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CASE STUDY 72986

Simplifying complexity in therapeutic protein drug development

A symphony of antibodies to attack cancer

Symphogen is a clinical-stage biopharmaceutical company near Copenhagen, Demark. Symphogen has a uniquely differentiated precision-medicine approach product pipeline based on monoclonal antibody (mAb) and mAb mixture drug candidates.

In addition to development of individual mAb drug candidates, Symphogen is further pioneering the application of mAb mixtures as a differentiated approach to cancer therapy that can overcome some of the limitations of conventional single-target mAbs. A mAb mixture is a combination of two or more systematically selected, well-characterized mAbs developed as a single drug product. The mAbs in the mixture may bind different positions on the same target or they may bind different targets. The targets can be on the same or on diverse cell types. Targets are typically cell-surface receptors or ligands. Symphogen has several clinical stage candidates each with high potential to provide cancer patients meaningful treatment options that are not currently available.

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"I consider myself a craftsman and like any craftsman I rely on having the right tools. The collaboration with Thermo Fisher Scientific will provide Symphogen with the right, powerful tools for reaching our end goals"

— Dr. Dan Bach Kristensen
Principal Investigator, Symphogen

The mAb mixtures are complex and include

- Sym004 – A mixture of two mAbs inhibiting EGFR
- Sym015 – A mixture of two mAbs inhibiting MET
- Sym013 – A multi-targeting pan-HER mAb mixture consisting of six mAbs

The challenge of complexity

Bringing a single-mAb-based drug product to market is challenging enough. Multi-mAb symphony treatments represent an exponential increase in complexity.

Why not just develop them as separate products? This approach requires multiple parallel development programs and independent clinical testing; which is inevitably costlier, potentially prohibitively so.

Consequently, the mixed-mAb development approach has significant time and cost benefits, providing the challenge of protein characterization and control can be overcome for these complex mAb mixtures.

Simplifying complexity with native mass spectrometry

Recently, Symphogen has implemented intact mass spectrometry under 'native' conditions to determine molecular mass information on some of their most complex therapeutic products. Analysing samples under non-denaturing conditions allows characterisation of the molecule closer to its native biological (therapeutic) form and can provide some complimentary structural insights.

Previously this information would be gained using conventional approaches often incorporating multi-step isolation and purification of individual antibodies, for example using ion exchange chromatography. These methods were cumbersome, labour intensive and required substantial amounts of valuable product. Furthermore, these methods also required fractionation and subsequent concentration steps which could in themselves impact product quality.

With native MS analysis many drug quality attributes can now be directly assessed with minimal need for sample preparation regimes, rapidly providing MS spectra for all

Monoclonal Antibodies (mAbs)

Antibodies are Y-shaped proteins, circa 150 kDa in mass, produced by specialized cells of the body's immune system. Antibodies can identify and bind disease-specific antigens found on bacteria, viruses, and cancer cells. Once attached to the antigen, antibodies can block signalling or recruit other parts of the immune system to help neutralize the cells containing the antigen. The place on the antigen where the antibody binds is called an epitope. Researchers can design antibodies to target a certain antigen. Antibodies can be produced recombinantly in large amounts as monoclonal antibodies. Monoclonal antibodies are a well-established drug class today used to treat many diseases, including cancer. Antibodies are manufactured using mammalian cell culture processes and are heterogenous mixtures of immunoglobin proteins, with heterogeneity stemming from genetic variations as well as environmental conditions of the cell culture process. Understanding and controlling the heterogeneity and structure of these complex proteins is critical to ensure drugs are both efficacious and safe. Characterization of a single antibody is a challenging process involving multiple analytical protocols and techniques.

"CVA-MS application method worked beautifully on our most complicated product from the very first tests"

— Dr. Dan Bach Kristensen | Principal Investigator, Symphogen

component antibodies. Each mAb in a multi-mAb antibody mixture drug product has the potential to exhibit differential pharmacokinetics (PK) and/or pharmacodynamics (PD) profiles, thus native MS analysis provides an important means to mitigate some of the analytical challenges that these complex molecules pose.

