

## EUROLAB adopts Orbitrap high-resolution accurate-mass (HRAM) solution for both targeted and untargeted contaminant analysis

“Our mission is the analysis of contaminants in environmental samples and consumer goods. As part of this mission, we use two Orbitrap HRAM LC-MS systems for screening, unknown identification, and quantitation. There are no operating difficulties with this mass spectrometer, only advantages”

—Dr. Massimo Peruzzo  
Partner and CEO, EUROLAB, Srl  
San Giuseppe di Cassola (VI), Italy

### Need for comprehensive analytical methods

EUROLAB, Srl focuses on the analysis of client samples for contaminants that impact product safety and environmental waters. Traditional contaminant monitoring strategies have involved targeted screening and quantitation of specific compounds. However, targeted methods address only a small portion of the potential universe of contaminants present in the environment and consumer products. The rise in unknown compounds that can have toxic effects is driving the need for advanced monitoring strategies that go beyond targeted screening and quantitation to comprehensive methods capable of revealing all compounds present in a sample. In addition to the challenges of comprehensive sample characterization, regulatory agencies continually lower contaminant levels and add new contaminants to monitor as toxicological evidence expands.



**Q Exactive Focus mass spectrometer**

“The advantages of using Orbitrap HRAM technology are time and cost savings—it doesn’t need an SPE column—and fewer errors are made because we don’t need an SPE pre-concentration step. Dirty samples typically require dilution, which compromises detection limits. However, using the Orbitrap solution we do not see a loss in sensitivity with diluted samples.”

—Dr. Massimo Peruzzo

### **Orbitrap HRAM solution expands and enhances analytical capabilities**

To address comprehensive sample analysis and dynamic regulatory requirements, EUROLAB, Srl relies on Thermo Scientific™ Orbitrap™ HRAM mass spectrometers and Thermo Scientific™ Compound Discoverer™ software. Using this powerful solution, the laboratory has expanded their capabilities beyond targeted analyses to include unknown identification with untargeted screening and retrospective data analysis. As a result, the laboratory can now address the increasing number and complexity of possible contaminants and matrices, with lower limits of detection and fewer false positives. Retrospective analysis of data allows the lab to investigate the presence of contaminants in samples collected and analyzed years ago. In a single injection, only Orbitrap technology provides the ultra-high-quality HRAM data needed to perform retrospective analysis.

### **HRAM technology provides lower detection limits for targeted analysis of compounds in complex matrices**

Prior to deploying Orbitrap instruments—Thermo Scientific™ Q Exactive Focus™ mass spectrometers—the laboratory used triple quadrupole LC-MS/MS methods for targeted screening and quantification. These triple-quadrupole methods easily met analytical needs when the contaminant limits were not too low, nor the sample matrices too complex. According to Dr. Peruzzo, Partner and CEO, EUROLAB, Srl, “In 2006, the European Reach Regulation (CE 1907/2006) required mandatory testing to determine if perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid PFOA are present at concentrations greater than 0.1%

w/w in matrices like textiles. Though this was the first time we faced the analysis, the regulatory limits at that time allowed LC-MS/MS to perform the method.”

However, in 2013 this all changed when water polluted with per- and polyfluoroalkyl substances (PFAS) put 250,000 Venetians at risk. “The regional authority set PFAS limits in drinking water, which were difficult to reach with triple-quadrupole sensitivity without using SPE enrichment,” said Peruzzo. SPE sample concentration is not only time-consuming, costly, and error-prone, it does not effectively capture short-chain PFAS.” Adding to the challenge, Venetian authorities went on to apply the same low limits—PFOS < 30 ng/L, PFOA < 500 ng/L, and the sum of other PFAS < 500 ng/L—to industrial wastewaters, a much more difficult-to-analyze matrix than drinking water.

Unlike drinking water, industrial wastes could contain organic solvent, which causes pre-concentration problems because the short chain PFAS (PFBA and PFBS in particular) aren’t captured on the SPE cartridges and are thus lost. However, many PFAS used today are those with short chains. “We not only had to meet the lower detection limits, we could not use SPE to pre-concentrate these samples. “The solution was to buy an Orbitrap instrument,” noted Peruzzo. The additional sensitivity of the Q Exactive Focus mass spectrometer method enables the laboratory to meet challenging contaminant monitoring requirements, even when samples are diluted up to 50 times. By avoiding SPE sample concentration, the laboratory reduces analysis time by about ten minutes (about 20%) per sample.

