CASE STUDY 10750

Versatile, highly sensitive and reproducible: How Orbitrap Exploris GC 240 strengthens metabolomics

Introduction

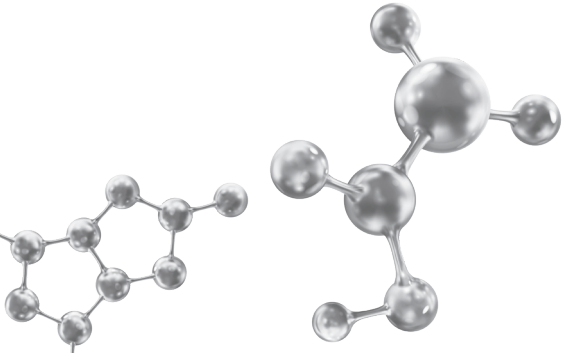
Over the last decade, metabolomics has demonstrated immense potential in accelerating biomarker research in human health and disease. The metabolome, influenced by both genetic and environmental factors, closely represents the underlying molecular phenotype, having direct applications towards both basic and translational research. Dr. John Bowden's work at the University of Florida takes the 'omics' approach to study biomarkers associated with pollutant exposure. His laboratory focuses on two main research questions: (1) what effect does the environment have on human and wildlife health, and (2) what effect does human activity have on environmental health. To tackle these research questions, the laboratory develops and employs mass spectrometric workflows to measure the presence of environmental pollutants as well as metabolomic workflows to identify biomarkers related to exposure. As industry experts in mass spectrometry (MS) techniques, the Bowden laboratory is prolific in its diverse MS-based metabolomics applications.

Recently, the team added a high-resolution accurate mass (HRAM) gas chromatography-mass spectrometry (GC-MS) system to take advantage of its higher separation power and unparalleled sensitivity for identifying and quantifying metabolites. Here, we discuss how the Bowden laboratory successfully expanded its capability with GC-MS and highlight key factors to consider for setting up a robust metabolomics platform.

A versatile, multi-platform approach to metabolomics

The expansive portfolio in the Bowden laboratory includes translational applications, such as human and wildlife exposure projects, pollutant measurements and the development of novel analytical methods. Running multiple parallel projects with a wide variety of applications is only made possible with instrument versatility. Centered around mass spectrometry, Dr. Bowden's laboratory is equipped with an orthogonal assortment of instruments to tackle these studies from varying perspectives. "In analyzing any sample, I want the capability to interrogate the widest chemical landscape possible," says Dr. Bowden.

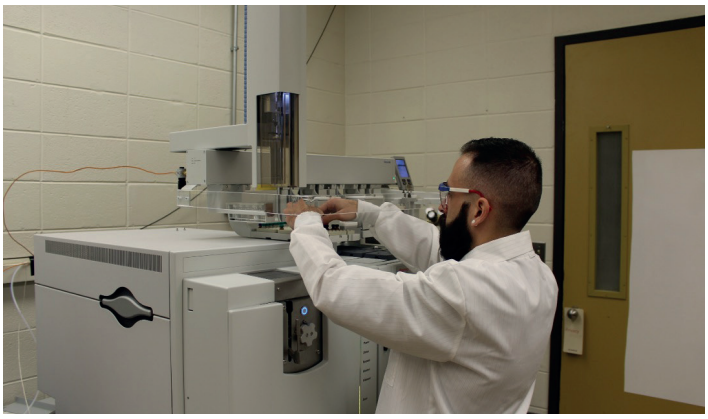
The instrumentation suite for metabolomics is diverse and includes the Thermo Scientific Orbitrap Exploris GC 240. This technology has provided the research group with the necessary workflow to develop robust methods, perform compound identification, analyze structures and obtain (semi-) quantitation across a variety of chemical classes within a diverse array of matrices. "It has made us a one-stop-shop to perform exposure-related studies," says Dr. Bowden. "We can analyze both the external measures of exposure (the pollutants) as well as internal measures of exposure (biomarkers) using the available instrumentation."



