Waters™

LC-MS Upgrade Bolsters Metabolic Phenotyping Services

The National Phenome Centre recently upgraded its Waters Quadrupole Time-of-flight (QTof) Mass Spectrometers to enhance the vast data generated by the institution's cutting-edge multi-technology platform.

METABOLIC PROFILING AT THE NATIONAL PHENOME CENTRE

Initially funded in 2012 by the Medical Research Council (MRC) and the National Institute for Health Research (NIHR) and now part-supported by the NIHR Imperial College Biomedical Research Centre (BRC), the National Phenome Centre (NPC) in London, UK, delivers access to world-class metabolic phenotyping that benefits the entire UK translational medicine community. The Centre's establishment took advantage of an unprecedented opportunity offered by the legacy of its 2012 Olympics state-of-the-art drug testing and analytical laboratory. Today, the NPC's vision is to harness the vast data generated by its cutting-edge multi-technology platform to inform patient diagnosis and therapy, bringing data from the bench to the bedside.

NPC sits within the Division of Systems Medicine in the Department of Metabolism, Digestion and Reproduction at Imperial College London, allowing it to draw upon decades of experience in metabolomics. The Centre has collaborated academically across a broad range of application areas ranging from rare diseases and niche specialisms to global and societal challenges. NPC is open to all UK academic and industry researchers and offers services from broad profiling untargeted assays to targeted quantitative assays.

The National Phenome Centre (NPC) delivers its world-class molecular-phenotyping services using a mix of analytical platforms, to gain unparalleled insight into the molecular composition of samples.

WORKING WITH WATERS

The NPC began the process of upgrading the Centre's original 10 Waters™ Xevo™ G2-S QTof Mass Spectrometers in December 2022 as the instruments began to reach end-of-life for Waters support. The decision to upgrade, however, has as much to do with the Centre's long-term relationship with the Waters team as the technical improvements of the new Waters Xevo G3 instruments. Dr. Gómez-Romero explains:

"We have service contracts with all our Waters instruments as it's the best, easiest, and most efficient way for maintenance and support. Over the years, we've developed a very close relationship with Waters. Many of the upgrades to the Xevo G3 came from the collaboration and development work we've done with Waters, and they've considered some of our feedback on the new instruments. While we considered other vendors, Waters was the right choice for what we wanted to achieve."

The Centre delivers its world-class molecular-phenotyping services using a complimentary mix of analytical platforms, combining nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography coupled to mass spectrometry (LC-MS) to give unparalleled insight into the molecular composition of samples. Dr. María Gómez-Romero, who started with NPC in 2017 and currently serves as the Mass Spectrometry and Chromatography Manager, describes the Centre's evolution:

"This year marks the NPC's 10th year anniversary. Our legacy began in the cutting-edge drug testing facility created for the 2012 London Olympic Games. Initially the Centre was designed to undertake large scale metabolomics with urine and blood samples. In 2018, we merged with the Clinical Phenotyping Centre (CPC) of the Imperial BRC, which specialized in smaller clinical studies with any kind of samples, and so now we are also able to support analysis for smaller cohorts and of many different types of biofluids, microorganism, tissue, or cell extracts, etc."

The NPC's established platform for metabolic profiling has been made open in its entirety, including complete protocols, methods, software, and metabolite annotations.[1,2] The open platform leverages the experience of dedicated metabolomics practitioners, providing both a foundation for cooperation among like-minded experts and a turnkey solution for novice users.

To continue to meet the demands of the rapidly evolving metabolic phenotyping field, the NPC recently upgraded its line of Waters instrumentation — in particular, the new Waters Xevo G3 QTof Mass Spectrometers — to help with the qualitative and quantitative analyses of challenging molecules. The lab's focus on streamlining its entire workflow and overall testing processes meant that the Centre also acquired the Waters ACQUITY™ Premier UPLC™ System, and plans to upgrade its columns to improve liquid chromatography capabilities.

UNIQUELY TAILORED PLATFORM FOR METABOLIC PROFILING

Metabolic profiling as a technology can be described as "disease blind," making it possible to apply it to a multitude of diseases, disease states and general population assessment. The main goal of the field is to understand the metabolic changes that occur in response to specific stimuli, such as diseases, environmental factors, drugs, or genetic modifications. By comparing the metabolic profiles of different samples, researchers can identify patterns, biomarkers, and potential metabolic pathways associated with conditions or physiological states.