Flexible separation approaches with native mass spectrometry

Conventional reverse phase chromatography approaches used with MS incorporate organic solvents, and/or acids at high temperatures. Symphogen has experienced analysis related sample degradation with some of their products, witnessing fragmentation of acid labile sites, using these harsh chromatography conditions. Native separations typically include room temperature conditions with physiologically pH relevant solvents. Innovations in mass spectrometry hardware and developments in volatile chromatography buffers are now making this possible.

Every drug candidate is unique and often a development laboratory will need an array of chromatographic techniques at their disposal. Thermo Fisher Scientific provides native MS analysis workflows with a variety of chromatography techniques. Symphogen welcomes this versatility and ability to use ion exchange, protein affinity, and size exclusion chromatography (SEC) hyphenated to mass spectrometry.

A variety of native MS chromatography approaches are proving beneficial to drug product development. Protein A capture coupled to native MS is conveniently used to monitor product quality during upstream fermentation. Harvest samples can be loaded directly into the system via a protein-A column, quickly revealing any changes to the glycosylation pattern and thus assessing product quality. The combination of native MS analysis with size exclusion chromatography provides high throughput characterization with its short analysis time, and quick desalting.

Multi-mAb products contain antibodies which are highly similar and can be challenging to separate, with the most complex mixtures requiring chromatography approaches that are traditionally incompatible with MS. Applying the Thermo Scientific™ Charge Variant Analysis Mass Spectrometry (CVA-MS) application method, Symphogen have been able to harness the combination of high resolution separations and high resolution, accurate mass Orbitrap-based mass spectrometry to characterize their most challenging products.

With 15 minutes turnaround time CVA-MS analysis provides unrivalled performance benefits and it's now something the company rely extensively on for characterization.

Built For Biopharma – Thermo Scientific Q Exactive Biopharma Platform and Thermo Scientific Vanquish Horizon UHPLC System

The indispensable ability to carry out Native MS analysis is facilitated by the Thermo Scientific™ Vanquish™ Horizon UHPLC System coupled to the Thermo Scientific™ Q Exactive™ BioPharma platform, which benefits from an extended mass range up to 8000 m/z to accommodate native MS analysis of mAbs and mulit-mAb products. Symphogen was early adopters of the Vanquish Horizon UHPLC System receiving the system in 2015. Immediately after installation the system exhibited outstanding chromatography performance in terms of both resolution and selectivity and has now established itself as an essential part of their internal LC and MS laboratories (having 4 and 3 systems, respectively), as well externally in the QC lab at preferred CMO (2 systems).

Symphogen has found that the Q Exactive BioPharma Platform provides superior spectral data quality. Dr. Dan Bach Kristensen explains; when you compare RAW, non-deconvoluted data from Time of Flight (ToF) and Orbitrap instruments, the spectral data quality is strikingly different. Unlike on ToF data, deconvolution algorithms

"The thing that struck me was the difference in quality of spectral data when comparing TOF vs Orbitrap data"

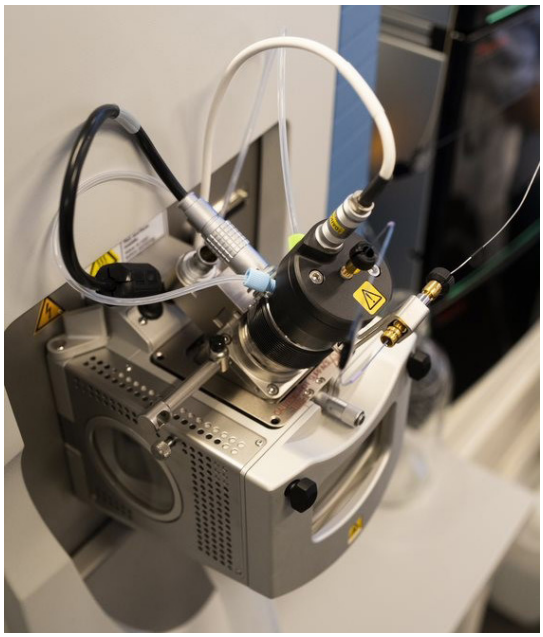
— Dr. Dan Bach Kristensen
Principal Investigator, Symphogen