ThermoFisher SCIENTIFIC

"For metabolomics experiments, the capability to achieve such high selectivity and maintain sensitivity is revolutionary for our research; having easy access to this data certainty and such wide coverage opens up new research avenues for us"

– Dr. Bowden



The Thermo Scientific Orbitrap Exploris GC 240 system in the Bowden laboratory

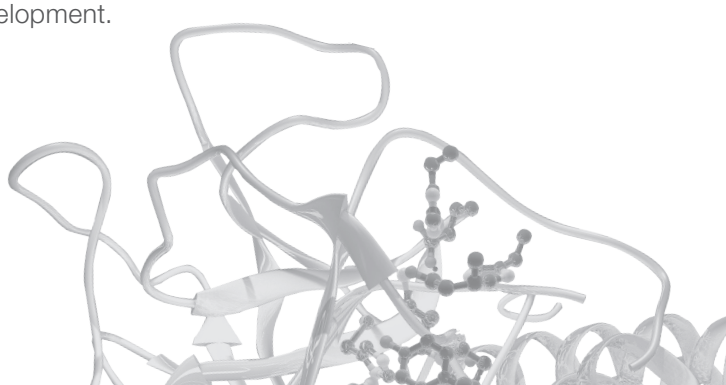
Prior to acquiring the Orbitrap Exploris GC 240, the team performed metabolomics studies solely using LC-MS/MS workflows on the Q-Exactive system. "The utilization of GC for metabolomics is gradually starting to receive the same level of attention as LC-MS-based approaches," says Dr. Bowden. "Incorporating both LC and GC into our metabolomics workflow has immensely broadened our compound identification capability. We can now identify a multitude of compounds ranging from volatile to nonvolatile, stable to thermally unstable and polar to nonpolar, providing us with a more comprehensive profile to interrogate."

With limitless potential on metabolite analyses, the research team put the technology suite to work to help execute multiple 'exposomics' and metabolomics projects.

How the comprehensive 'omics' setup serves multiple projects

The Bowden laboratory extends its metabolomics approach towards a wide variety of projects, breaking new ground with novel research questions and the use of unusual samples. Below are a handful of ongoing projects:

- **Measuring pollutants within biological systems:** Products of anthropogenic pollution, volatile per- and polyfluoroalkyl substances (PFAS) are known to infiltrate and persist in biological systems due to their high bioaccumulation potential. Using the Orbitrap Exploris GC 240, this study investigates volatile variants of PFAS present in both environmental and biological matrices of a wide variety of animal species, such as manatees. These studies will set new frontiers for PFAS research and trigger future high-profile studies involving other emerging areas of concern, such as pollutants in landfill emissions and indoor air.
- **The heterogeneity of lupus:** A part of a large longitudinal study, this project aims to understand the genetic and environmental mechanisms behind lupus. Metabolomics and lipidomics strategies are applied towards plasma and urine samples collected from a cohort of Gullah African American patients, a subpopulation with a high risk for lupus. To better understand the heterogeneity of lupus pathophysiology, the study will examine associations between lupus subtypes, environmental contaminants, diet, genetic and auto-immune risks, and the generated metabolomic and lipidomic profiles.
- **Wildlife exposure studies:** The goal of this project is to determine the extent to which wildlife populations are affected by environmental stressors, providing potential clues towards assessing human health risks. By adapting omics-based strategies typically employed in human health research to measure adverse health consequences in marine mammals, fish, crocodilians and apex predators, these projects challenge the status quo of metabolomics and venture into a vastly unexplored area in both biomarker discovery and method development.



Using GC-MS to expand metabolomic readouts

Choosing the right GC-MS system largely depends on the types of samples being measured, the level of expertise in the laboratory, and the availability of other complementary instruments to aid downstream or upstream experiments. As such, success with GC-MS is dictated by producing high-quality data which includes the number of compounds the research team can confidently identify, while ensuring all quality assurance and quality control requirements have been met.

