis of 500 samples per liter of solvent. With the automated sample preparation workflow, we are heading in the right direction, saving about 50–100 times on solvent consumption and drastically reducing exposure risks," explained Pintonello.

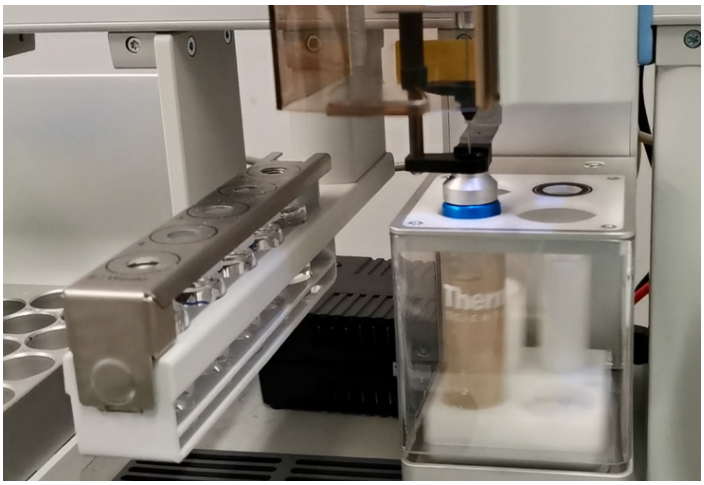


Figure 2. Vortex Mixing tool for automated in-vial liquid-liquid extraction (LLE)

The automated LLE workflow reduced the amount of sample required per test from 1 L to only 10 mL, resulting in reduced sample storage and transportation costs, including costs related to refrigeration, since in transport or storage all samples must be held between 2 and 7 °C. Said Pintonello, "We improved our logistics since the space and cost needed to transport and store samples is much smaller."

"On one hand the sample volumes are reduced, on the other, the required sensitivity is maintained thanks to the ability to inject more. With the PTV injector it is possible to inject 50 µL or more of extract into the GC-MS/MS system. Additionally, the ionization capability of the Advanced EI source on the TSQ 9000 GC-MS/MS system assures reliable detection at very low concentration levels."

— Gilberto Pintonello

Large-volume PTV injector with TSQ 9000 GC-MS/MS analysis provides ultra-sensitivity

The iConnect PTV Injector is ideal for trace-level analyses and suitable for thermally labile compounds. For the automated LLE GC-MS/MS SVOCs analysis workflow, the iConnect PTV Injector enables large-volume injection of 40–50 µL in the Solvent Split mode, instead of 1–2 µL, compensating for the reduced sample and solvent used while achieving the required sensitivity and compliance with imposed limits. The injector's modular design is free of tubes or cables, making maintenance procedures easy.

The workflow's sensitivity is also enhanced by the ionization efficiency of the TSQ 9000 GC-MS/MS system's Advanced Electron Ionization (AEI) Source. The AEI source offers robust, ultimate EI sensitivity for reproducible low-level quantitation of target compounds in challenging matrices (Figure 3). The positioning of the source filaments allows for inline sample ionization, significantly increasing ionization, while generating a tightly focused ion beam. Describing the source's benefits Pintonello said, "The enhanced ionization increases sensitivity, the highly focused ion beam makes the source extremely robust against contamination, and maintenance frequency is significantly reduced even when analyzing difficult matrices."

