

## RAPID TURNAROUND OF HIGH-QUALITY METABOLIC PROFILING STUDIES WITH A UPLC/QTOF AND METABOLYNX XS-BASED WORKFLOW

Combining metabolism expertise with the best exact mass LC/MS system available allows one CRO to deliver more complete metabolic structural information to their clients in less time.

### BACKGROUND

MicroConstants is a GLP-compliant CRO based in San Diego, California that provides GLP bio-analytical services, IND-enabling drug metabolism (DMPK) assays, immunoassay development, and pharmacokinetic analysis services to pharmaceutical and biotech companies worldwide. MicroConstants is the largest bioanalytical LC/MS/MS service provider on the West Coast, specializing in method development, method validation, and sample analysis to support drug discovery and development programs.

Dedicated to becoming a recognized leader in bioanalytical and pharmacokinetic analysis, they understand that the only way to achieve this goal is by providing only the highest quality data and exceptional client services. Their team of skilled professionals recognizes that by combining expertise with state-of-art LC/MS systems, the best, most comprehensive results can be delivered to their clients on time, every time.

### CHALLENGE

In pharmaceutical development, safety studies are an integral part of Investigational New Drug (IND) applications to the FDA seeking approval to begin Phase I clinical trials in humans. Since safety studies are conducted in animals, it is important to select a species that exhibits a metabolite profile as close as possible to the profile generated in humans. Importantly, unique or disproportionate metabolites may signal a need to conduct costly additional toxicological studies, as described by the FDA guidance on Metabolites in Safety Testing.

Metabolite profiling studies are generally conducted by incubating the test article with liver hepatocytes from human and a few animal species that are commonly used in toxicological studies. Samples from the incubations are analyzed by LC/MS, and the data sets are searched for the presence of metabolites. The resulting metabolite profile can then be reviewed to determine which animal species most closely matches human. Species that do not produce a major human metabolite can be avoided. If unique or disproportionate human metabolites are unavoidable, steps can be taken to test the safety of those metabolites far earlier in the development process, saving valuable time.

Since critical decisions are based on the metabolite profile information, the quality of the data is of paramount importance. Like many in the industry, MicroConstants used to use a tandem quadrupole mass spectrometer to analyze the hepatocyte incubations. In addition to full scan data, parent ion and neutral loss scans were analyzed to search for metabolites. Product ion spectra were then collected to characterize and identify the metabolites.



“UPLC with QToF and MetaboLynx XS provides an empowering platform for our metabolism scientists...With this complete workflow, we can routinely see more metabolites in a single run, and present a more definitive metabolic pathway picture for our clients in less time.”

**DR. DAVID JOHNSON,**  
DIRECTOR OF DMPK,  
MICROCONSTANTS

While this workflow produced good quality data, there were a number of shortcomings. Full scan data collection was not very sensitive, and few points per peak could be acquired. Thus, only abundant metabolites could be detected, and the reproducibility of the areas was in need of improvement. Also, the nominal mass data was not able to distinguish metabolites with similar mass shifts. Lastly, collection of the product ion spectra required re-injection of the sample for each metabolite. This necessity consumed a significant amount of instrument time, and test articles with complex metabolic pathways could not be properly characterized due to lack of sample volume.

To solve for the analytical and operational inefficiencies experienced in the laboratory and to provide better quality results for clients, MicroConstants acquired a new QToF MS to have a more fit-for-purpose UPLC®/MS solution for their drug metabolism experts, and have subsequently been able to expand their overall services portfolio in this area as a result.

## THE WATERS ADVANTAGE

The Waters QToF mass spectrometer technology enables MicroConstants to achieve simple exact mass measurements of precursor and fragment ions, via a powerful QToF function known as MS<sup>E</sup>, with maximum sensitivity to yield high-confidence structural elucidation. When paired with ACQUITY UPLC®, the system delivers unmatched resolution with 1.7 µm particle size columns. Dr. David Johnson, Director of DMPK, states, “The completeness of the UPLC/QToF MS<sup>E</sup> data set is impressive. You can’t fully appreciate it until you’ve solved a major problem with it. We simply didn’t have access to these kinds of results before. What really pulls it all together is MetaboLynx™ XS software.”

MetaboLynx XS, a MassLynx™ Application Manager, facilitates easy, visual interpretation of full scan data sets and the identification of potential metabolites. The software also includes isotope modeling and fragmentation simulation tools for detailed structure analysis and assignment.

Dr. Johnson continues, “MetaboLynx XS is amazingly good software. We are now able to provide more definitive metabolite profiling and identification for *in vitro* experiments such as hepatocyte and microsomes incubations for our clients. Without it, we would just have a lot of data, and not a lot of information. Additionally, with the entire UPLC/QToF solution, we now have the sensitivity to extend our studies to plasma samples from dosed animals or humans.”

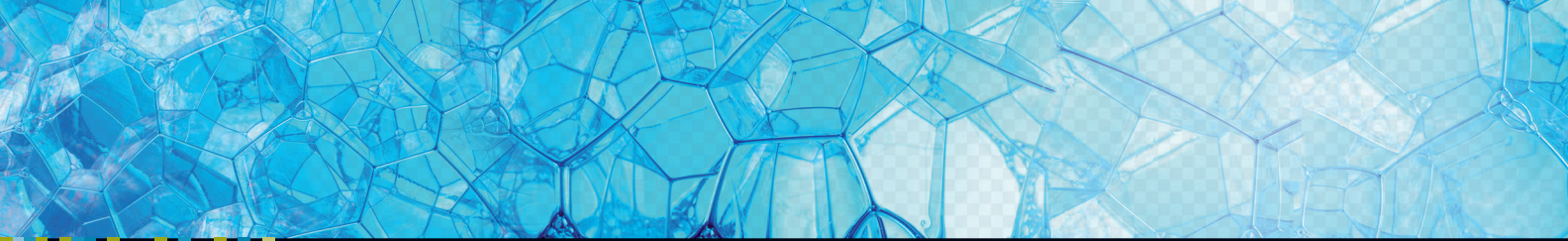
## BUSINESS BENEFIT

In one particular instance, a client had been working on a challenging metabolic pathway project for months, and finally sent their samples to MicroConstants. “With the UPLC/QToF solution, we provided them with far more data, much better structural elucidation on their metabolites – all in less than a week’s time. The client was truly astonished, and now sends all of their metabolic profiling work to us. Time is probably one of the biggest single cost drivers in the pharmaceutical industry, and with this solution, we’re able to really provide savings for them – along with the benefit of our expertise.”

## KEY POINTS FOR EFFECTIVE PRACTICE

MicroConstants was able to refine their own metabolic profiling workflows and arrived at a solution that addressed performance and productivity simultaneously, to provide rapid turnaround times for quality client data. Dr. Johnson concluded, “We realize how important a powerful software tool is to analyze large, complex, information-rich data sets. This, combined with the accurate mass and retention time precision of the UPLC/QToF system, allows our scientists to present a much more complete metabolic pathway picture for clients. The solution simply enables our metabolic profiling experts to do their jobs as well as they possibly can.

Match the scientist with the right LC/MS solution and the outcome is outstanding.”



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