

## Using simplified walk-up software to bring UHPLC-MS to the organic synthesis lab



Michael Tidwell, Research Scientist in Medicinal Chemistry at the University of Texas at San Antonio, Center for Innovative Drug Discovery (CIDD)

### Introduction

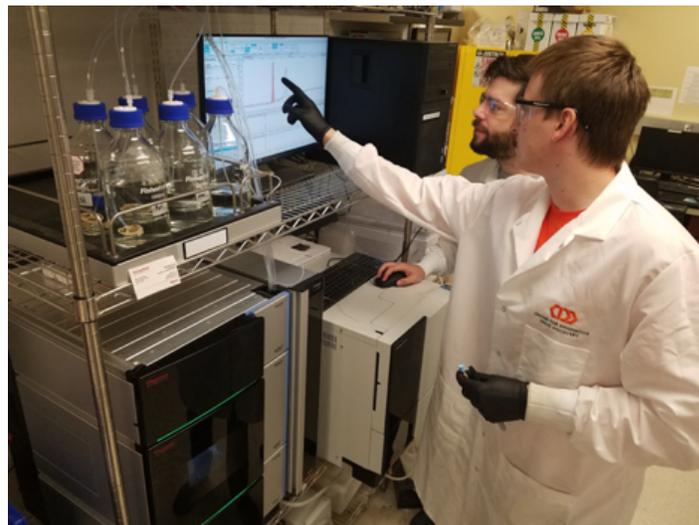
Medicinal chemists in the Center for Innovative Drug Discovery at the University of Texas at San Antonio are speeding up drug discovery by getting mass spectrometry data without ever leaving the lab. The combination of easy-to-use Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) with the Chromeleon XPS Open Access software has allowed the chemists to utilize Ultra-High Performance Liquid Chromatography Mass Spectrometry (UHPLC-MS) to cut by two-thirds the time it takes to obtain the information they need to help deliver their target compounds.

The Center for Innovative Drug Discover (CIDD) is a joint venture between the University of Texas at San Antonio (UTSA) and UT Health San Antonio. Their goal is to provide high quality, industry-level facilities and expertise to companies that wish to take their basic scientific discovery and transform it into a pre-clinical drug candidate, and, hopefully someday, a clinical therapy. The CIDD has two facilities to help make that happen – a High-Throughput

Screening facility at UT Health San Antonio, and the Medicinal Chemistry and Synthesis Core Facility located at UTSA. The Medicinal Chemistry and Synthesis Core Facility is a 2,000 sq. ft. technology center containing all of the necessary equipment and services to provide custom synthesis and medicinal chemistry research for small molecule drug discovery projects in any therapeutic area.

Michael Tidwell is a Research Scientist in Medicinal Chemistry at the CIDD facility. For the past two years, Michael has been tasked with the implementation and optimization of the Thermo Scientific™ Vanquish™ Flex UHPLC system coupled to the Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer to meet the group's needs. Primarily, the group focuses on structure-activity-relationship development for various programs within CIDD. Michael described CIDD's role in drug development as usually occurring through custom synthesis (the customer has a specific molecule they want synthesized), lead optimization (the customer wants to make improvements in their synthesis route, biological activity, or physiochemical properties), or by utilizing CIDD's HTS screening facility to mine for lead molecules for a specific disease. When asked about their customers, Michael responded, "We have a diverse array of clients. Many are small drug companies, biotechs or startups. A significant amount are related to academic (other professors or researchers) that are researching a disease and want to introduce some medicinal chemistry to their project."

The group at CIDD consists of 10 members that primarily perform organic synthesis of target compounds. A typical synthesis involves (1) combining the starting reagents, (2) monitoring the reaction as it takes place, (3) purification of the resulting product and then (4) confirmation that the product is the target compound. These reactions take place on a small scale, with the goal being 100-200mg of the purified target compound.



**Ph. D. candidates Michaels Glassman (left) and Hans De Kraker (right) at the University of Texas San Antonio, Center for Innovative Drug Discovery (CIDD) evaluating chromatograms obtained with UHPLC-MS**

### **Analytical challenges in medicinal chemistry labs**

During the synthesis steps, the reaction is monitored to ensure it is proceeding as expected. The monitoring looks for the loss of starting materials in order to ensure they are being consumed in the reaction, and for the appearance of a new product. In many cases, the target compounds have no published synthesis route, or may never have been synthesized before, so monitoring needs to be performed more frequently.

Thin Layer Chromatography (TLC) is the traditional method for monitoring small-scale synthesis reactions. TLC requires minimal training and equipment, which is the reason why it has historically been used in synthesis labs. However, the process is time-consuming, and the data that can be provided is limited. In most instances, the only information that can be obtained from a TLC analysis is a visual confirmation of the presence (or absence) of a spot, with quantitative information limited to how dark the spot is.

