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# A FULLY AUTOMATED LC-MS WORKFLOW FOR IDENTIFYING TARGETS IN CHEMICAL REACTIONS

*Darren Barrington-Light • Product Marketing Manager for Chromeleon*

**"WE HAVE USED THE SYSTEM NOW FOR FIVE MONTHS AND IT WORKS VERY WELL. IN TOTAL WE HAVE ANALYZED OVER 9,000 SAMPLES SO FAR AND IN 90% OF CASES WHERE THE TARGET MASS WAS IN THE REACTION, WE HAVE FOUND IT AUTOMATICALLY."**

**— RENE SPANG, HEAD OF LABORATORY MASS SPECTROMETRY SERVICE BAYER PHARMA AG, WUPPERTAL, GERMANY**

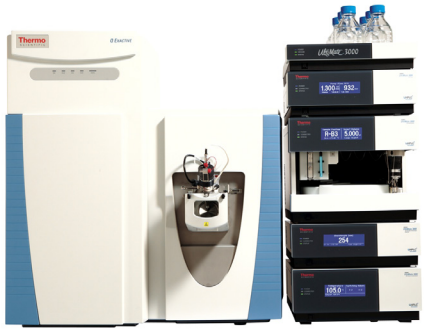

Figure 1. Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system and the Thermo Scientific™ Exactive™ Plus Orbitrap™ Mass Spectrometer.

End-to-end automation of an integrated liquid chromatography-mass spectrometry (LC-MS) workflow for targeted screening applications is readily achievable and yields substantial advantages. An automated, robust platform that combines high quality target mass interpretation with fast analysis times can boost the efficiency and productivity of drug discovery laboratories. Thermo Fisher Scientific has designed and implemented an automated LC-MS workflow optimized for accurate target mass interpretation. Under the control of the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, this customized, fully automated workflow is in operation at Bayer Pharma AG, with a reported success of finding the target mass in about 90% of more than 9,000 reaction controls studied to date.

Herein we describe a robust and efficient LC-MS workflow for high-throughput target mass analysis. It combines ultra-high performance liquid chromatography (UHPLC) and high resolution MS capable of accurate mass analysis and rapid compound identification and quantification (Figure 1). State-of-the-art Chromeleon CDS software synchronizes and controls the system components, ensuring a seamless workflow from the set-up and initiation of a screening run through data analysis and results reporting.

Thermo Fisher tailored the design and features of the workflow described here to meet the specific requirements of a targeted screening application developed by scientists in the research laboratory of Rene Spang at Bayer. We provide a detailed description of the laboratory's requirements, the criteria used to select the most appropriate LC-MS technology for the application, and the design and implementation of the workflow. Rene Spang provides an in-depth account of his lab's early experience with the system, highlighting the advantages of its ease of use, daily quality control checks, automated start-up, data analysis and reporting functions.

## THE APPLICATION: TARGET MASS INTERPRETATION

Bayer provided Thermo Fisher with several requirements for the proposed target screening system. The overall goal of the lab was to establish a fully automated workflow for finding the target mass in a chemical reaction with accurate mass interpretation in both positive and negative polarity modes (Figure 2). Additional requirements included the following:

- High quality hit rate
- Fast analysis time—a run time of approximately 3 minutes and a maximum turnaround time for the complete analysis of 15 minutes
- High capacity—screening of at least 140 samples per day and more than 35,000 per year
- Automated reporting of the results directly to the lab