Dr. Gómez-Romero (pictured) and her colleagues are preparing to delve into the new possibilities presented by the Xevo G3 QTof's increased levels of analytical sensitivity and specificity.

With active projects in cancer research, gut health, cardiovascular disease, diabetes, dementia, nutrition and more, the NPC has developed a uniquely tailored platform to facilitate a deeper understanding of the phenome and a wide coverage of the metabolome; high fidelity identification and quantitation of metabolites; an accurately curated database of metabolites; and data interpretation and modeling infrastructure. NPC's collaborators include academia, industry, charities, small and medium-sized enterprises (SMEs) and start-ups. The Centre's services are set up to deliver on academic collaborations, fee-for-service projects, longitudinal studies, long-term strategic partnerships, and a range of other collaboration options such as bioinformatics and metabolite identification.

NPC's precision profiling assay suite uses a multi-technology approach to provide deep analytical and chemical insight to complex samples that allows for the identification and quantification of metabolites in complex biological samples. These techniques generate large amounts of data, which are then processed using statistical and bioinformatics tools to extract meaningful information and interpret the metabolic changes (Figure 1). Dr. Gómez-Romero explains:

"Multiple technologies and assays give us increased molecular coverage. We use ultra-performance (UPLC)-MS and NMR for untargeted analyte measurement, discovery profiling and targeted metabolite data extraction. We then use UPLC-MS/MS for targeted analyte and metabolic pathway measurements."

The National Phenome Centre platform for metabolomics

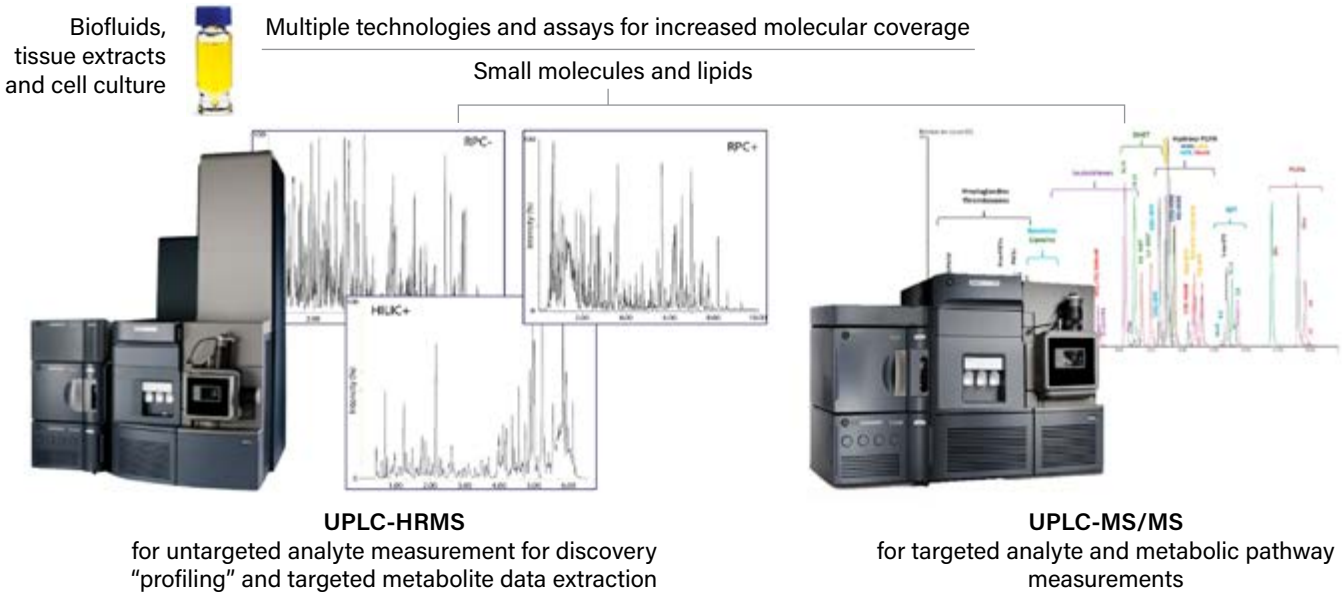


Figure 1. NPC's precision profiling assay suite uses a multi-technology approach to provide deep analytical and chemical insight to complex samples.

