Case study | 003406

Food safety

Nestlé develops an Orbitrap Exploris 480 mass spectrometer method for wide-scope analysis of pesticides and natural toxins in foods

Introduction

Nestlé is committed to ensuring the highest standards of food quality and safety through rigorous research and innovation. Located in Lausanne, Switzerland, Nestlé Research plays a pivotal role in developing analytical methods for monitoring chemical contaminants in food products. These robust methodologies are implemented across Nestlé's global laboratory network to screen, identify, and ensure compliance with food safety standards.

In response to the challenges posed by climate change, evolving regulations, and shifting consumer preferences, Nestlé Research has developed a QuEChERS-based liquid chromatography high-resolution mass spectrometry (LC-HRMS) workflow. This innovative approach allows for the simultaneous analysis of over 1,100 contaminants, including pesticide residues, mycotoxins, and plant toxins, across various food matrices such as cereals, fruits, and vegetables. The method's sensitivity and low quantification limits ensure compliance with regulatory standards while facilitating the exploration of additional compounds to preemptively address future food safety risks.

> “The LC-HRMS workflow is built to be a fully confirmatory approach in one single injection thanks to full-scan and fragmentation experiments.”
>
> –Thomas Bessaire, Scientist, Nestlé Research

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“We develop the analytical methods that support the company in ensuring safety and quality of food products and develop tools that prepare us for the future through research and knowledge creation. High-resolution mass spectrometry is a key player in achieving these goals.”

—Dr. Thierry Delatour, Group Leader, Nestlé Research

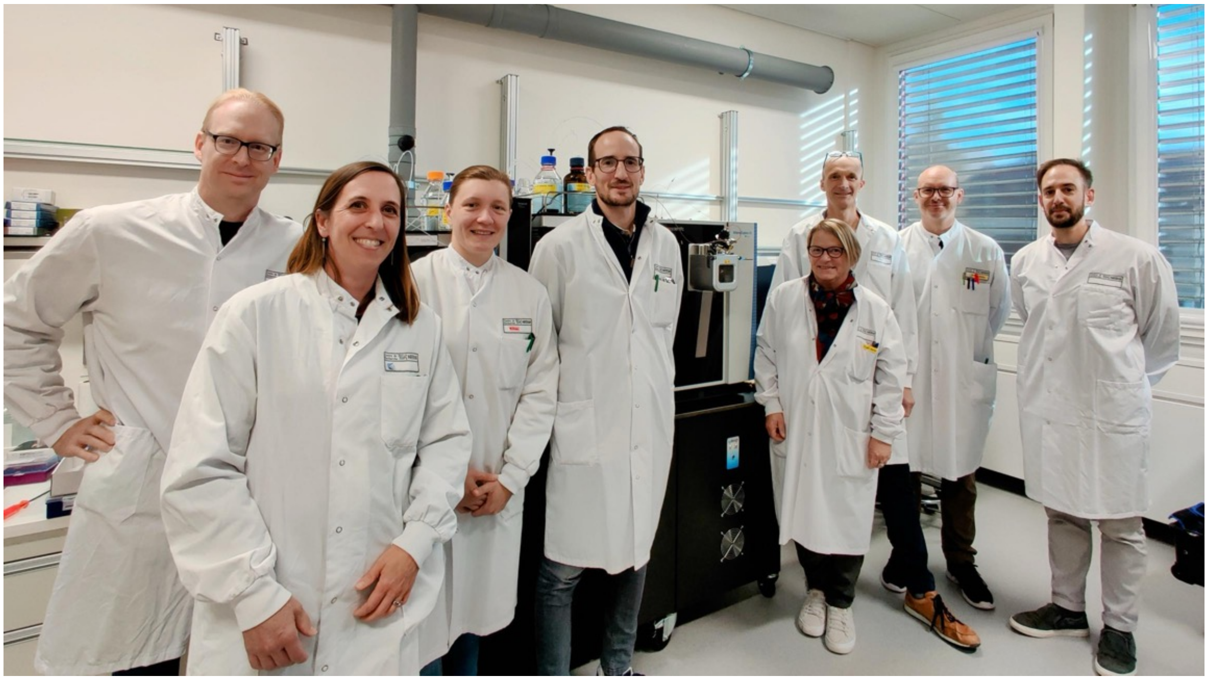

Figure 1. LC-HRMS method development team at Nestlé Research in their laboratory in Lausanne, Switzerland, with the Orbitrap Exploris 480 mass spectrometer. Photo courtesy of Nestlé.