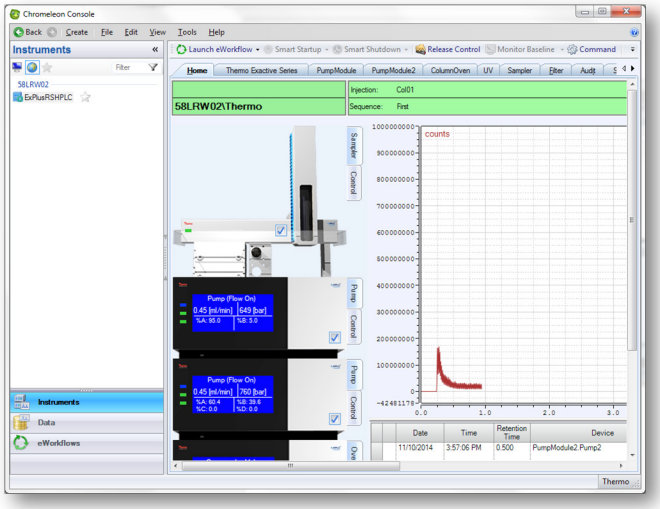


**Figure 2. Chromeleon 7.2 CDS provides seamless control of both LC and MS and simplifies the scientific workflow. This interface displays real-time pump pressure and flow rates.**

Another important capability of the system was to "find the target mass at low concentrations" (<1% in a matrix), said Rene Spang.

More specifically, the Bayer group wanted the interpretation of the reaction controls to be automated, with the analysis supported by accurate mass information. Additionally, the workflow should be compatible with UHPLC. The capability for rapid polarity switching would allow for virtually simultaneous data capture in positive and negative modes in a single run (Figure 3).

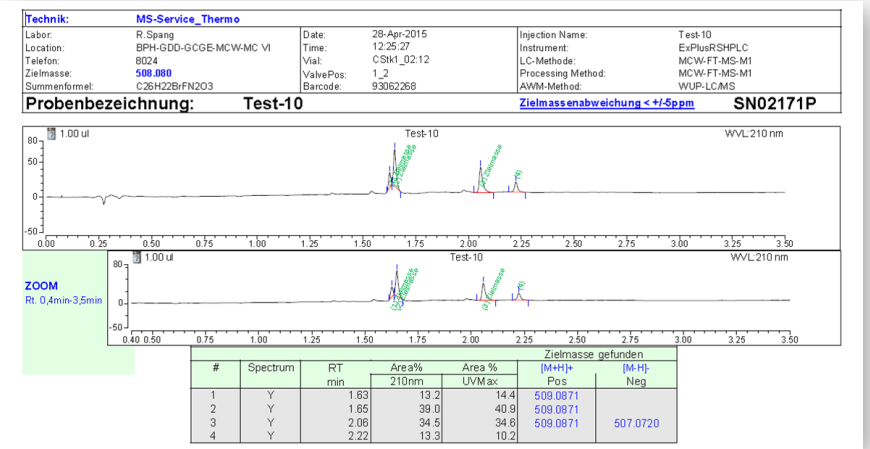


**Figure 3. Chromeleon CDS processes data in real time and automatically calculates the results, simultaneously evaluating positive and negative scans for target mass.**

## THE WORKFLOW: CDS & LC-MS

### CDS

The Chromeleon CDS facilitates the integration of UHPLC and MS into a quantitative workflow for targeted screening. It combines intelligent functionality, operational simplicity, strong quantitative capabilities, and comprehensive auditing and data management, making it broadly applicable, easy to use, and ideal for applications requiring regulatory compliance. Chromeleon 7.2 CDS, released in 2012, was the first CDS to control multi-vendor gas, ion, and liquid chromatography and mass spectrometry instruments in an enterprise or client/server environment. Thermo Fisher developed the updated version of the Chromeleon CDS software to overcome the limitations of available MS software platforms, which, according to users, included the following: the inability to work remotely; the need to store data locally; back-up being a tedious, manual procedure; limited data reporting options, often dependent on the use of Excel spreadsheets only; and regulatory compliance being difficult if not impossible to achieve.

**"I LIKE THE FACT THAT, WITH THE FLEXIBILITY AND CAPABILITIES OF CHROMELEON, WE NOW HAVE THE POTENTIAL TO DEVELOP NEW APPLICATIONS IN THE FUTURE."**

**— RENE SPANG HEAD OF LABORATORY MASS SPECTROMETRY SERVICE BAYER PHARMA AG, WUPPERTAL, GERMANY**

At the heart of Chromeleon's design and functionality are principles of Operational Simplicity™ driven by three main rules that are applied to every feature developed for the software:

- Minimize the number of steps needed to perform any task
- Make all steps easy to understand and perform
- Minimize the time needed to carry out any task

The client's application workflow should begin with entry of the target screening experiments in the scientist's lab journal, which links with the CDS through the laboratory information management system (LIMS) (Figure 4). Automated data reporting from the CDS via the LIMS means that analysis results are delivered directly to the scientist. The LIMS also manages application-related reporting, storage, and communication functions such as document security and email notifications.