Since its inception in 2012, the NPC has been central to many projects focusing on a variety of applications, giving it a unique perspective on the metabolic profiling landscape that has contributed to its development of its SOPs, assays, and platform. The type of research projects also has evolved, particularly when NPC merged with the CPC in 2018 and added smaller, clinical studies to its service offerings. That change resulted in an expansion of the types of samples the Centre could handle, as well as some new methodologies to meet the needs of these collaborators. Dr. Gómez-Romero explains:

"The CPC was getting more requests for projects and didn't have the capacity. Whereas, NPC had a large facility prepared to run large cohorts. So, the strategic decision was made to merge. As a result, we started getting projects with different types of biological samples. In clinical studies, the volumes are much smaller and more precious. While our chromatography methods have stayed mostly the same, we've needed to adapt our sample preparation for new sample types, such as tissue, feces, cells, microorganisms, to meet the needs of these clinical projects."

MULTI-TECHNOLOGY APPROACH

NPC's multi-technology approach serves as the basis for the Centre's ground-breaking work. The team's recent work includes collection of urine fractions into a "fraction bank" suitable for long-term laboratory storage. The team developed a workflow that enables the decoupling of purified metabolites from the pipeline at each stage, which can then be sent to a combination of downstream analytical technologies for increased confidence in structural elucidation.

The pipeline is particularly relevant where analytical standards are not commercially available (for example, with bio-transformed metabolites, such as glucuronides). The protocol is most suited for instances where structure elucidation and metabolite annotation are critical for the downstream biological interpretation of metabolic phenotyping studies.[3]

"We have a very strong pipeline for metabolite identification, and we've created a bank of reusable preconcentrated urinary fractions that are stored in the freezer and have been profiled. If the feature of interest from any project is present in the collected fractions, we can then purify it from the fractions, and elucidate the structure of the compounds of interest by additional MS and NMR experiments, without having to work with the precious samples of the clinical study. We can always go back and work with the already collected fractions."

DR. MARÍA GÓMEZ-ROMERO
Mass Spectrometry and Chromatography Manager

In a related project, the NPC team chromatographically isolated interfering metabolites from human urine, responsible for matrix effects in both RP and HILIC high-throughput LC-MS metabolic profiling assays, and unambiguously identified them via de novo structure elucidation as two separate proline-containing dipeptides. This workflow used LC-MS/MS, MRM-MS and NMR spectroscopy for the full chemical and spectroscopic characterization of these metabolites and for the establishment of the coexistence of cis and trans isomers of both dipeptides in solution.[4]

NPC's research has also contributed to the use of metabolic profiling to aid in clinical decision making and in the development of diagnostic tools. For example, NPC recently published research where the team used untargeted high-resolution LC-MS to compare the admission serum metabolome of emergency department patients with viral infections (including COVID-19), bacterial infections, inflammatory conditions, and healthy controls. These findings highlight a future diagnostic role for antiviral metabolites in viral diagnosis, pandemic preparedness, and acute infection management.[5] Dr. Gómez-Romero explains how the classical metabolomics approach in biomedical research is evolving in the NPC:

"In global profiling, the generated datasets represent tables of features corresponding to mass-to-charge ratios of ions detected at specific retention times. For the features resulting as statistically significant following data modeling, metabolite annotation and identification is sought, which can be a time and resource-consuming process. In some occasions, it can be a quick match to characterize database entries, while in other cases, especially in the absence of commercially available standards, there is a need for more tedious and costly procedures to be implemented to isolate compounds of interest and perform its full structural elucidation. Our clinical collaborators want everything – discovery of novel biomarkers, to know the identities of all features and even their quantities – and we have found a way to maximize the untargeted data generated. A first approach can be to run a much more targeted, semi quantitative analysis with the QTof. A second approach is to provide annotated datasets extracted from the profiling data. Because we've run thousands of standards, we've created a database and specific lists of well-characterized and pre-annotated features for all our assays that could be detected and integrated in any project we acquire. So, we can provide different datasets to our collaborators, depending on their needs."

WATERS XEVO G3 QTOF MASS SPECTROMETER

LC-MS stands as the primary powerhouse in the field of metabolomics due to its exceptional levels of analytical sensitivity and specificity when examining the wide range of chemical compounds within intricate biological samples. The NPC's decision to incorporate the Waters Xevo G3 QTof mass spectrometer into its platform will provide the Centre with opportunities for improved qualitative and quantitative analyses of challenging molecules, particularly because the G3 is capable of up to 10X more sensitivity than its predecessor (Figure 2).