Harnessing innovative technology for broader surveillance of chemical contaminants in food

Dr. Thierry Delatour, Group Leader, Nestlé Research explained, “One of our major responsibilities is to develop analytical methods that will be deployed for routine testing. Here we make sure the scope and performance of our methods meet regulatory requirements. We also work on specific R&D projects to understand chemical contaminants across the food chain, from farm to plate, to understand the occurrence of emerging safety and quality risks. For these investigations, we think more broadly about preventing future chemical safety risks. We develop analytical methods that support the company in ensuring safety and quality of food products and develop tools that prepare us for the future through research and knowledge creation. High-resolution mass spectrometry is a key player in achieving these goals.”

Another important part of the Nestlé Research mission is to share best practices and knowledge throughout the scientific community. For this reason, its staff regularly publish methods and research in peer-reviewed publications and participate in method-standardization efforts. Dr. Delatour said, “Nestlé publishes on a regular basis for two reasons. The first is that food safety should not be a competitive advantage. Anything that will improve the safety of food products should be shared to help others likewise improve the safety of their products. The other reason is to prevent misalignment by ensuring that reliable science-based testing methods can be harmonized across different labs and the food industry. We also contribute to method-standardization programs to make sure that there is no misalignment in methods and results. If the same methods are used, the risks of inconsistent results are reduced.”

> "The occurrence and management of contaminants is evolving and becoming more complex. We need tools that cover a broad range of contaminants in a single analysis, not only to assure compliance to regulations but to anticipate food safety risks. This is the evolving landscape that has prompted us to think differently and develop a broad method in terms of compounds and matrices."
>
> —Thomas Bessaire

Complexity drives needs for wider scope methods

Climate change, continuously evolving regulations, and shifts in consumer diets are increasing the number and diversity of compounds and matrices that must be considered during method development. Thomas Bessaire, Scientist, Nestlé Research, described the drivers that are changing the occurrence and management of chemical contaminants. "Climate change—higher temperatures, intense rainfalls and drought periods, and others—are expected to contribute to an increase in pest populations that will likewise require increased pesticide use. Climate change is also known to modify the distribution of fungi, and thus the distribution and occurrence of mycotoxins in crops. New agricultural practices like organics and regenerative agriculture also have an impact on the occurrence of chemical contaminants in food. Then there are consumer trends. We are seeing an increased demand for plant-based food, and therefore we must consider the natural toxins that are present in certain plant-based ingredients. Add on top of these factors, global free trade where ingredients move from one country to another where regulations differ."

This evolving complexity, along with the fact the current GC-MS/MS and LC-MS/MS targeted triple quadrupole methods are limited to a few hundred contaminants per method, drove Nestlé Research to seek a new approach. Said Bessaire, "We need tools that can analyze a broad range of contaminants in a single analysis to assure compliance and to anticipate the food safety risks. This is the evolving landscape that prompted us to think differently and to develop a broad method in terms of compounds and matrices."

Rigorous method development and validation

Maximizing the target list, compound identification reliability, and quantification capacity from a single injection of food extract for routine analysis required a rigorous method-development and validation process. Figure 2 depicts the steps used for method development and validation. Further details are in the article, "Enhanced Surveillance of >1100 Pesticides and Natural Toxins in Food: Harnessing the Capabilities of LC-HRMS for Reliable Identification and Quantification."[1]