This end-to-end software solution ensures that the set-up, operation, and quality control functions are fully automated. During screening, interpretation of the reaction controls is also automated, with the analysis supported by accurate mass information. When the system has completed all measurements, it automatically transmits the results to the researchers in the lab, drastically reducing response times.

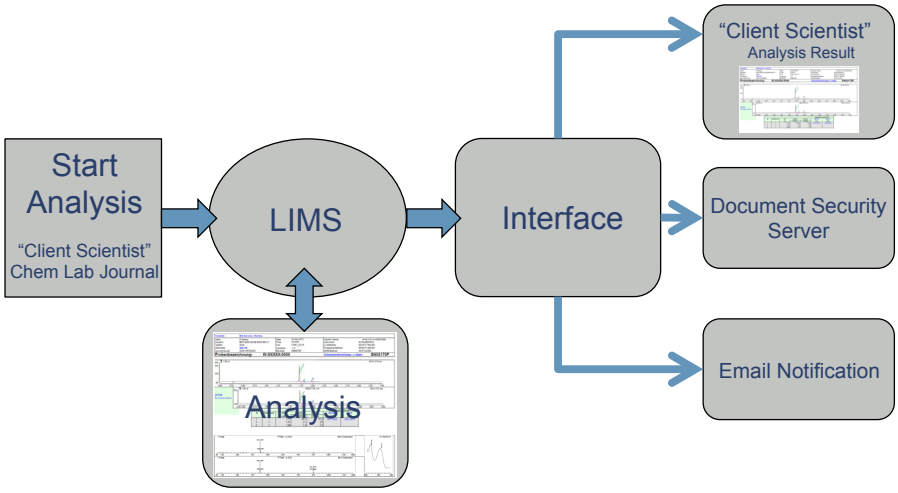


**Figure 4. An overview of the application workflow. The scientist's lab notebook links with the CDS through the LIMS. Data reporting back to the scientist is fully automated.**

### LC-MS

Selection of the hardware components of the system best suited for the application presented by Bayer focused on comparing the features of the available open access UHPLC-MS platforms. The comparison included three UHPLC-MS systems:

- Single quadrupole MS
- AM-TOF
- AM-Orbitrap

All three are good, robust options and both the AM-Orbitrap and single quadrupole MS offer fast analysis times. The AM-TOF system, however, requires longer analysis times and is not capable of automatic switching to scan in positive/negative polarity modes, making it less suitable for this application. The AM-Orbitrap and the AM-TOF provide excellent interpretation capability for both accurate mass and isotope; whereas the single quadrupole MS system is restricted in its target mass interpretation workflow due to the lack of accurate mass data. The ideal set-up optionfor the Bayer lab, therefore, was the UHPLC-MS/AM-Orbitrap.

## SYSTEM DESIGN

### SET-UP

Figure 5 illustrates the complete system as installed in the lab at Bayer. The Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system can be seen with the pump (far right) set here at 0.9 ml/min, or 479 bar, the diode array detector (middle bottom), and an autosampler (middle top) with three drawers holding 96-well plates for high throughput sample processing. This system is an ideal choice

for rapid, high resolution UHPLC separations and offers exceptional flexibility for different column formats. On the far left is the Thermo Scientific™ Exactive™ Plus Orbitrap™ Mass Spectrometer, which provides >1 ppm mass accuracy, fast scanning to support UHPLC applications, and rapid polarity switching to maximize the information obtained in a single run. These characteristics make the Orbitrap particularly well-suited for screening applications that depend on accurate target mass and the identification and quantification of compounds in complex samples.

