

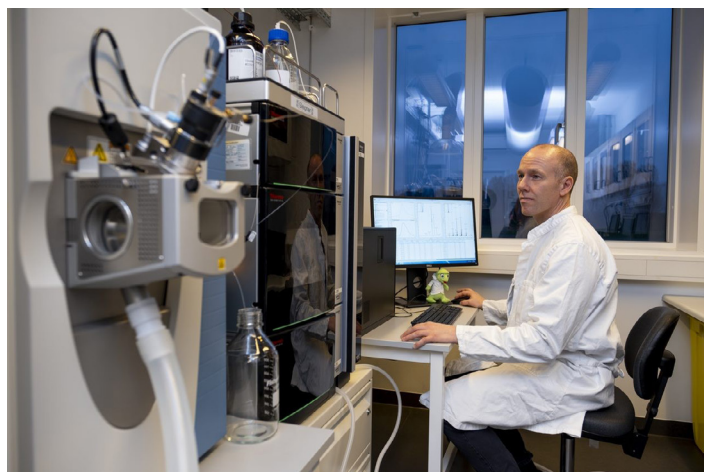
Boosting productivity of monoclonal antibody development using Chromeleon CDS

Symphogen, a subsidiary of Servier and antibody center of excellence

Single software platform streamlines operations and supports powerful mass spectrometry (MS) workflows

Symphogen is an antibody oncology-focused company, pioneering discovery and early development of highly differentiated monoclonal antibody (mAb) therapeutics. To advance these efforts, Symphogen develops validated analytical liquid chromatography (LC)-MS workflows for characterizing and monitoring therapeutic proteins. Of these workflows, native size exclusion chromatography (SEC)-MS using the Thermo Scientific™ Q Exactive™ mass spectrometer and the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer have become indispensable for rapidly obtaining accurate molecular mass (from MS data) and aggregation information (from chromatographic separation and UV trace) for mAb lead selection studies (Figure 1). Both instruments provide high-resolution accurate-mass (HRAM) data that enables Symphogen to accurately and confidently characterize mAbs in their native state.

The challenge to be first to market with urgently needed therapies means technologies that accelerate and increase the efficiency of LC-MS workflows are highly desirable. Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) is one such technology. Used by



Symphogen for over 20 years, the software provides an easy-to-use, unified platform that streamlines laboratory operations, including acquisition, processing, review, and reporting of data obtained from chromatography instruments and Thermo Scientific™ mass spectrometers. In addition, seamless data and method transfer with extensive compliance features facilitates moving candidate molecules to contract research and contract manufacturing organizations (CROs and CMOs) for the next phase of drug development.



“To process so many samples, it’s really nice to have a solid unified platform for acquiring, processing, and reviewing data, which we have with Chromeleon CDS. When we do our lead selection studies, the first thing is to determine if our candidate molecules are aggregating and their identities. We can do that in a single experiment with native SEC-UV-MS and Chromeleon software, and it’s a matter of seconds screening through the results.”