"The Waters Xevo G3 Mass Spectrometers are more sensitive, which is great for us because the less sample needed the better, especially for clinical studies. Waters also has a new option to filter out some background noise that isn't recorded in the data file. That's useful because sample sets are extensive and can generate large data packages. They also have an option for multiple reaction monitoring (MRM) transition, which will be very interesting for developing semi-quantitative methods because monitoring the fragmentation gives an extra level of confidence about the metabolite you're quantifying."

DR. MARÍA GÓMEZ-ROMERO
Mass Spectrometry and Chromatography Manager

Waters engineered the Xevo G3 QTof system to reliably give scientists both reproducible and accurate qualitative and quantitative information about molecules in their samples whether in very small or very large amounts. NPC is particularly interested in seeing how the new instrument will affect its recently introduced cellular metabolism service offerings. By comparing the metabolic profiles of healthy and diseased cells, researchers can identify unique metabolic signatures associated with specific conditions.

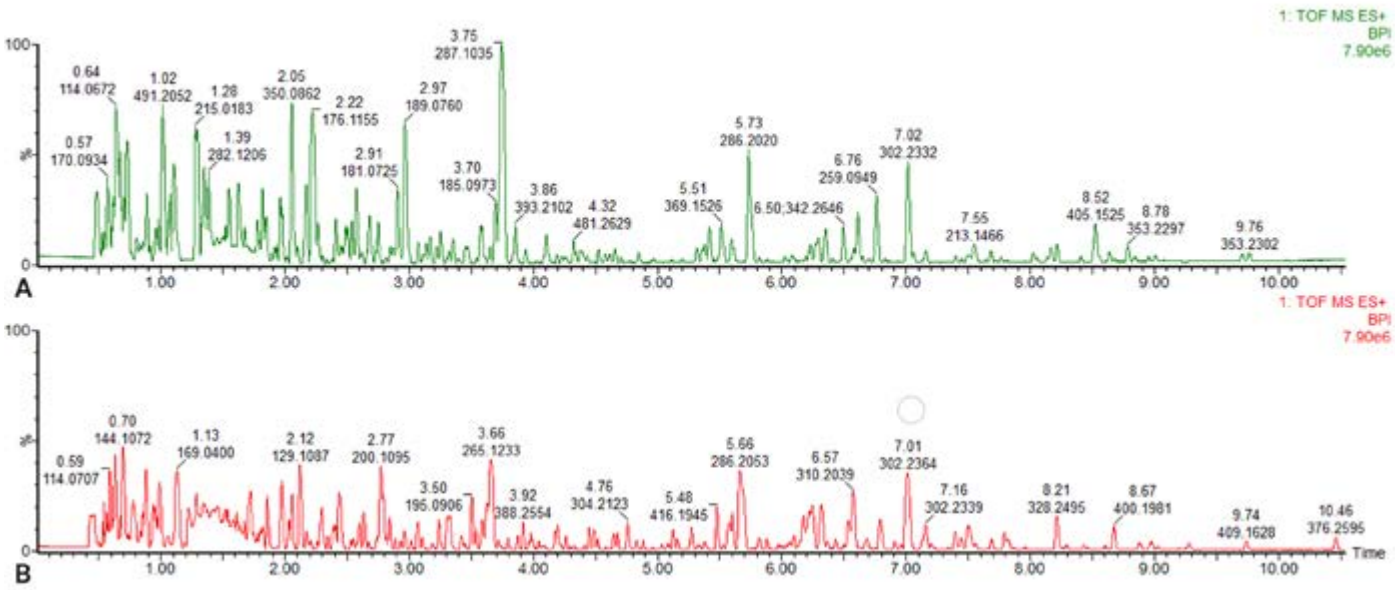


Figure 2. Comparison of RPC (C_{18}) ESI(+) ToF-MS data. Base peak intensity (BPI) of the NPC's urine long term reference (LTR) sample with A) ACQUITY Premier UPLC Xevo G3 QTof, ongoing optimization (1 µL injection), and B) ACQUITY UPLC Xevo G2-S QTof with optimized parameters for small molecule detection (2 µL injection).