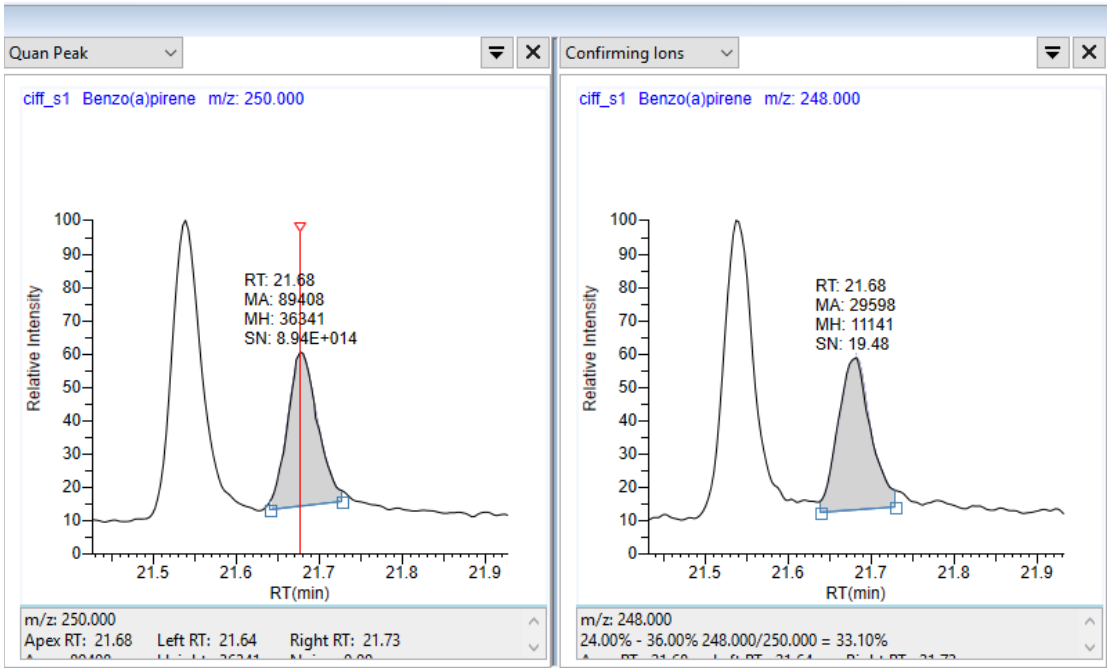


Figure 3. Sensitivity of the automated LLE GC-MS/MS SVOCs analysis workflow for benzo(a)pyrene at 0.0025 µg/L. The AEI Source together with large-volume injection facilitate compliance with challenging regulatory requirements.

"Our main challenge is timely response to customers, who continually ask for more tests to be done in less time. Automation is helping us achieve customer satisfaction. It extends analysis overnight and over the weekend, resulting in a significant increase in sample throughput. Add to that, the sensitivity of the TSQ 9000 GC-MS/MS system with large-volume injections enables full compliance with the regulatory limits. The system is very robust for the analysis of our most critical samples with minimum maintenance."

— Gilberto Pintonello

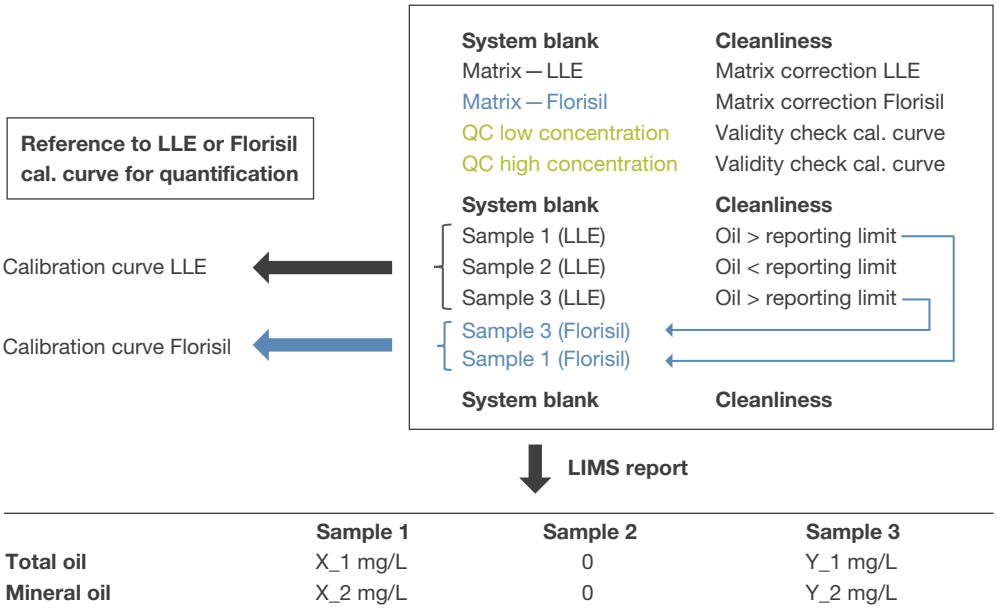
"An important benefit is the much reduced exposure to hazardous solvent, which reduces safety risks in the lab and is better for my well-being."