– Dr. Dan Bach Kristensen,
Principal Scientist, Symphogen

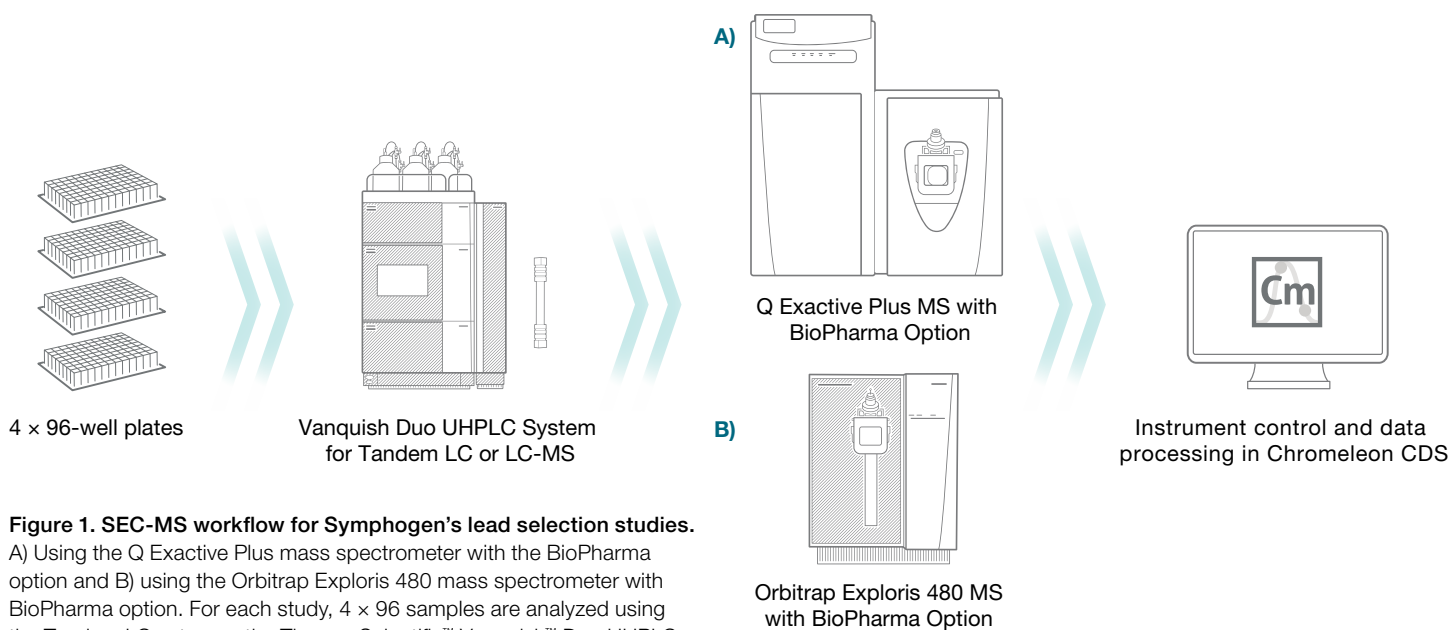


Figure 1. SEC-MS workflow for Symphogen’s lead selection studies.

A) Using the Q Exactive Plus mass spectrometer with the BioPharma option and B) using the Orbitrap Exploris 480 mass spectrometer with BioPharma option. For each study, 4 x 96 samples are analyzed using the Tandem LC setup on the Thermo Scientific™ Vanquish™ Duo UHPLC system. Instrument control, data processing, and reporting are performed in Chromeleon CDS.

Integrates chromatography and mass spectrometry

With increasing demand for powerful MS workflows, Chromeleon CDS delivers a single platform solution that offers control, data acquisition, data processing, and reporting for Thermo Scientific chromatography and MS instruments. Continued software development has extended these capabilities to HRAM instrumentation, including MS-specific data visualization and compliant tuning and calibration. For the native SEC-MS workflow, the software supports intact protein deconvolution (IPD) data processing, allowing screening and characterization of intact proteins with increased productivity and confidence.

Two complementary deconvolution algorithms optimized for isotopically resolved (Xtract) and unresolved data (ReSpect™) take full advantage of the high-quality HRAM data produced by Thermo Scientific™ Orbitrap™ mass spectrometers. MS data processing parameters are saved as part of the Chromeleon Processing Method, ensuring traceability and completeness, and creating a template for future use. In addition, support for HRAM MS instrument control has enabled Symphogen to seamlessly transfer their SEC-MS analyses from the Q Exactive instrument to the Orbitrap Exploris 480 instrument.

“Chromeleon CDS is a very efficient platform for acquiring, processing, and reviewing data. Despite having a large number of samples, we can quickly review results comparing the theoretical masses with the measured masses of our lead candidates. It’s very easy to visually process a lot of samples in a very short time.”

– Dr. Dan Bach Kristensen.