"When a laboratory decides to expand its metabolomics workflow with GC-MS, there are a few key characteristics that need to be considered," notes Dr. Bowden. "At the top of the list are versatility, sensitivity, ease of use and rigorous ID capabilities." With rare and scarce samples at the heart of numerous projects, the Bowden laboratory needs to reliably extract the most information they can within each sample in one run. Dr. Bowden continues: "For laboratories like ours, where it's important to maximize the amount of information obtained from a single injection, acquiring systems that exceed the above characteristics is crucial."

With the installation of the Orbitrap Exploris GC 240 system, the Bowden laboratory is now equipped with a set of technologies to examine any metabolite. One of the distinguishing benefits of the Orbitrap Exploris GC 240 system is its superior resolution that allows compounds to be identified against a complex chemical background. Moreover, the flexibility of tandem MS enables structural investigations and the identification of unknowns, a helpful tool when investigating profiles generated with non-model species. Unlike time-of-flight technology, that calls for a compromise between resolving power and sensitivity, the Orbitrap Exploris GC 240 system provides an unprecedented depth of analysis by offering both high selectivity and sensitivity at the same time.

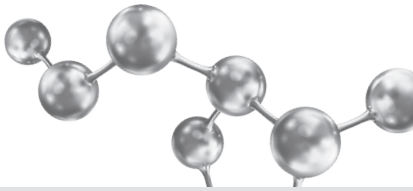
Additionally, the system itself is designed to facilitate easy and routine use by all members of the analytical team, irrespective of their level of expertise or training. Straightforward and uncomplicated workflows eliminate repeated user adjustments, thereby providing consistent and reproducible results, even across multiple users. If desired, laboratories can further minimize analyst variability by incorporating automated sample prep. Once raw data is obtained, the user-friendly software simplifies and speeds-up data processing and analyses.

Members of the Bowden laboratory generally use non-targeted approaches for initial pilots, exploratory studies or to generate new hypotheses. The high resolving power of the Orbitrap Exploris GC 240 is particularly well-suited for non-targeted studies. Its mass accuracy of <1 ppm boosts confidence in metabolite identification, even during exploration. Targeted methods, on the other hand, are applied towards established hypotheses or routine analyses that require higher specificity and reproducibility. More often than not, broad nontargeted studies in the Bowden laboratory feed into specific, targeted studies. "Targeted or not, either way, we can use mass spectrometry to interrogate both pollutants and biological molecules with a simple change in sample preparation, chromatography and scanning strategies," says Dr. Bowden.

Key factors for setting up a robust metabolomics platform

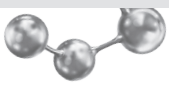
Optimizing a reproducible workflow

From sample prep to data analysis, each step of the workflow needs to be optimized for individual matrices or sample types to deliver accurate, reproducible results. "The optimization of the entire metabolomics workflow, irrespective of LC- or GC-based separation, needs to be accomplished to provide a level of confidence in the method and ease with the process," advises Dr. Bowden. Every stage of the method warrants meticulous optimization, starting from the initial experimental design and sample preparatory steps, such as extraction and derivatization, and extending to mass spectrometric scanning strategies, data analysis and interpretation.



"For laboratories like ours, where it's important to maximize the amount of information obtained from a single injection, acquiring systems that exceed the above characteristics is crucial."

– Dr. Bowden



Method-based studies or application notes outlining optimized workflows are helpful but need to be repeated from scratch in each laboratory and for every matrix type to ensure optimal translation. To better support the community, the Bowden laboratory is currently working towards building and disseminating a more practical approach to optimize metabolomics workflows. The team utilizes commercially available Standard Reference Materials (SRM), such as plasma, urine, stool and so on, to evaluate their in-house methods, and assess their robustness and reproducibility over a long period of continuous operation. The homogenous nature of the SRM overrides any variability arising from the starting sample, while the intricacies of the method itself get undivided focus for optimization. "Our ultimate goal would be to produce 'sample-method-data' products, where laboratories new to metabolomics can optimize their own methods by purchasing the same SRM we used, testing them with our methods that are available online and finally, validating their results with our published data," informs Dr. Bowden.