One of the biggest business drivers in custom organic synthesis is cost per compound. In order to be competitive in that field, the lab must be able to keep the cost low to attract customers and maintain profitability. A major factor in cost per compound is the amount of time it takes to synthesize and purify the compound; the analyses required to verify the correct compound has been synthesized and to determine its purity, are all part of that. Typically, the group at CIDD performs anywhere from 20-100 analyses per day, so this time can become significant. In order to lower the lead cost per compound, the group at CIDD needed to find a solution that provides all of the required data and to deliver it in a quick and efficient manner.

### **Overcoming the challenges by utilizing LC-MS**

Additional analyses are often needed to determine what the purity of the compound is, and to identify if the correct compound is being synthesized. These analyses are typically performed using Liquid Chromatography (LC), Liquid Chromatography Mass Spectrometry (LC-MS) and Nuclear Magnetic Resonance (NMR) techniques. Modern chromatographic systems, such as UHPLC-MS, are able to provide much more information than TLC, and can deliver it in a fraction of the time. However, due to specialized software and the need for interpretation of the data into meaningful results, scientists trained in chromatography and mass spectrometry are usually required. As a result, many organic synthesis labs will either send samples to another lab for analysis (which can add significant time and cost) or will purchase their own system, which is the route CIDD chose.

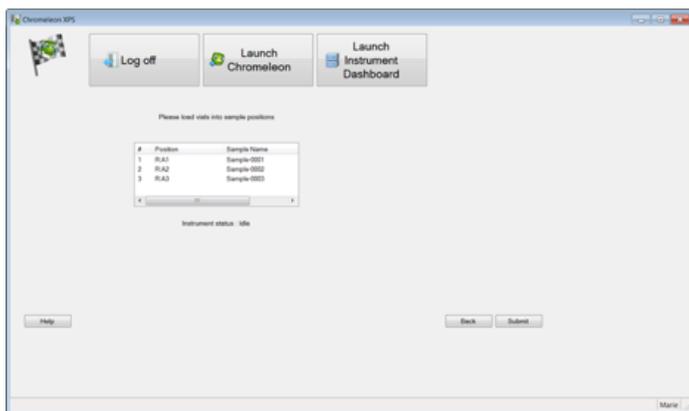
Scientists at the Medicinal Chemistry and Synthesis Core Facility at CIDD reached out to Thermo Fisher Scientific to help. They needed a solution that would allow them to generate LC-MS results right in their lab, and because the scientists at CIDD are all synthetic organic chemists, with no analytical chemists on staff, needed it to be user-friendly. The solution was a Vanquish Flex UHPLC system with a ISQ-EC single quadrupole mass spectrometer detector both controlled by Chromeleon XPS.

The Vanquish Flex UHPLC system provides a rapid analysis time with very small sample requirements. At CIDD, this means that from a single drop of solution, they can have all the information they need about their

reaction in under two minutes. The Vanquish Flex UHPLC system was also designed to be simple-to-operate and easy-to-maintain, perfect for the lab's needs. Add in the simplicity of Chromeleon XPS and they can have their results all presented in a customized report with just a few mouse-clicks. The team often utilizes the simplicity of overlaying chromatograms in Chromeleon CDS in order to visually observe the progression of the reaction, noting the decrease in starting material peaks as they are consumed and increase in new peaks as products are formed. This also allows for the observation of transient intermediate products that otherwise may not be seen.

The ability to get a full understanding of their reaction is aided by the easy-to-use ISQ-EC single quadrupole mass spectrometer. It seamlessly integrates with the Vanquish Flex UHPLC and Chromeleon software and has a mass range up to  $m/z$  1250, making it able to detect small and mid-size molecules. Since the chemists at CIDD are using this for small molecules, where the charge of the molecule is usually 1, so the mass-to-charge ratio is equal to the mass +  $H^+$ , the MS results essentially provide the mass of each compound observed in the chromatogram. With this information the chemists are able to monitor their reactions by observing the reduction in starting materials (as they are consumed in the reaction), appearance of new compounds (target or intermediates) and confirmation of the intended product by mass. While NMR is still required to obtain the information necessary for applying for a patent in order to confirm the compound has the correct structure, the ability to obtain the mass of the new compounds eliminates the need to routinely send samples to another lab for analysis.

The group at CIDD had previously tried an LC-MS solution from another vendor. While it was down for a repair they started using the Thermo Scientific system and never looked back. Michael figures the switch to the Vanquish Flex UHPLC system, ISQ-EC single quadrupole MS and Chromeleon XPS software saves CIDD about two-thirds the amount of time they traditionally would have needed prior to utilizing this setup. Michael noted "Our savings are more due to the faster run times (2 min or less) compared with a standard 7 minute run on the previous system, as well as the faster log in of samples using the walk up XPS mode."