| Summary | | | | | | |
|---------------------------------|-----------------|---------------------|------------------|---------------------------|------------|--------------------------|
| Sequence name: | | | | | | |
| SEC and Intact Mass results for | | Intact Mass Results | | Delta Mass | Delta Mass | Delta Mass |
| Inj. No | Injection Name | Intact Mass Da | Expected_Mass Da | Abs(Expected-Intact Mass) | >5 Da | Complies/Does not comply |
| 5 | ASS_23480 | 147368.3 | 147368.8 | 0.50 | | Complies |
| 6 | ASS_23482 | 147476.6 | 147477.3 | 0.67 | | Complies |
| 7 | ASS_23483 | 148407.4 | 148408.4 | 1.05 | | Complies |
| 8 | ASS_23484 | 147264.2 | 147265.1 | 0.86 | | Complies |
| 9 | ASS_23487 | 148435.6 | 148436.4 | 0.76 | | Complies |
| 10 | ASS_23488 | 147397.6 | 147397.1 | 0.54 | | Complies |
| 11 | ASS_23490 | 147251.8 | 147252.9 | 1.07 | | Complies |
| 12 | ASS_23491 | 147321.8 | 147321.1 | 0.68 | | Complies |
| 13 | ASS_23492 | 147655.4 | 147655.9 | 0.51 | | Complies |
| 14 | ASS_23493 | 148933.9 | 148934.9 | 0.99 | | Complies |
| 15 | ASS_23494 | 149388.1 | 149389.5 | 1.36 | | Complies |
| 16 | ASS_23496 | 148131.2 | 148132.1 | 0.95 | | Complies |
| 17 | ASS_23499 | 148126.3 | 148126.3 | 0.03 | | Complies |
| 18 | ASS_23501 | 147927.3 | 147927.7 | 0.45 | | Complies |
| 19 | ASS_23502 | 147400.9 | 147401.1 | 0.17 | | Complies |
| 20 | ASS_23503 | 147945.3 | 147945.9 | 0.63 | | Complies |
| 21 | ASS_23505 | 147771.0 | 147771.7 | 0.69 | | Complies |
| 22 | ASS_23507 | 150485.5 | 150486.6 | 1.13 | | Complies |
| 83 | ASS_23622 | 147199.6 | 147200.9 | 1.36 | | Complies |
| 84 | ASS_23624 | 147603.4 | 147603.4 | 0.08 | | Complies |
| 85 | ASS_23626 | 147312.6 | 147312.9 | 0.31 | | Complies |
| 86 | ASS_23627 | 147739.4 | 147739.6 | 0.20 | | Complies |
| 87 | ASS_23628 | 148011.5 | 148011.7 | 0.19 | | Complies |
| 88 | ASS_23629 | 147270.6 | 147271.0 | 0.41 | | Complies |
| 89 | ASS_23631 | 148811.3 | 148812.4 | 1.17 | | Complies |
| 90 | ASS_23632 | 147459.2 | 147459.1 | 0.14 | | Complies |
| 91 | ASS_23633 | 148092.8 | 148227.7 | 134.93 | 134.9 | Does not comply |
| 92 | ASS_23636 | 147190.5 | 147190.9 | 0.49 | | Complies |
| 93 | ASS_23637 | 149673.3 | 149673.6 | 0.34 | | Complies |
| 94 | ASS_23638 | 147983.2 | 147983.6 | 0.43 | | Complies |
| 95 | ASS_23640 | 148280.0 | 148280.3 | 0.33 | | Complies |
| 96 | ASS_23641 | 148762.0 | 148762.4 | 0.46 | | Complies |
| 97 | ASS_23643 | 147325.7 | 147327.3 | 1.58 | | Complies |
| 98 | ASS_23646 | 147973.3 | 147973.7 | 0.49 | | Complies |
| 99 | ASS_23650 | 148486.5 | 148486.4 | 0.13 | | Complies |
| 100 | ASS_23656 | 147931.4 | 147931.6 | 0.18 | | Complies |
| 101 | ASS_23658 | 147176.8 | 147177.3 | 0.53 | | Complies |
| 102 | ASS_23661 | 148348.0 | 148348.2 | 0.16 | | Complies |
| 103 | ASS_23663 | 147488.8 | 147489.0 | 0.20 | | Complies |
| 104 | ASS_23665 | 147909.8 | 147909.6 | 0.29 | | Complies |
| 105 | ASS_23483_Reinj | 149387.9 | 148408.4 | 979.50 | 979.5 | Does not comply |
| 108 | ASS_23666 | 147937.4 | 147937.6 | 0.19 | | Complies |

Figure 2. Rapid visualization of native SEC-MS results in Chromeleon software, with clear indication of outliers.