“When we changed from our triple-quadrupole mass spectrometer to the Orbitrap instrument, the percentage of false positives decreased dramatically. We were astonished. The reason was its high resolution.”

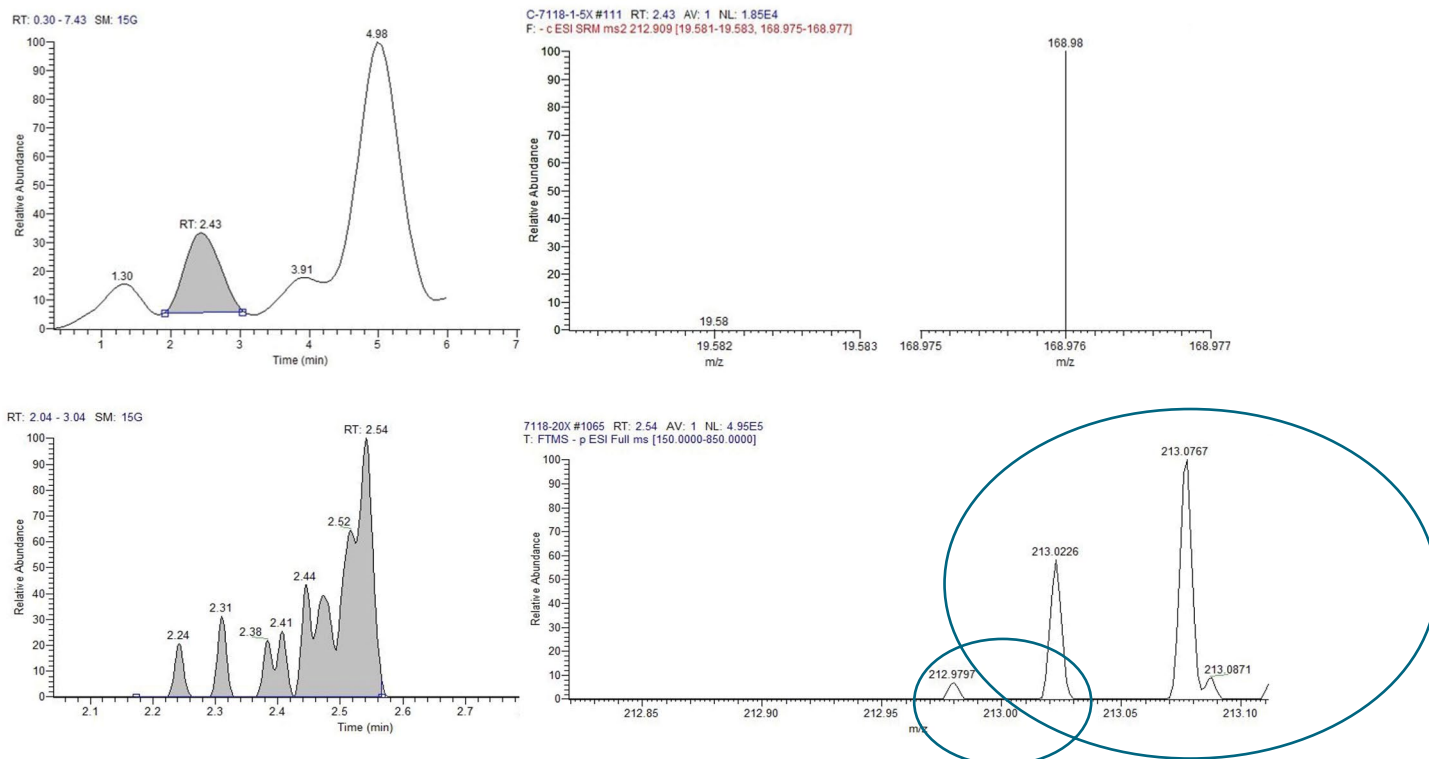
—Dr. Massimo Peruzzo

### Orbitrap HRAM technology reduces false positives

Another benefit of Orbitrap HRAM methods is fewer false positives. According to Peruzzo, “switching from triple-quadrupole to Orbitrap technology, we found that the percentage of positives in wastewater samples decreased from 60% to about 30% for the short chain PFAS.” Unlike other technologies, Orbitrap instruments generate HRAM data, which provides well-resolved exact mass measurements, enabling detection of compounds with very similar masses (Figure 1).