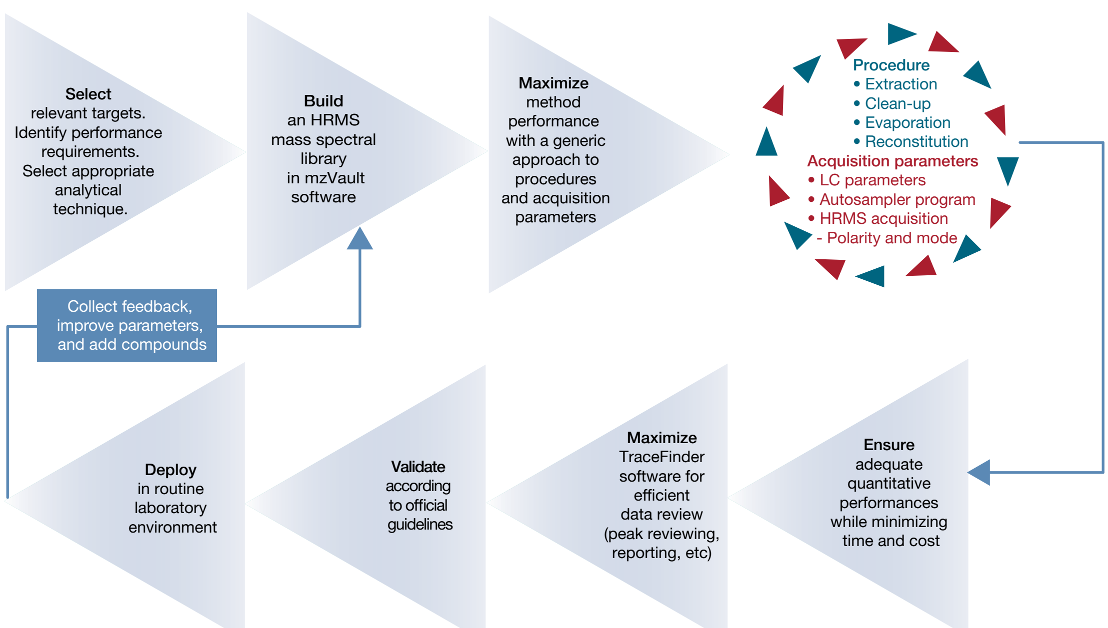

Figure 2. Overview of the steps used to develop and validate the wide-scope, multi-contaminant method

“Because of the number of analytes to measure, high-resolution technology with accurate mass is an advantage. In theory, full-scan high-resolution accurate-mass acquisition can analyze an unlimited number of analytes.”

—Dr. Nicolas Christinat, Scientist, Nestlé Research

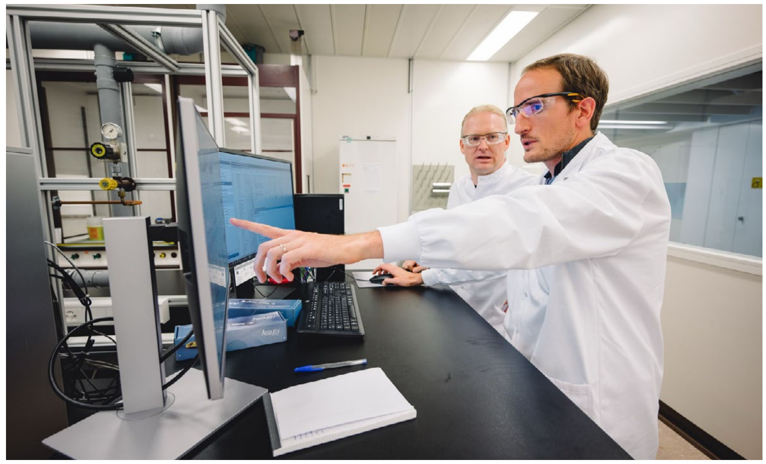

Figure 3, Dr. Nicolas Christinat and Thomas Bessaire review data in TraceFinder software. Photo courtesy of Nestlé

Desired compound scope

The starting point was to establish a comprehensive list of pertinent target analytes. Natural toxins for which maximum levels are already in place or under discussion were considered, as well as toxins regularly or recently mentioned in scientific articles and in European Food Safety Authority (EFSA) calls for occurrence data. For pesticides, an extensive data review focusing on EU regulations, pesticides monitoring programs, scientific articles, and recently registered pesticides were considered among other sources.

Technology selection

Next, Nestlé Research scientists aimed to identify the most suitable technology to address the challenges associated with broad-scope contaminant analysis. The versatility and high selectivity of LC-HRMS for screening a broad range of compounds offered a solution. Theoretically, in full-scan mode, HRMS instruments can monitor an unlimited number of molecules simultaneously, opening up avenues for continuous extension of the method’s scope. The high-quality, high-resolution accurate-mass (HRAM) data provided by the Thermo Scientific™ Orbitrap™ mass analyzer can help differentiate between compounds with similar masses and isobaric interferences from coextracted matrix components, reducing the chance of false positives. High-resolution mass spectrometry (HRMS) systems offer the capability to perform nontargeted or retrospective analyses by leveraging existing data and potentially identify novel contaminants.

Due to its sensitivity and HRAM data quality, the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer was evaluated and ultimately selected for workflow development. The developed method uses the full-scan MS/data-dependent MS^2 (FS-ddMS^2) acquisition mode, which combines a full scan with MS^2 scans on the targets of interest measured in the full scan.

Necessary compromises

Though HRMS technology theoretically offers an unlimited scope of analysis, the true scope of analysis remains limited by the sample preparation method, chromatographic conditions, and MS ionization efficiency. According to Bessaire, “We needed to compromise on sample preparation and extraction, the LC method—the column and the gradient—and MS ionization mode because not all organic molecules can be analyzed using a single ionization mode. The scope of compounds that we dreamt about at the beginning was not the same as what we had at the end.”