**Figure 1: Submitting samples for analysis using Chromeleon XPS software.**

### Software for any scientist

Chromeleon CDS provides full integration of Thermo Scientific gas chromatography, liquid chromatography, and ion chromatography instruments with many Thermo Scientific MS detectors, giving it wide-ranging potential for the type of analyses it can run. One potential problem for synthetic organic chemistry labs is the lack of analytical chemistry experience with the chromatography and mass spectrometry techniques that are traditionally needed to operate the equipment and generate the mass spectrum. This was the case with the lab at CIDD, as they do not have analytical chemists on staff. Chromeleon XPS Open Access software makes it easy for anyone in the lab to get results, and the staff at CIDD found the software easy to learn due to its user friendly interface. It does this by allowing users to simply walk up and use a very straightforward interface to run chromatography samples, exploiting the full power of Chromeleon CDS without the need to worry about all of the details. The full capabilities of the Chromeleon software, such as the instrument control, data processing, reporting and compliance tools, are available if needed, but their use is not necessary, and are not part of the interface in order to maintain ease-of-use. The walk-up interface is the preferred choice for analysts at CIDD according to Michael, "Chromeleon XPS is simply faster, and within only a few clicks, your analysis is running. Being able to quickly access a walk-up login window allows lab members to be more efficient and complete more target molecules in a shorter amount of time."

Chromeleon XPS is able to make chromatography simple through the use of eWorkflow™ procedures which contain a set of rules that guide the analysis, including which instrument parameters to use, how to evaluate the resulting chromatogram and how to report the results. CIDD researchers can setup an eWorkflow to automatically evaluate the chromatogram to report the retention time (similar to a retention factor in TLC), the mass and the chromatographic purity of the compound, and they will not need to perform any calculations on their own.

eWorkflow procedures can be created a number of different ways to fit into the way the lab works. The scientists at CIDD use a different eWorkflow for each project, which allows the data to easily be grouped together and found when needed. They have procedures for monitoring reactions and purification, evaluating the LC-MS system performance and even for identifying individual compounds obtained by extracting from a spot on a TLC plate. Since the different eWorkflows are all executed from the same Chromeleon XPS interface, it doesn't matter who initiates the sample analysis, the correct instrument parameters will be used and the data will all be organized the same way. Michael summed up the functionality this way, "Chromeleon XPS provides a quick and easy way to access eWorkflows and log in samples for analysis. Since the group uses different eWorkflows for each project, the XPS acts as an efficient bridge allowing multiple users to log in and run samples."

All of the data generated in the Chromeleon software is stored in a relational database. When additional insights to their data is required, the database allows CIDD to easily query their data in order to find exactly what a scientist is looking for, whether it is a peak with a specific mass, an injection performed on a certain date, or any of the other hundreds of possibilities. The query function can even be used to build charts to trend results over time. With the large number of compounds that CIDD has synthesized, the query function becomes very useful. Michael said "The ability to perform data queries is also really nice and saves a lot of time compared to other software packages I've used in the past."

## Next steps

With the Vanquish UHPLC, ISQ EC MS and Chromeleon XPS software solution working so well for their everyday routines, Michael plans to continue using the system and explore what the software can do to help them even more. One area of interest is to use 96 well plates in the autosampler in order to verify compound purity or to screen multiple reactions during optimization studies. Michael envisions injecting samples without a column in place in order to rapidly detect if the mass of the compound of interest is present. If the mass of interest is detected, the sample would then be injected on a separate flow path containing a chromatography column so that information regarding impurities/starting materials could be obtained. Michael is sure that with the help of the Thermo Fisher engineers they will be able to make this happen, "They have done a world class job of helping us at every turn. From the simplest software questions to more involved maintenance operations, they have been there."

## Summary

All synthetic chemistry labs rely on analytical testing methodologies to provide insight into their reactions and the compounds they are producing. Modern technology is available that allows many labs to forgo traditional methods in order to get much more information from a single sample. Unfortunately, many of these labs do not have personnel who are trained in current analytical technologies so they need to send samples to another lab for testing – a process that adds delays and cost to each compound they synthesize. Chromeleon XPS Open Access software allows any analyst, regardless of their chromatographic knowledge, to run samples on powerful instruments such as UHPLC-MS, with just a few mouse clicks. The ability to perform the analysis in-house saves time (by as much as two-thirds) as well as expense for the lab, allowing them to meet their goal of delivering the assigned target compounds on time and within budget.



Find out more at [thermofisher.com/chromeleon](https://thermofisher.com/chromeleon)

**ThermoFisher**  
SCIENTIFIC