Industrial waste matrix



**Figure 1. Top: Triple quadrupole LC-MS/MS analysis of a wastewater sample.** At 2.4 min there was a peak ( $m/z$  213) with a fragment at  $m/z$  169 determined to be about 3 ppb PFBA. To check the result, the sample was fortified with 20 ppb of PFBA and the LC-MS/MS analysis repeated. The retention time and the fragmentation did not change, so the result appeared to be confirmed. Bottom: When running the sample on the Orbitrap mass spectrometer, four different compounds with a mass of about 213 were detected, but only one with the exact mass of PFBA ( $m/z$  212.9797).

“Using the Orbitrap instrument in full scan mode allows us to collect all the data, and then we can go back to the chromatogram and look for additional compounds if our clients request it. It’s faster and cheaper than rerunning samples”

— Dr. Gianluca Coppola

Research and Development Specialist, EUROLAB, Srl

## HRAM technology allows retrospective data analysis for added regulated and concerning compounds

In July 2018, a new pollutant of concern arose in Veneto: the potentially carcinogenic GenX—a fluorinated surfactant used to replace PFOA. With data collected using the Orbitrap mass spectrometers, the laboratory was able to perform retrospective analysis of samples previously

analyzed to search for GenX (Figure 2). “With triple-quadrupole mass spectrometer data there was no way to check if GenX was present in samples analyzed in the past. There was not a “historical” record to go back to look for compounds not targeted in analyses already performed,” said Peruzzo.