With these compromises in mind, a QuEChERS-based sample preparation followed by dispersive solid-phase extraction (dSPE) cleanup was chosen due to its effectiveness for a wide range of sample types and analyte chemistries. LC method development was carried out on the Thermo Scientific™ Vanquish™ Horizon UPHLC system. A 22 minute LC run using acidic mobile phases on a 15 cm reversed phase (RP) C18 column with a smooth gradient slope was chosen to retain a broad range of chemicals with various polarities, while separating a maximum number of isomers and isobaric compounds within an acceptable run time. The generic sample preparation approach has advantages as Dr. Flavia Nagy (-Badoud), Scientist, Nestlé Research noted, “With the generic multiresidue method, we can reduce the use of solvents and work towards greener chemistry.”

> "Building the mzVault spectral library is the best advice we can give, starting from a solid foundation using reference analytical standards. It's a lot of time to invest and a lot of money for the standards, but in the end, it produces very good quality data and from there you can build solid methods."
>
> —Dr. Nicolas Christinat

In-house HRAM mass spectral library maximizes compound identification

To maximize the method's capability to unequivocally confirm the presence or absence of numerous compounds from a single injection, the group developed an in-house HRAM mass spectral library populated in Thermo Scientific™ mzVault™ software. Creating the library was time-consuming and involved injection and analysis of individual standard solutions for each compound. Dr. Nicolas Christinat, Scientist, Chemical Contaminants, Nestlé Research described the library creation process, "We relied on analytical standards for each molecule. That's a lot of individual injections and we acquired multiple parameters for each, including different fragments at different collision energies, at different polarities, and for different adducts. All this information was stored in our mzVault spectral library, and we can use that information to build targeted methods that use the most relevant collision energy, most abundant ions, and the best polarity. Bessaire added, "Our internal mass spectral library was key to maximizing the identification capabilities of our method."

Ensuring adequate quantitative performance

After extensive work to maximize the scope of the targeted molecules that could be identified in food extracts with high reliability, Nestlé Research scientists tackled the challenges of multiresidue quantification. Bessaire noted, "It was a compromise between the cost of the analysis, the sample throughput for efficient turn-around times in high routine environments, and the method performance that we wanted to reach." In this case, the use of an external calibration curve was perceived to be the best compromise, as evidenced by the overall method performance characteristics.

Validating overall method performance

The method was validated per SANTE/11312/2021 version 2 and its ruggedness assessed with the strong support of Nestlé's laboratory in Singapore, which was equipped with the same instrument setup. The validation determined that 92% and 98% of the target compounds met quantification criteria at the lowest validated level in the cereal, fruit, and vegetable matrices. The labs also applied the method to explore the occurrence of various target contaminants in 205 real-world cereal and grain samples collected worldwide. The results of these studies are presented in the article.[1]