Robust HRAM data processing and rapid data review

Symphogen typically analyzes 400 samples per study by native SEC-MS, generating substantial amounts of data that must be efficiently processed and reviewed to confirm identity and to check for mAb aggregation. According to Dr. Dan Bach Kristensen, Principal Scientist at Symphogen, “We need a system that’s robust and efficient not only for acquiring data, but also for processing data, which can be time-consuming with MS.” Chromeleon CDS provides a single software workflow to view and report identity confirmation and aggregation. Once data are acquired, the software automatically compares the theoretical mass entered by the user with the experimentally determined mass and presents the results in an easy-to-visualize format (Figure 2). This data visualization approach makes it very easy to review numerous samples in a matter of seconds.

Seamless method transfer to contract organizations using the same CDS

GMP production of material for clinical trials is performed by CROs and CMOs. Transferring biopharmaceutical methods between systems in the same lab, between labs, or to an external partner lab can be challenging. Symphogen’s CMO also uses Chromeleon software, allowing for seamless exchange of data and methods. Said Trine Meiborg Sloth, Senior Technician, Analytics and Formulation at Symphogen, “We can send the entire sequence with the instrument methods and data, and the CMO can easily open them.” The software packages up the data, methods and associated files, such as the report template and creates a CMBX file, which can be directly imported back into the software. Because only Chromeleon CDS recognizes the CMBX file, the package is secure.



“Using the enterprise version for all the instruments makes remote reviewing and approving easier. There are 23 users of our enterprise installation and our MS systems will be a part of that.”

– Trine Meiborg Sloth,
Senior Technician, Analytics and Formulation, Symphogen

Full connectivity for all laboratory operations with one CDS

Chromeleon software addresses analyses of hundreds of samples throughout Symphogen’s operations, not only in the MS lab, but also in the chromatography lab where it’s used as the platform for acquiring and processing LC-UV instrument data. In addition, data from other instruments such as Beckman™ capillary electrophoresis (CE) and ProteinSimple® iCE™ systems are imported and managed in the software. Standardizing on a single platform offers considerable efficiency gains, including reduced training effort and implementation time, and centralized access to data and results from any location using client/server architecture. Enterprise deployment makes it easy for staff to review and approve data remotely, without interrupting work at the lab bench. For this reason, Symphogen plans to move their MS system control to the enterprise setup, which will allow them to take full advantage of the benefits it can provide.

Simplified processes with predefined templates and customized reporting

Designed with scientists and technicians in mind, the software delivers customizable method templates that simplify repetitive tasks, reduce errors and help labs to achieve more ‘right first-time’ analyses. Its workflows deliver ease-of-use, comprehensive instrument control, automated data analysis, and flexible reporting to provide useful insights into data.

According to Trine Meiborg Sloth, “In our HPLC lab, we have created our own sequence template for Symphogen’s projects and associated analytical methods. They contain the predefined known sample information and all the instrument and processing methods together with the report template. When analyzing daily samples, we just choose the sequence template for the analysis and copy it into the defined folder path. We have also created custom fields, so we only have to type in the sample concentration and unknown sample information and then we are ready to go.”