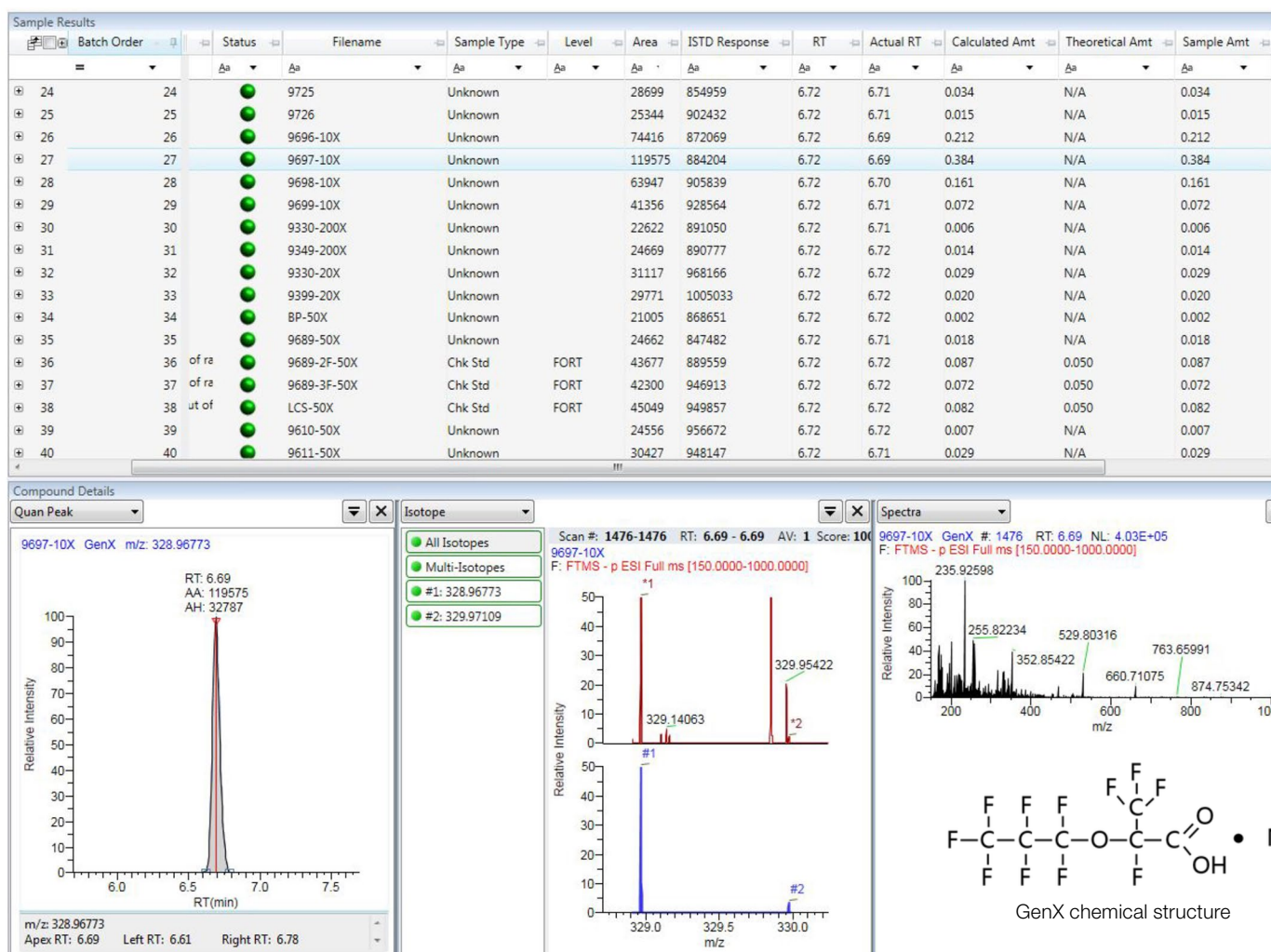


Figure 2. Retrospective analysis of Orbitrap HRAM instrument data shows that GenX was found in many data files collected from samples run at various times in the past. Retrospective analysis is not possible using MS/MS or Q-TOF data.



“Our customers want to know what is inside their samples—without a target list.”

— Dr. Massimo Peruzzo

### Full-scan methods minimize method setup

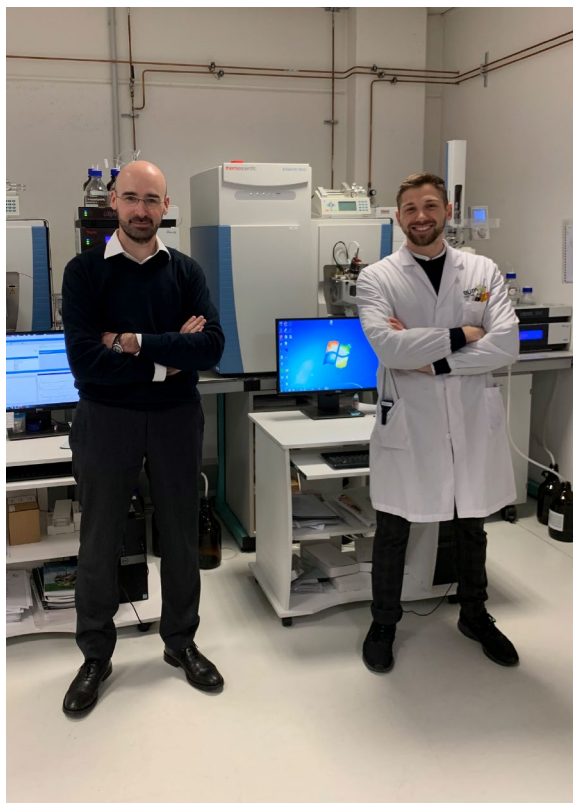
Setting up an Orbitrap HRAM method is remarkably easy. Unlike triple-quadrupole mass spectrometer methods that require users to specify retention-time windows and SRM transitions for each compound targeted, Orbitrap HRAM methods only require specifying the mass range to monitor. In addition, because Full-scan-mode methods do not depend on retention time windows, changes in

chromatography such as retention time shifts resulting from columns do not require rerunning samples. According Dr. Gianluca Coppola, Research and Development Specialist, EUROLAB Srl, “The Orbitrap mass spectrometer is very easy to use—just choose your desired mass range for full scan—and you can use normal chromatography. It’s very, very easy.”

### Orbitrap HRAM instruments provide a solution for unknown identification

The laboratory’s clients often request an assessment of all contaminants in a sample without specifying specific compounds or compound classes. The advantages of full-scan HRAM data for comprehensive sample characterization are unmatched. Though triple-quadrupole mass spectrometers provide selective monitoring of known (targeted) compounds with Selected Reaction Monitoring (SRM), their capabilities are limited when it comes to unknown identification.

Compound Discoverer software capabilities, such as mass spectral library searching against the online Thermo Scientific™ mzCloud spectral library, laboratory-developed Thermo Scientific™ mzVault™ spectral libraries, and third-party databases like ChemSpider™, streamline unknown identification. As Coppola described, “We use Compound Discoverer software for all unknown analyses. There is no comparison to the other software products I’ve tried. It is much better for its user-friendly interface, the quality of the data, and its use of custom or pre-defined workflows.” If the unknown compound is not identified using a library or database search, the user can identify the best candidates using spectral similarity searches and sub-structural elucidation in silico.