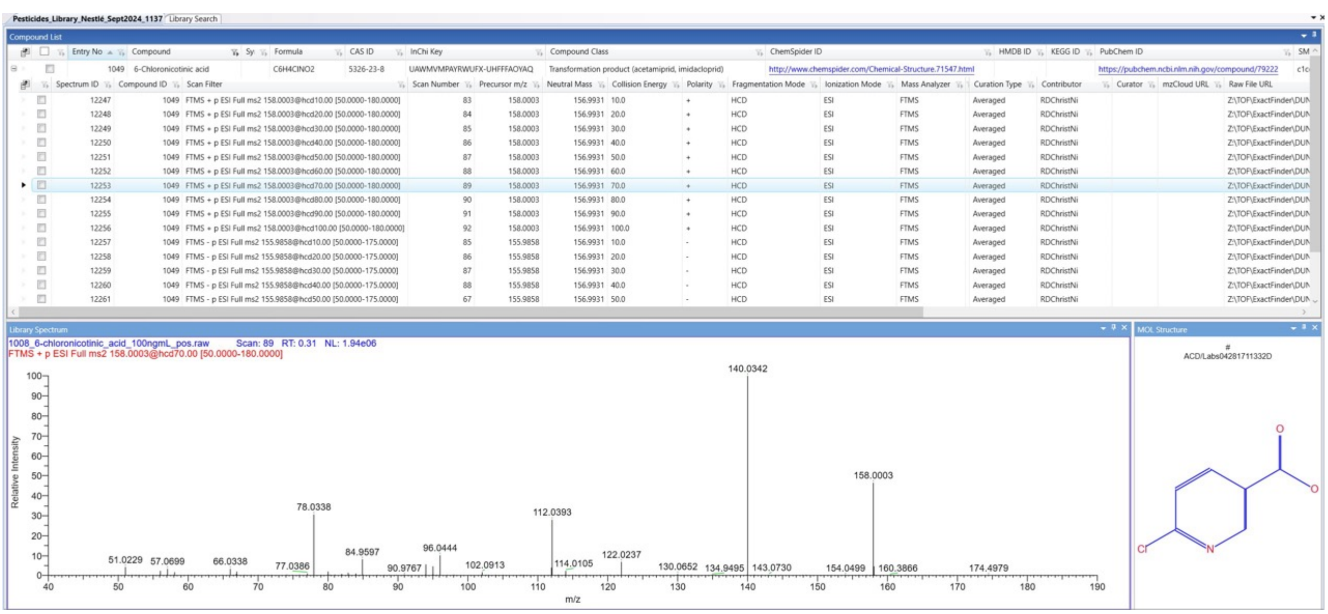

Figure 4. mzVault software library entry for 6-chloronicotinic acid, collected in positive electrospray ionization mode. Image courtesy of Nestlé.

"We are fully convinced that the data quality is there. In one working day, we can deliver a fully confirmatory analysis for more than one thousand molecules in one single injection. This is the most important advantage of this platform. There is a huge value for a very low effort."

—Thomas Bessaire

Orbitrap Exploris 480 mass spectrometer essential capabilities

The Orbitrap Exploris 480 mass spectrometer platform provides capabilities essential to developing methods that provide reliable identification and quantification.

Sensitivity

First, the platform offers good sensitivity compared to other HRMS instruments. Aurélien Desmarchelier, Scientist, Nestlé Research explained, "We need state-of-the-art instruments when we develop new methods. We evaluated the Orbitrap Exploris 240 and Orbitrap Exploris 480 mass spectrometers. The Orbitrap Exploris 480 system's data pointed to a bit more sensitivity and that's why we selected and are recommending it to the laboratories that will be implementing the method, so we are aligned." Dr. Nagy (-Badoud) added, "Contaminant analyses are always at low levels—parts per billion normally—so it is very important that we have a sensitive instrument. That's mostly why in the past HRMS was not considered and contaminant analysis was done by triple quadrupole instruments with targeted approaches. One of the most important criteria was that we reached the required sensitivity."

HRAM data

Orbitrap mass analyzer technology produces true high mass resolution and mass accuracy in one compact, easy-to-use instrument that reliably and routinely achieves sub-ppb sensitivity. Desmarchelier noted, "Unlike LC-MS/MS methods that require extensive optimization prior to the first injections, LC-HRMS methods can be run in a matter of minutes with minimal input, such as generic source conditions, ionization mode, and a mass range for full scan analysis. The high mass accuracy in MS^1 enables direct searches for specific masses. Additionally, when paired with MS^2 experiments, the precision achieved on fragment ions further enhances the versatility of HRMS, allowing it to address target, suspect, and non-target analyses." Bessaire continued, "We are fully convinced that the data quality is there. In one working day, we are able to deliver a fully confirmatory analysis for more than one thousand molecules in one single injection. This is the most important advantage of this platform. There is a huge value for a very low effort."

Software's role

The large amount of information available during method development and generated during sample analysis requires substantial data processing. In this context, mzVault software and Thermo Scientific™ TraceFinder™ software provide advantages. Dr. Christinat commented, "With the system and the software, we get better information for the same amount of work. The mzVault software is very useful for library building and visualization. TraceFinder software also helps with visualization of results for pre-reporting—it's very flexible in that respect. We are also able to easily plug its Excel™-like tabular results into our R-script custom software[3] to get the specific results we want and that's convenient."

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Retrospective analysis

Full-scan HRAM data enables retrospective interrogation of existing data for compounds not initially targeted. Dr. Christinat described how this capability was used to address the evolving regulatory landscape, "We had a case where a new transformation product was mentioned related to the regulation of a specific pesticide. We used retrospective data analysis—we went back to our data to check if this transformation product was present in our samples, and we found it (Figure 5). And because we can find it, we were confident about including it in the method. That's another way we use LC-HRMS."