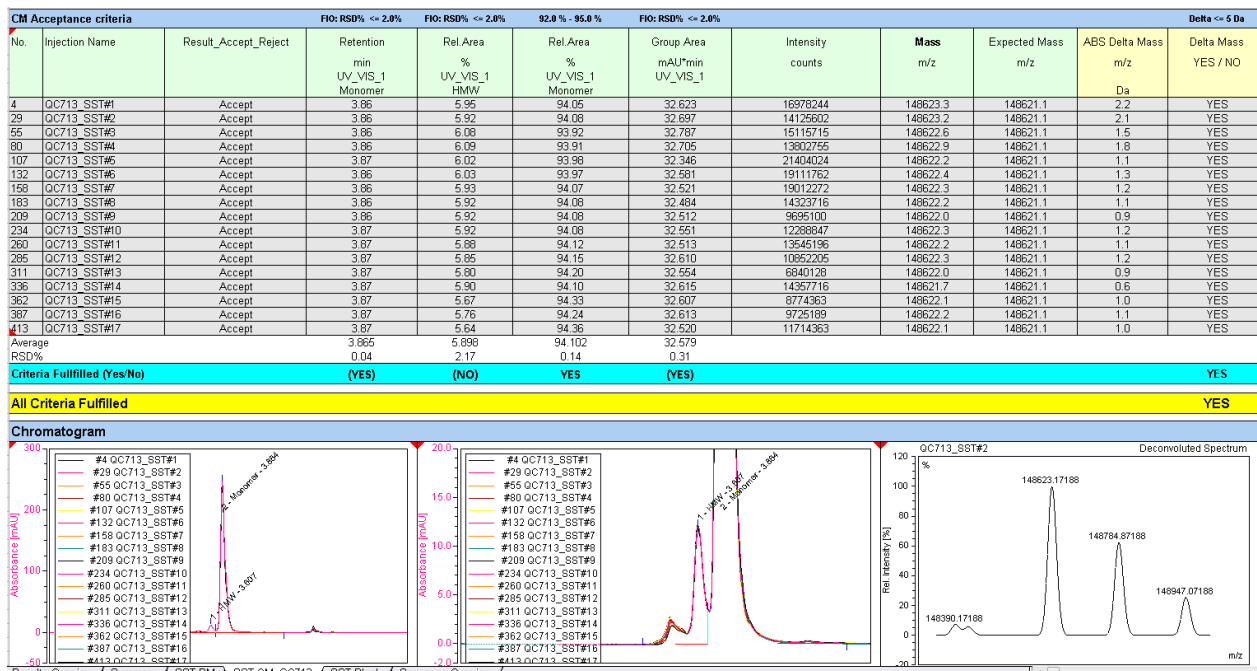
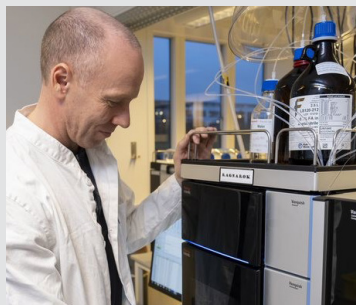


Figure 3. Customizable reports in Chromeleon CDS, tailored to specific methodologies.



“Exchange of data and methods is very easy. When we develop a method we have to transfer that to an external partner and that’s seamless when the same very well-known Chromeleon software is used.”

– Dr. Dan Bach Kristensen

Chromeleon CDS sequence templates are built the same way, regardless of technique and instrument, making the process of switching from one to the other straightforward. And with intelligent recognition, the appropriate method options are presented to the end user based on the instrument configured.

Customizable, built-in spreadsheet-based reporting options makes it easy to for Symphogen to tailor reports to specific methods containing different criteria. For example, to quickly monitor results for carry over, reference material, and control samples, and to present test sample results (Figure 3). Microsoft® Excel®-like reporting functionality means that calculations can be easily transferred or replicated within the CDS, simplifying the learning and reporting process for users. In addition, by performing

calculations in the software, data remain in a secure environment and the possibility of transcription errors is eliminated.

Conclusion

Using the right tools is of paramount importance in the effort to advance tomorrow’s therapies. That is why Symphogen has deployed Chromeleon CDS for both routine chromatography and cutting-edge MS workflows, and to integrate data across their operations in an intuitive software platform. From acquisition to processing to review of data, the software streamlines laboratory operations, while seamless data and methods transfer to CROs and CMOs makes it easy to move leads to the next phase of drug development.

A collaboration to move biopharma forward

Symphogen and Thermo Fisher Scientific have a long-standing working relationship dating back to 2000, when Symphogen was founded and entered an official collaborative relationship in 2018. This collaboration has been built and expanded on a foundation of knowledge sharing. Symphogen focuses attention on developing patient-focused products with unique modes of action, with the aim of moving the field forward and potentially making a huge difference in patient outcomes. These ambitions can only be realized with a best-in-class understanding of protein chemistry and the best suited analytical tools, to ensure that Symphogen remains at the forefront of biopharmaceutical techniques. The collaboration provides Symphogen with state-of-the-art technology for product development and knowledge sharing, relevant to the biopharmaceutical field.



For more information about the protein characterization workflows, technologies, and methods used by Symphogen, visit thermofisher.com/Symphogen.



Find out more at thermofisher.com/Symphogen

For General Laboratory Use. Not for diagnostic procedures. © 2021 Thermo Fisher Scientific Inc. All rights reserved. Beckman is a trademark of Beckman Coulter, Inc. ProteinSimple ICE is a trademark of ProteinSimple. ReSpec is a trademark of Positive Probability Ltd. Excel is a trademark of Microsoft Corporation. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. **CS73994-EN 0321M**

ThermoFisher
SCIENTIFIC