Dr. Massimo Peruzzo and Dr. Gianluca Coppola

## Untargeted Orbitrap HRAM method characterizes micro-pollutants in wastewater

In collaboration with the Water Research Institute-National Research Council of Italy, the University of Insubria, Italy, and Thermo Fisher Scientific, the laboratory applied an untargeted Orbitrap HRAM method to wastewater samples to characterize micro-pollutants removed, persisting, and formed during treatment processes.<sup>1</sup> HRAM data were processed in Compound Discoverer software using peak picking and integration, retention time alignment, isotope and adduct peak grouping, unknown compound grouping

(to form suspect hits), and grouping of suspect hits across samples (Figure 3). Possible elemental compositions were predicted for each suspect hit and compound search using ChemSpider and the mzCloud mass spectral library. Their work found the presence of untargeted contaminants including antiretroviral drugs in sewage from a hospital, poorly removed surfactants and their homologues and transformation products, and triphenylphosphine oxide (TPPO), a toxic compound found in pharmaceutical industry effluents.

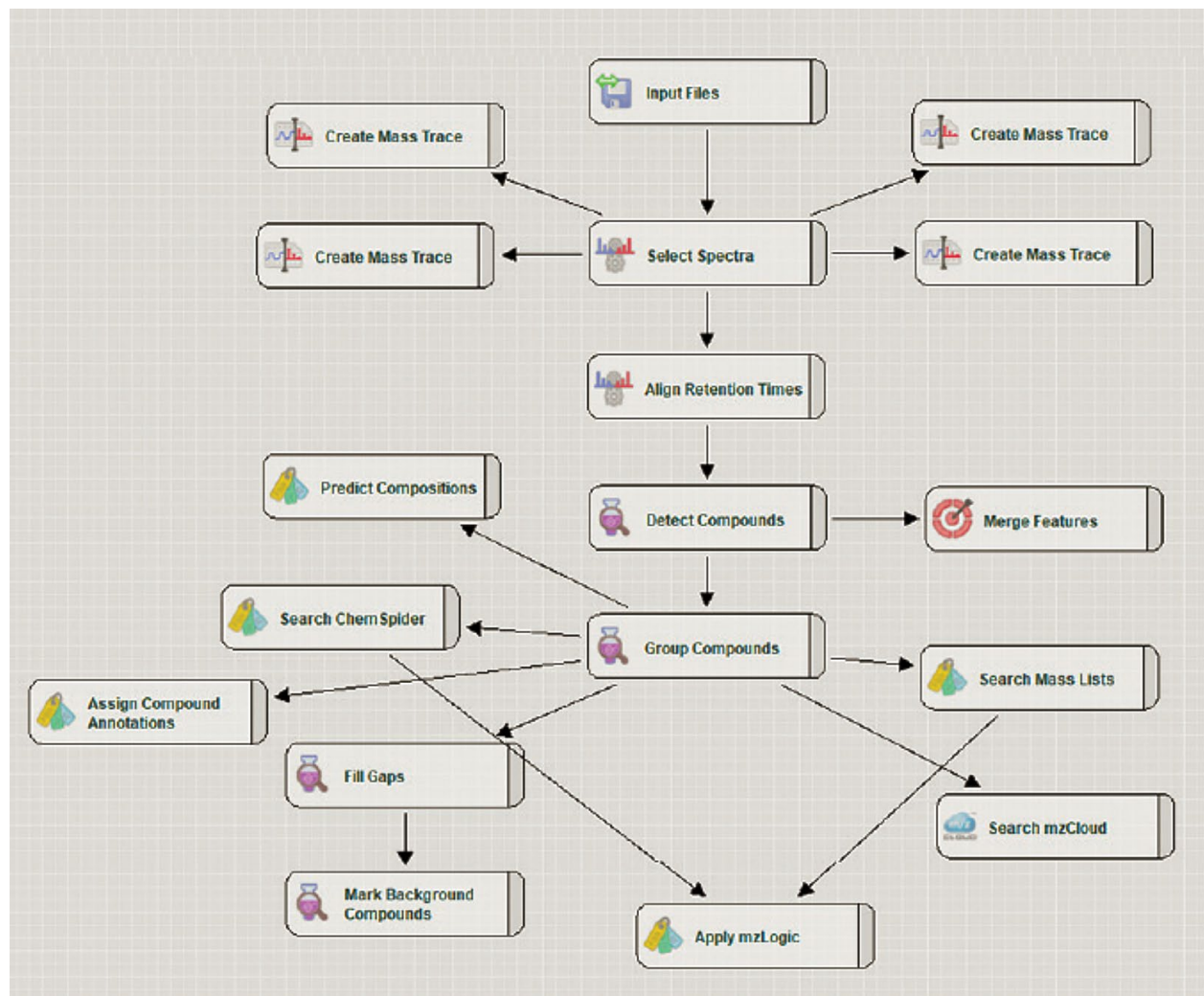


Figure 3. Compound Discoverer software data-processing workflow for the wastewater samples studied

## Conclusion

With the ever increasing numbers of contaminants that must be screened and quantified at increasingly lower concentrations, and in a wider variety of sample matrices, the need for powerful and accurate high-resolution LC-MS instruments will continue to rise. Though targeted contaminants can be detected and quantified using triple-quadrupole mass spectrometer methods, comprehensive characterization of samples for unknown emerging contaminants requires high-resolution full-scan LC-MS methods. Orbitrap HRAM methods not only increase the potential targets monitored, they reduce false positives and per-sample costs, save time, provide more confidence in compound identifications, and allow retrospective data analysis.