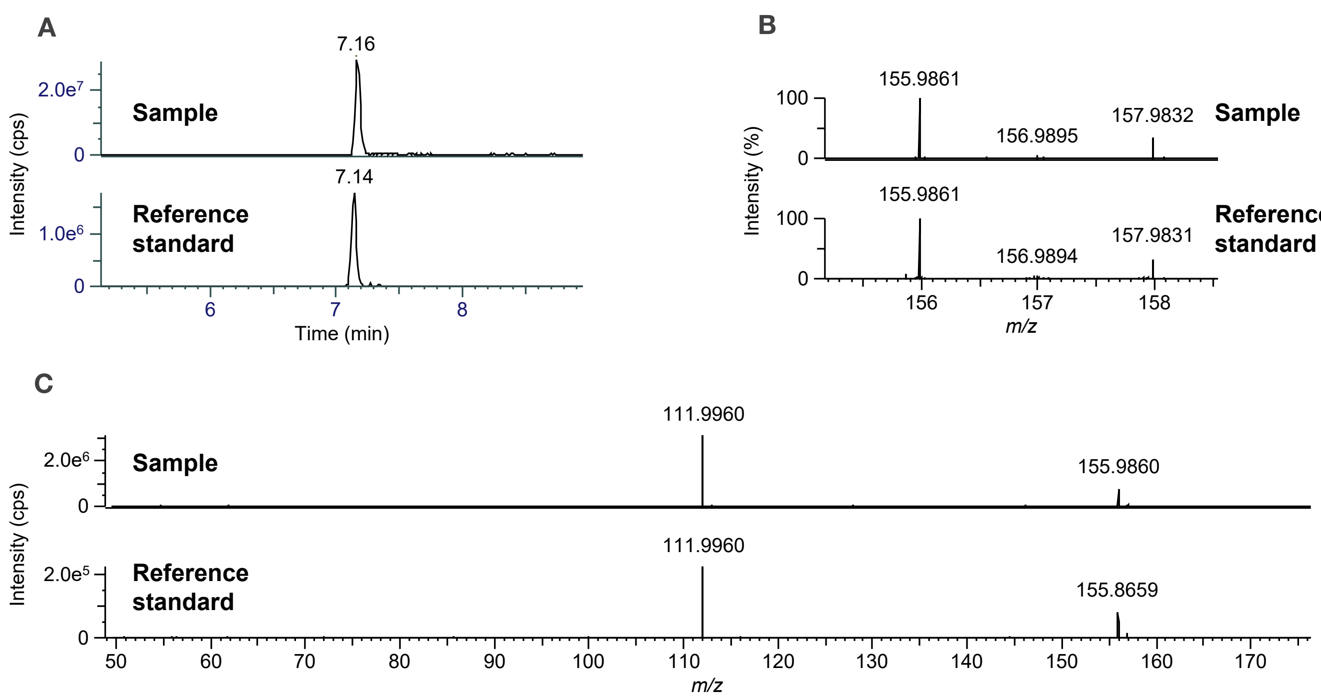

Figure 5. Retrospective analysis of 6-chloronicotinic acid, a transformation product of imidacloprid. (A) Extracted ion chromatogram (*m/z* 155.9858) in ESI negative. (B) Corresponding spectrum in full scan. (C) Fragmentation spectra from precursor ion *m/z* 155.9858 at 41 normalized collision energy.

> “There are compounds that will need specific sample prep, ultra-sensitivity, and LC-MS/MS analyses using dedicated methods to demonstrate the full compliance of samples. HRMS generates a lot of additional information to help optimize our existing portfolio of LC-MS/MS methods, and, very likely, we will decrease the number of analyses performed using LC-MS/MS in favor of HRMS. It allows us to optimize the work in the lab to be more effective and efficient.”
>
> —Dr. Thierry Delatour

Figure 6. Dr. Nicolas Christinat and Thomas Bessaire review data in TraceFinder software.
Photo courtesy of Nestlé.

Complementary HRMS and LC-MS/MS technologies enable optimization of analytical work

HRMS and LC-MS/MS technologies are considered complementary because while LC-MS/MS excels at highly selective quantification of known compounds at very low levels, LC-HRMS enables extensive multi-residue analyses and offers high mass accuracy in both MS^1 and MS^2 modes, making it suitable for suspect and non-target analyses. The complementary nature of the two technologies provides labs with opportunities to optimize their analytical workflows. Dr. Delatour explained, “We see high resolution as both an opportunity to do quality assurance and generate more data for investigations. The way we will use it in our labs depends on the request. There are some compounds that will need specific sample prep, ultra-sensitivity, and LC-MS/MS analyses to demonstrate the full compliance of samples. HRMS generates a lot of additional information that will help optimize our existing portfolio of LC-MS/MS methods, and, very likely, we will decrease the number of analyses performed using LC-MS/MS in favor of HRMS. We see lots of opportunities to use HRMS to optimize the work in the lab to be more effective and efficient.”

Bessaire added, “We don’t claim that such an LC-HRMS method will replace all LC-MS/MS methods at this stage. There are specific cases where dedicated and traditional methods are still important. There are compounds that require dedicated conditions for which the extraction or HPLC separation is totally different than what is in the method. All the GC-amenable compounds will never fit in the method. The other example is the need for low sensitivity. The method will not work for that analysis because a dedicated cleanup step is needed to enrich the final extract to achieve those levels.” Desmarchelier summarized, “For pesticide analysis and plant toxins, we saw an opportunity to go beyond what was done before using LC-MS/MS and switch to LC-HRMS.”