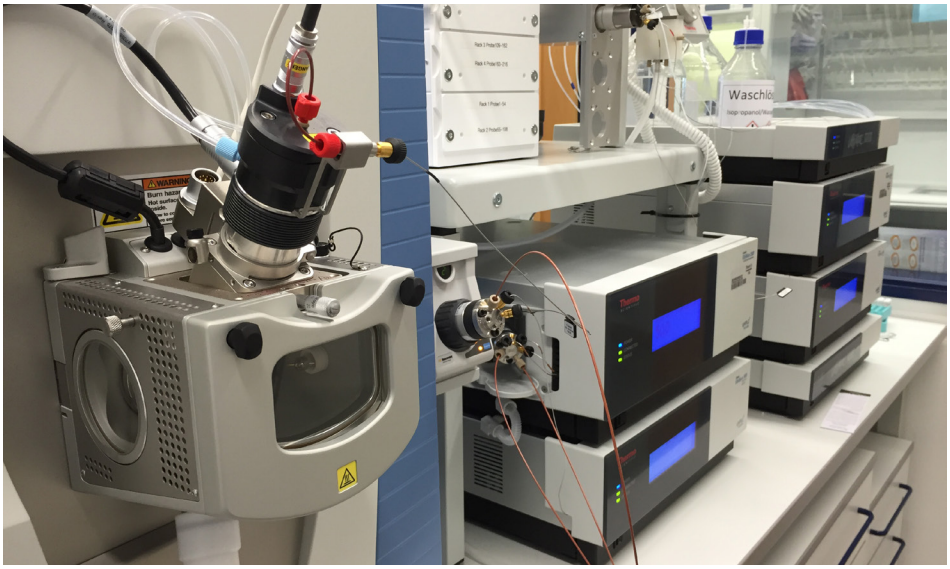

**Figure 5. The system installed at Bayer includes the Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system and the Thermo Scientific™ Exactive™ Plus Orbitrap™ Mass Spectrometer.**

## WORKFLOW

Chromeleon 7.2 CDS synchronizes and controls all of the system components. Chromeleon eWorkflows™ allow for fast, accurate sequence set-up (Figure 6). In the morning, scientists can select the samples to be analyzed and readily create a full day's sequence for screening, specifying processing methods, custom variables, and results reporting. Metadata entry, including sample number, target mass, and chemical mass requires only the use of a barcode scanner. Adduct selection can be made from a dropdown box if required.

The system simultaneously captures and analyzes UV and MS data—both positive and negative polarity— in one run (Figure 7). Analysis time is only 3.5 minutes per sample. Injection overlap—preparation of the next sample as the previous one is being analyzed—ensures that no time is wasted between runs.

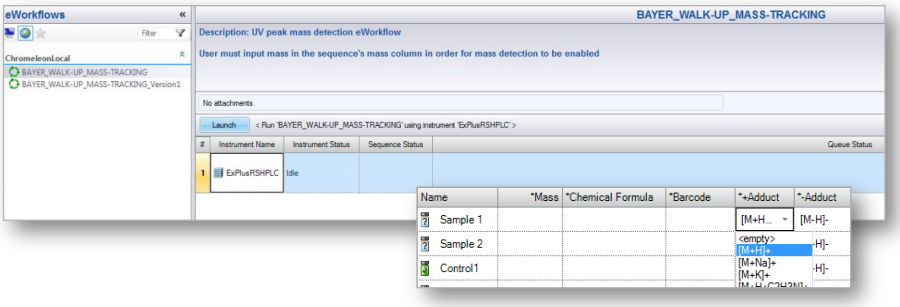


**Figure 6. Scientists use eWorkflows™ to set up a full day's sequence of screens, selecting the samples, processing methods, custom variables, and results reporting methods.**

The Chromeleon Intelligent Run Control feature automatically extracts a UV optimum integration path, creating a chromatogram with a maximum response for each peak. All data generated are immediately available for analysis, and data processing is fully automated. Chromeleon CDS processes the data and calculates the results.