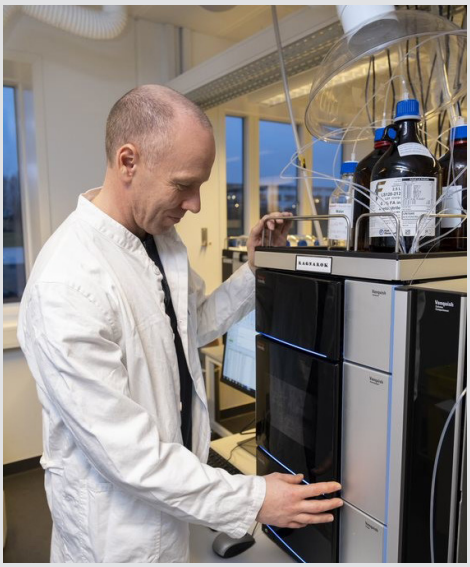
have very little impact on native Orbitrap data. With Orbitrap data, if you take a single charge state in the raw spectrum and compare that to the deconvoluted spectrum with respect to the glycan distribution, you will see that glycan distributions are highly similar. The increase in spectral data quality observed on Orbitrap systems provide confidence with respect to precision, accuracy and reproducibility of the results. This is a clear advantage when submitting data in regulatory filings.

Having extensively utilized Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software within their LC laboratory the transition to Native MS workflows within Chromeleon CDS was simple. What's more, Chromeleon CDS provides a compliant environment for native MS analysis which is essential for their plans to transition this approach into more routine environments, including with external CRO partners. The Q Exactive Plus Biopharma is an ideal choice as Chromeleon CDS can be used from data acquisition to data processing, assuring data integrity needs for MS methods implemented in the QC Laboratory. With exceptional hardware and software capabilities Thermo Fisher Scientific is an ideal partner in Symphogen's efforts to transition MS analysis into routine QC environments.

Native MS analysis has quickly become the method of choice and a workhorse platform for intact MS analysis in Symphogen's development laboratory, a transition that was driven by the versatility, gentleness, robustness and superior spectral data quality obtained by native MS on the Orbitrap platform.

In addition, native SEC-MS method has become the preferred identity test, as it, in addition to advantages mentioned above, offers significant time saving when hundreds of samples need to be analysed in a lead selection study. After extensive use of native SEC MS, Symphogen has found that instrument performance is robust and stable over the course of a typical lead selection package (~400 samples analysed consecutively).





"The collaboration with Thermo Fisher Scientific provides know-how, the ability to work with state-of-the-art equipment and get the best out of it, which is a big advantage for us"

— Mads Laustsen
Chief Manufacturing Officer, Symphogen

Symphogen & Thermo Fisher Scientific – a partnership built for biopharma

Thermo Fisher Scientific and Symphogen have a long-standing collaboration dating back to 2000 when Symphogen was founded. The collaboration has been built and expanded upon by a foundation of knowledge sharing. Symphogen resists any efforts to produce 'me-too' products and focus attention on developing patient focused products with unique modes of actions with the aim of moving the field forward and potentially making a huge difference to a patient's life. These ambitions can only be realised via a best in-class understanding of protein chemistry and the correct tools to go with it. It is therefore essential that Symphogen remain at the forefront of biopharmaceutical development technology. The partnership between Thermo Fisher Scientific and Symphogen provides state-of-the-art technology for drug product development as well as knowledge sharing relevant to the biopharmaceutical field.

Symphogen – Copenhagen, Denmark

For more information on the workflows, technologies, and methods employed by Symphogen for characterization of complex protein mixtures please visit thermofisher.com/Symphogen.

Find out more at **thermofisher.com/QEBP**

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