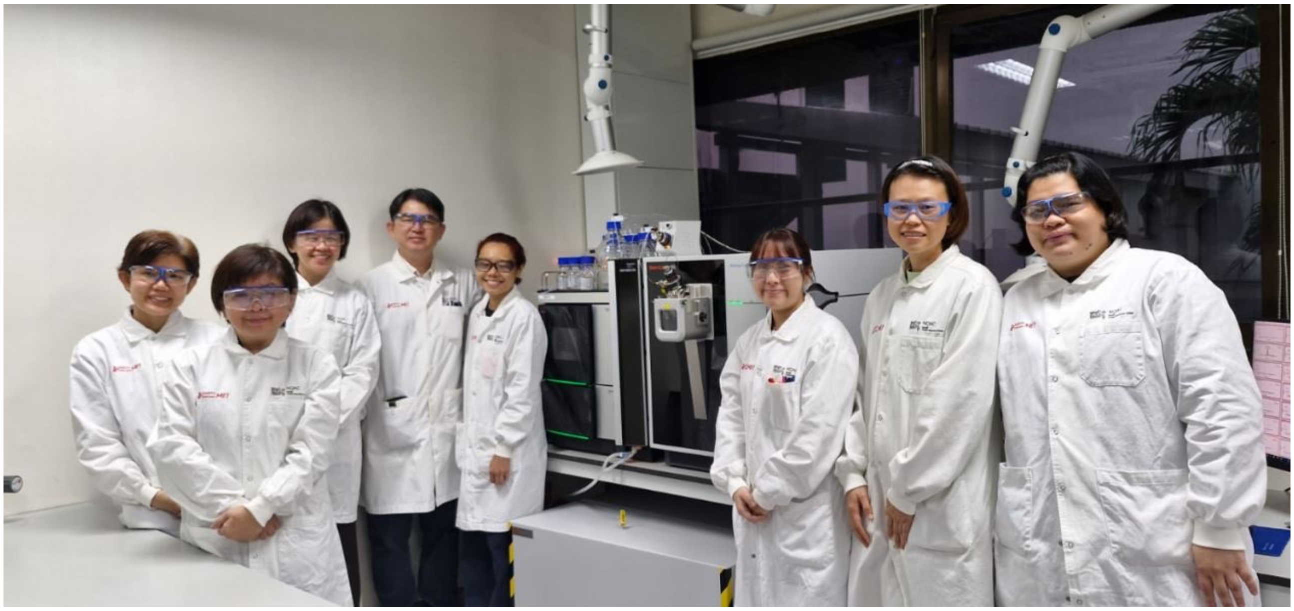

Figure 7. Nestlé Quality Assurance Center in Singapore. The analytical team with the Orbitrap Exploris 480 mass spectrometer used to run the LC-HRMS wide-scope, multi-contaminant method. Photo courtesy of Nestlé.

Future plans

In the future, scientists at Nestlé Research will continue to improve the method based on feedback from the labs that have deployed it on this journey in Singapore and Cergy. Bessaire noted, "As of today, two routine labs have embarked on this journey. Our lab in Singapore is fully operational and runs samples daily on the platform. Now we are in the process of implementing it at our lab in France, and they should be ready to perform routine analyses in the coming weeks." The successful transfer of this method across multiple sites has not only demonstrated the technology's robustness but also its capability to adapt within laboratories that have traditionally relied on classical LC-MS/MS platforms for their analytical needs. However, to fully harness the advanced capabilities of LC-HRMS technology, Nestlé Research foresees additional needs for specialized training and skills development. The ability to monitor many pesticides and natural toxins using the Orbitrap Exploris 480 mass spectrometer is worth the effort since it offers more comprehensive analyses and the potential for discovering novel compounds, positioning labs at the forefront of analytical science.

Already identified as an area for improvement, the group is looking for ways to streamline data processing. Said Bessaire, "Decreasing the time required to interpret and report the data is where we have focused our work over the past few months. It's where we push TraceFinder software to its maximum and then we plot it with our own internal R-script to help the operator to focus on what is important—what went well, what went wrong, what is present, and what is absent—because otherwise they could spend days on the data. With filtering options and color-coding, we are able to dig into what is needed. What we need to do in the next year is to improve the software to generate the final report as quickly as possible."