These biomarkers can be used for early disease detection, monitoring treatment response, and understanding disease mechanisms. Dr. Gómez-Romero describes how the new Xevo G3 Mass Spectrometer will impact these types of projects:

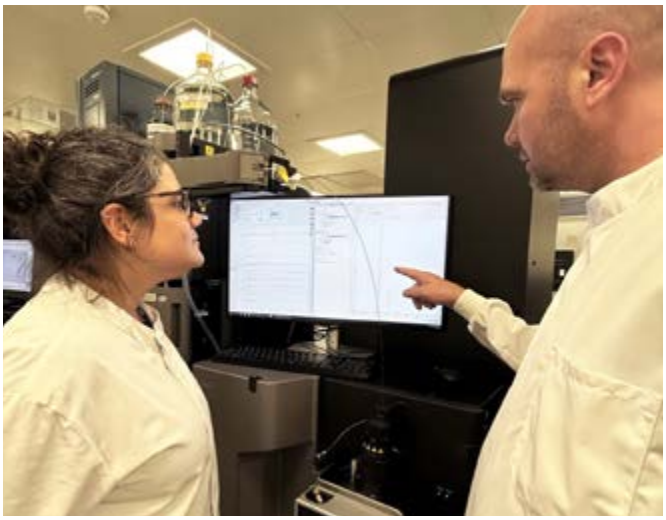
"Cellular metabolism is a slightly different world to biofluid analysis, each cell line or scraped cells from tissues differing from each other. We had to develop a specialized chromatographic method and adapt our workflow and protocols because we rely on quality controls and feature filtering based on a pool sample of the whole study and its dilution series. The more samples you have, the less volume you must take from each sample. When it's a large cohort, volume per sample to create a pool is not normally an issue. But when you have about 15 samples, which is typical in these cellular studies, that becomes a problem. So, we've had to work on different solutions for quality control because we always want to provide the best data quality to our collaborators, particularly filtering out as many of the background and contaminants as possible and only keeping features reliably measured, with low coefficients of variation and correlating to dilution. We had already run into projects where sensitivity was an issue for the cellular metabolism pathways. We've found the G3 can find metabolites in cell samples that we couldn't detect before. We had a workaround, but now the G3s are offering a solution at the right time. It's really good timing."

NEXT STEPS

In the near future, Dr. Gómez-Romero and the NPC team are focused on fully transitioning to the new Waters Xevo G3 QTof mass spectrometers, which they expect will be fully operational in late 2023. The Centre's next step includes thoroughly testing the new instrumentation and comparing the results against the older G2s to ensure everything is working smoothly before running projects.

"We're moving to the G3s as quickly as possible. We run thousands of samples, and we run them continuously in one go. So, the system must be very stable. We're planning on running one cohort on both platforms, passing them through the whole pipeline, and then comparing the results. It's not only the LC-MS details that need to be tweaked, but also our data preprocessing and data filtering. This adaptation period ensures we're super comfortable and happy with the entire workflow."

DR. MARÍA GÓMEZ-ROMERO
Mass Spectrometry and Chromatography Manager



Stephane Camuzeaux (right) lead the efforts to get the Waters G3 systems up and running in the lab.

Dr. Gómez-Romero and her colleagues are also preparing to delve into the new possibilities presented by the Xevo G3 QTof's increased levels of analytical sensitivity and specificity, which could impact the NPC's clinical studies in particular. She explains:

"We're looking at how much more we can do with this new instrumentation. We've done as much as we can with the G2s, but we're excited about where the sensitivity of the G3 can take us. Targeted assays are very sensitive but, for some projects, when we are trying to quantify over 50 metabolites from the same class, their concentration ranges can vary greatly in the samples - this is the case of bile acids in fecal samples - and for a more complete coverage we would need to run different dilutions of the same sample. We find that the semi-quantification methods on the time-of-flight allows for a bigger dynamic range and to capture very different concentrations in a sample."

Throughout this transition, the NPC plans on continuing to rely on Waters support personnel, as it has so often in the past.

"We've worked with many Waters personnel during this upgrade, and everyone is super nice and helpful. That's made these changes much easier. Again, it's a strong relationship. It's already bringing new and exciting things for our team."

DR. MARÍA GÓMEZ-ROMERO
Mass Spectrometry and Chromatography Manager

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