Standardizing analytical measurements

Even with an optimized method, external or inherent variables, such as the standards used or sample stability, can result in differential outputs with the same protocol. Inconsistencies stemming from these variables can inadvertently generate false reports in this emerging field and negatively impact the metabolomics community rather than advancing it. A community-wide standardization of analytical measurements is, therefore, essential to foster collaboration and data sharing in the long run. One way to achieve this is to incorporate modern instruments, such as the Orbitrap Exploris GC 240 system, that are fitted with 'intelligent run controls,' and other smart functionality tools to help minimize errors and achieve reproducible, high-quality data.

Having access to expansive databases

Working with non-model species and unique matrices requires having comprehensive databases to identify compounds. "We often encounter peaks that we're unable to identify due to the lack of available databases. This is especially common when moving away from mammalian species, and is further compounded by the lack of standards and variable derivatization methods," notes Dr. Bowden.

To eliminate database-related limitations from novel exploratory studies, the Orbitrap Exploris GC 240 uses an extensive breadth of libraries to generate candidate compounds. Researchers can tap into both commercially available nominal mass libraries, such as NIST or Wiley, as well as the Orbitrap GC-MS HRAM metabolomics library (Figure 1). In the case of cutting-edge studies, it's also possible to construct one's own library. A faster and more confident route to compound identification is supported by the Compound Discoverer software built into the Orbitrap Exploris GC 240. Its deconvolution and library searching capabilities extract meaningful information from large datasets.

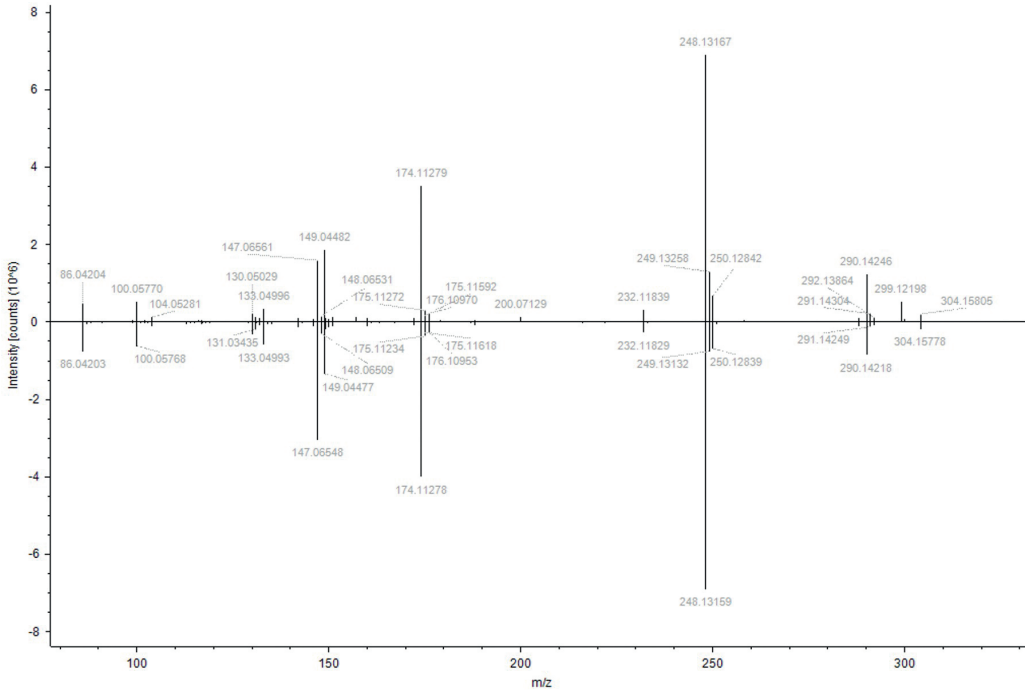


Figure 1. The deconvoluted spectrum of L-Alanine 3TMS mirrored with the library spectrum from the Thermo Scientific Orbitrap GC-MS HRAM metabolomics library. Search index score of 909 forward fit and 934 reverse search index.