Orbitrap HRAM instruments provide a one-instrument solution for unknown identification, untargeted and targeted screening, and targeted quantitation that is accurate with a simplified user experience that does not

require complicated, time-consuming method development and sample preparation. It is for these advantages that EUROLAB, Srl relies on their Q Exactive Focus mass spectrometers and Compound Discoverer software.

## About EUROLAB

EUROLAB Srl is focused on analysis of consumer goods, environmental samples, textile samples, and Extractables and Leachables analysis. All the analysis methods used are recognized worldwide. Its laboratories exist to meet customer demands in a high-quality, fast, accurate, and reliable way using state-of-the-art equipment, and experienced staff.

## Acknowledgements

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### About Dr. Massimo Peruzzo and Dr. Gianluca Coppola

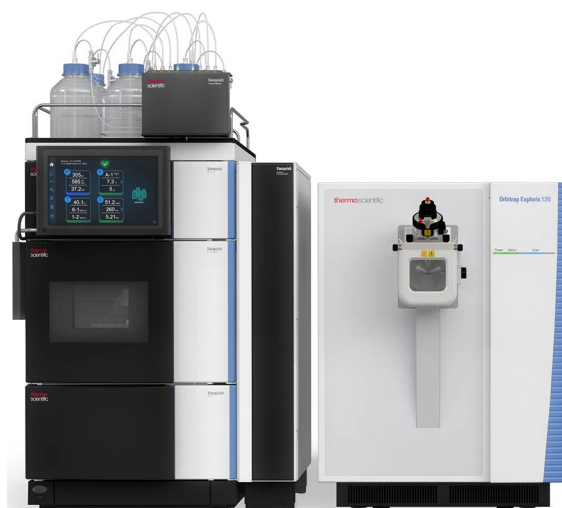
Dr. Massimo Peruzzo is Partner and CEO of EUROLAB Srl, having been with the company since 2004. Dr. Peruzzo has a degree in Chemistry from Padua University and MBA from CUOA Business School.



Dr. Gianluca Coppola is the Research and Development Specialist of EUROLAB Srl since 2019. Dr. Coppola has a Master Degree in Food Science and Technology from University of Udine and multiple years of experience in LC and GC-MS method development.

#### References and additional resources

1. Identification of Parent and TP Compounds in Wastewater Effluents by non-Target Analysis. Francesca Cappelli *et al.*
2. Perfluoroalkyl acids concentrations in liquid wastes: a survey campaign and implications for waste disposal. Massimo Peruzzo *et al.* Norman Bulletin | Issue 6 | October 2019 (<http://www.norman-network.net>)
3. From Sample to Knowledge: Strategies for Overcoming Challenges of Emerging Contaminants - PFC and Surfactants. Thermo Scientific Webinar. <https://www.thermofisher.com/us/en/home/global/forms/industrial/sample-knowledge-strategies-overcoming-challenges-emerging-contaminants-pfc-surfactants.html>



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