—Pietro Leinardi

Flexible automation applicable to enhancing the efficiency of many laboratory workflows

Quantitation of mineral oil hydrocarbons, also referred to Hydrocarbon Index or H53, in water, soil and sediment samples is also a typical task for environmental testing laboratories. Preparation of water samples for analysis includes LLE extraction, which is followed by a cleanup step with Florisil® to remove more polar co-extracted components such as lipids. The purified extract can then be concentrated further or can be directly injected for GC-FID analysis.

After experiencing the benefits of the automated workflow for SVOCs analyses, VERITAS purchased a second TriPlus RSH autosampler solution to similarly automate LLE for GC-FID analysis of mineral oils in water. In this application, the robotic system works together with the intelligent sequencing capabilities of Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software to only perform the Florisil cleanup step on positive samples, accelerating sample throughput and conserving reagents. Data from Chromeleon CDS are automatically sent to the laboratory information management (LIMS) system for data management (Figure 4). Pintonello added, "Over time the TriPlus RSH solution has shown itself to be flexible, for example, we switched from using pentane to hexane, we modified the programming according to the prevalent type of samples analyzed, and we added racks to load a greater number of samples so analysis can continue on weekends."



	Sample 1	Sample 2	Sample 3
Total oil	X_1 mg/L	0	Y_1 mg/L
Mineral oil	X_2 mg/L	0	Y_2 mg/L

Figure 4. Schematic overview of the data structure and workflow using Chromeleon CDS Intelligent sequence. Samples highlighted in light blue are automatically generated in the sequence according to the System Suitability Test (SST) results.

Conclusion

For most laboratories, manual sample handling and preparation is a bottleneck that requires extensive time, labor, and costs, while adding complexity to sample and operating logistics. Automating procedures like LLE is possible using state-of-the-art TriPlus RSH autosampler technology for unattended operations that offer relief from high sample loads and tedious work. In addition, automated workflows reduce the variability associated with manual procedures and enable laboratories to scale down sample and solvent volumes and to use less hazardous chemicals, reducing operational costs and safety risks.

"Our relationship with Thermo Scientific is very good. An important aspect is the after-sales service specialists and technical assistance which help us work more efficiently, especially with state-of-the-art equipment, such as the autosampler with integrated LLE."

— Gilberto Pintonello

About Gilberto Pintonello

Gilberto Pintonello, is Supervisor of the Lab Chromatography Department at VERITAS S.p.A, an Italian provider of services in the environmental sector, focused on waste and drinking water testing. He has 40 years of experience in GC and GC-MS (VOC, PAH, and pesticides analysis), 30 years in ion chromatography (anions analysis) and almost 10 years in LC-high-resolution mass spectrometry (HRMS) (pesticides and PFAS analyses). He has also worked in pharmaceutical companies in analysis of active ingredients, and in other public companies in the area of drinking and wastewater analysis.

About Pietro Leinardi

Pietro Leinardi has been a Chemical Laboratory Technician at VERITAS Integrated Service Laboratory since 2003. He has more than ten years of experience in ion chromatography (anions analysis) and five years in GC and GC-MS analysis focused on nonhalogenated organics, PAHs, pesticides. During his career, Leinardi has also worked in food fraud control institutions, and in raw, tap and wastewater treatment laboratories.

About VERITAS Integrated Service Laboratory

VERITAS is a multi-utility provider serving the Veneto region of Italy, focused on waste collection and treatment and the integrated water cycle. The company is responsible for more than 5000 km of water network and more than 2000 km of sewerage, for a total of 120M cubic meters of distributed water and 92.5M cubic meters of treated wastewater. The company diligently controls and guarantees the quality of the water supplied to more than 790,000 residents and over 70,000 daily tourists in the region.

Accredia accredited since 1988, VERITAS Integrated Services Laboratory provides analytical support to the environmental chemical and biological sectors, focusing on wastewater, surface, and deep waters intended for human consumption. The laboratory produces more than 45,000 test reports per year and employs about 50 people, of which 25 are technicians performing chemical analysis. Active in the Permanent Water Study Committee of the Ministry of Health, the laboratory works on implementation and validation of new analytical methods to guarantee the utmost quality of the water distributed in the Veneto region. The laboratory is also a member of the ViveracquaLab Network, whose charter is to ensure operator compliance with water quality control legislation and support operators in the analytical control of discharges and purification plants by enhancing and sharing skills, knowhow, and resources, and to provide scientific and technical support for environmental and hygienic health issues.

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