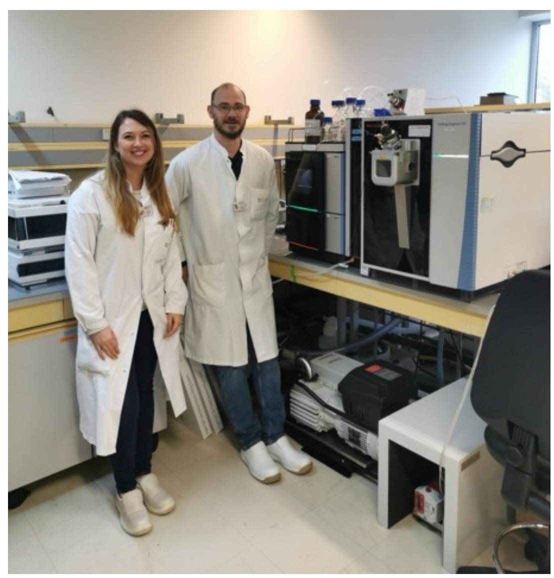

Figure 8. Nestlé Quality Assurance Center in Cergy, France. The analytical team with the Orbitrap Exploris 480 mass spectrometer used to run the LC-HRMS wide-scope, multi-contaminant method. Photo courtesy of Nestlé.

About Dr. Thierry Delatour

Dr. Thierry Delatour, Group Leader at Nestlé Research. His team is focused on projects for product innovation and scientific support and advice for routine laboratories in quality control. Currently, his team is devoted to wide screening of various chemical compounds by high-resolution mass spectrometry and mechanistic studies of process contaminants for deployment of effective mitigation strategies. Since joining Nestlé's research group in 2000, he has developed substantial expertise in the analysis of chemical contaminants and adulterants in food, particularly by liquid chromatography mass spectrometry. Delatour's research is focused on multi-residue analysis, root cause investigations, and mechanistic studies, and he interacts with AOAC Int., CEN, and ISO/IDF to promote reliable control of contaminants and adulterants in food products. He obtained his PhD at the 'Centre d'Études Nucléaires de Grenoble' (France) in 1998.

About Thomas Bessaire

Thomas Bessaire, Scientist at Nestlé Research. Currently, he supports Nestlé businesses by providing state-of-the-art analytical methods and scientific expertise for chemical contaminants analysis in foods including mycotoxins, plant toxins, veterinary drugs, process contaminants, pesticides, detergent residues, and dyes and food adulterants. Bessaire is a member of the European CEN Experts Panel on Biotoxins and AOAC Int. and earned his MSc in Analytical Chemistry from Montpellier Graduate School of Chemistry.

About Dr. Nicolas Christinat

Dr. Nicolas Christinat, Scientist at Nestlé Research. He joined Nestlé Research in 2013 as a lipidomics specialist before specializing in the analysis of chemical contaminants in food. He holds a PhD in Chemistry from the École Polytechnique Fédérale de Lausanne and has been working with high-resolution mass spectrometry instruments for more than 15 years.

About Aurélien Desmarchelier

Aurélien Desmarchelier, Scientist at Nestlé Research. A specialist in the analysis of chemical contaminants (veterinary drugs, natural toxins, pesticides) and process contaminants (acrylamide) in foods by LC-MS/MS and LC-HRMS, Desmarchelier joined Nestlé Research in 2007. He is the author of 30 peer-reviewed publications and a member of the AOAC. He earned his master's degree in environmental and industrial biotoxicology at Université Lille 2 in 2004.

About Dr. Flavia Nagy (-Badoud)

Dr. Flavia Nagy (-Badoud), Scientist at Nestlé Research. Dr. Nagy joined Nestlé in 2015, where she focused on developing innovative mass spectrometric approaches such as high-resolution, tandem mass spectrometry and isotope-labeled experiments to address food safety and quality topics spanning from lipid oxidation products, pesticides, plant toxins to environmental contaminants. Her educational background started in pharmaceutical sciences at the University of Geneva and Lausanne, where she also obtained her PhD by developing high-resolution mass spectrometric approaches for antidoping control. Her exceptional work during this period earned her a fellowship sponsored by the Swiss National Foundation, which allowed her to conduct her postdoctoral research in the field of nutrigenomics using transcriptomic and GC-MS/MS technologies at the University of Guelph in Canada.

Nestlé Research, Lausanne, Switzerland. Photo courtesy of Nestlé.

About Nestlé

Headquartered in Vevey, Switzerland, Nestlé's purpose is to unlock the power of food to enhance the quality of life for everyone, today and for generations to come. To achieve this, the company pursues growth through rapid innovation, operational efficiency, and disciplined resource and capital allocation. Nestlé has products for people at all life stages that can be consumed as part of a balanced diet. The company strives to contribute to people's health through nutrition and to help people of all ages live better. With 275,000 employees working in more than 340 factories in 77 countries, and products sold in 188 countries, Nestlé is truly a global company.

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