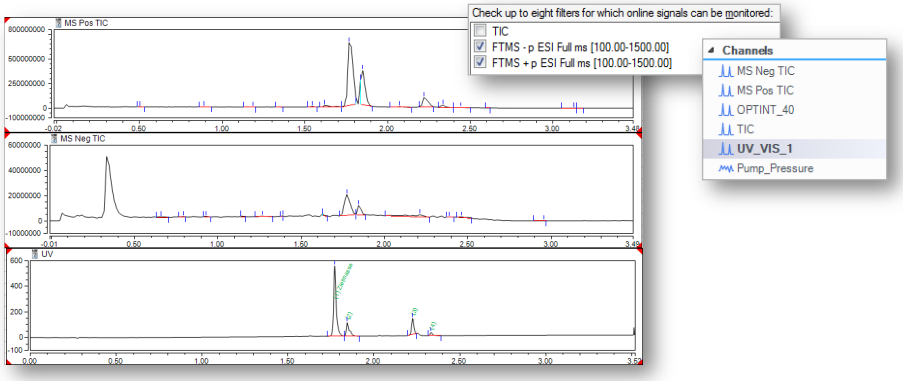


**Figure 7. Chromeleon 7.2 CDS simultaneously acquires and analyzes UV and MS data, in both positive and negative polarity, in a single run.**

The UV summary report brings together all of the metadata for analysis and shows the UV traces. The report identifies all of the peaks found in the chromatogram with their retention times and the target mass found in positive or negative traces. The UV and MS spectra are shown for each peak for which the target mass is found.

Chromeleon CDS exports the results to a pdf. The pages are labeled according to sample barcode, name, and test date. The software autofiles each report based on whether the target mass was detected (pass), not detected (fail), or blank or quality control sample.

## SYSTEM PERFORMANCE

After 8 months of operating the UHPLC-MS/AM-Orbitrap/Chromeleon CDS system in his laboratory, Rene Spang described the workflow as "very successful." As previously stated, his group had run more than 9,000 reaction controls, and in 90% of cases the system had identified the target mass. The researchers are especially enthusiastic about the quality control capabilities of the system, ease

of use, automatic system start-up feature, and the good resolution and fast analysis times the system provides. They are able to instruct the system to start up automatically at 6:30 am on workdays, so it is ready for use when his team arrives in the morning.

Thermo Fisher created a customized QC system for the application. The QC software checks the spectra and the chromatograms by peak capacity and performs whole system checks every 3 hours. Especially beneficial is an automatic daily QC system check to confirm calibration to +/- 4ppm. The system calibration has proven to be very stable over weeks. The report generated by the QC system gives "a good overview of the condition of the system," says Rene Spang.

## CONCLUSIONS

Thermo Fisher was able to deploy an integrated and fully automated UHPLC-MS workflow for a target screening application that meets and exceeds the original expectations and requirements of Bayer Pharma. The sample run time is 3.5 minutes, made possible by simultaneous positive/negative scanning through rapid polarity switching. The total end-to-end analysis time is less than 15 minutes per sample.

The UltiMate 3000 RSLC system gives robust, reproducible UHPLC separations. Its high sample capacity means users can load the autosampler in the morning with enough samples for a full day of runs. The Bayer group has confirmed the instrument's excellent long-term calibration stability. Key features of the Exactive Plus MS system—its speed, high resolution and accurate mass capability, and simultaneous polarity detection—make it ideal for a targeted screening application. Finally, Chromeleon CDS provides seamless control of the hardware components and full automation of sample analysis, accurate determination of target mass, and results reporting. It allows for customized QC protocols and easy sequence and injection set-up.

This automated workflow for target analysis in a chemical reaction can help identify high quality hits and accurately interpret target mass. The system meets the needs of a drug discovery laboratory for a robust, reliable, fast, and high capacity, high-throughput system. Experience operating the system in a real-world setting confirms the performance and advantages of the automated workflow for a targeted screening application.

**Reference**

Barrington-Light D., Spang R. (2015, August 26). A Fully Automated LC-MS Interpretation Workflow for Finding Targets in Chemical Reactions. [video file]. Retrieved from http://cen.acs.org/media/webinar/thermo_082615.html



AMERICAN CHEMICAL SOCIETY
1155 Sixteenth Street, NW,
Washington, DC 20036

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