> "We extensively communicated with the Thermo Fisher team before the instrument was delivered to make sure on-site requirements and specifications, such as electrical supply, gas inlet and ventilation, were met, they provided us with clear and precise instructions, which we were then able to pass on to our operations personnel."
>
> – Dr. Bowden

Reliable technical support

Incorporating new technology into current workflows isn't without technical and logistical challenges. Reliable technical support provided by manufacturers can preempt and alleviate some of the issues associated with installation and troubleshooting. Even before the Orbitrap Exploris GC 240 was installed, members of the Bowden laboratory worked in close collaboration with the Thermo Fisher technical support staff to ensure the new equipment seamlessly assimilated into the existing facility. "We extensively communicated with the Thermo Fisher team before the instrument was delivered to make sure on-site requirements and specifications, such as electrical supply, gas inlet and ventilation, were met," informs Dr. Bowden. "They provided us with clear and precise instructions, which we were then able to pass on to our operations personnel."

After delivery, the Bowden laboratory has continued collaborations with the Thermo Fisher staff to roll out the installation process and get the system running. Dr. Bowden continues: "The engineers had clear plans and defined goals for what they needed to accomplish on each day of the installation. They were good at thoroughly explaining every step to us and answering our questions. As a part of the training, they provided multiple resources, including manuals and videos, that clearly explained how to set up the instrument, perform calibrations, clean the filament and switch out the column, giving us the necessary foundation to start using the system."

Once installation was complete, seeking technical support to validate and troubleshoot workflows on the new system can eliminate hours of trial and error. "During the demo stage and throughout method development on our own system, we have worked closely with engineers, application scientists and other support staff at Thermo Fisher. The team has provided us with exceptional support, including recommendations for sample preparation, useful guidance for instrument maintenance, and tips for troubleshooting and working with software, such as Compound Discoverer," says Dr. Bowden. Collaborating with well-informed technical support staff before, during and after set-up undoubtedly shortens the learning curve for laboratory members and yields results faster.

Conclusion

Choosing the most appropriate technology and leveraging its capabilities to answer new research questions in metabolomics has catapulted the potential of the Bowden laboratory. The recent addition of the Orbitrap Exploris GC 240 to the laboratory's orthogonal assortment now offers technical flexibility to work on a range of metabolomics projects to answer both current and future research questions. This comprehensive metabolomics suite that is capable of tackling samples from mammalian and rare species alike, with both nontargeted and targeted approaches, all while consistently providing reliable data, has attracted fruitful collaborations and earned the laboratory additional grant funding to further advance the field of metabolomics.

> "We have worked closely with engineers, application scientists and other support staff at Thermo Fisher. The team has provided us with exceptional support, including recommendations for sample preparation, useful guidance for instrument maintenance, and tips for troubleshooting and working with software."
>
> – Dr. Bowden

thermo scientific

Dr. John A. Bowden

Dr. John A. Bowden is an Assistant Professor in the Department of Physiological Sciences in the College of Veterinary Medicine at the University of Florida (UF). He also has a joint appointment in the Department of Chemistry and has affiliations with the Center for Human and Environmental Toxicology and the Engineering School of Sustainable Infrastructure & Environment. He received his Ph.D. in Analytical Chemistry from UF under the guidance of Dr. Richard A. Yost in 2009. After a two-year postdoctoral fellowship at Saint Louis University, he joined the National Institute of Standards and Technology (NIST) as an NIST/NRC postdoctoral fellow in 2011, working at the Hollings Marine Laboratory in Charleston, SC, where he would later serve as a Research Chemist until 2018. In his current position, his research is focused on employing novel mass spectrometric methods at the chem/bio interface, with an expanding interest in the fields of metabolomics, lipidomics and exposomics.

Find out more at **thermofisher.com/OrbitrapExplorisGC240**

ThermoFisher SCIENTIFIC