

Complete biotherapeutic characterization

BioPharma Finder Software

thermo scientific

Transform MS data into actionable results with comprehensive, workflow-driven software

Thermo Scientific™ BioPharma Finder™ software powered by the Thermo Scientific™ Ardia™ Platform provides comprehensive, automated data-processing workflows for the characterization of biologics, including peptide mapping, HCP analysis, multi-attribute method (MAM) method development and non-QC MAM applications, oligonucleotide impurities and degradation products, identification of proteoforms using top- and middle-down analysis, and accurate intact mass measurements—all with certainty, speed, and ease. With industry-leading deconvolution and component detection algorithms, MS² data processing, and powerful data visualization, BioPharma Finder software helps you achieve confident biotherapeutic characterization.

• Simplify data-analysis workflows using one software solution

Streamline data processing and analysis to rapidly gain insights into your samples. From batch processing to in-depth analyses, flexible data analysis workflows guide both new and experienced users to set up, interpret, and visualize results without time-consuming manual steps.

Leverage HRAM data for highest confidence

For low-level chromatographic component identification in both denatured and native conditions, superior deconvolution algorithms make the most of high-quality high-resolution accurate-mass (HRAM) data produced by Thermo Scientific™ Orbitrap™ mass analyzers. The unique Sliding Window algorithm uses all of your data, not just a slice, for enhanced deconvolution, relative quantitation of isoforms, and drug-to-antibody ratio (DAR) accuracy. Automated MS² data processing provides unprecedented certainty in peptide and oligonucleotide sequences.

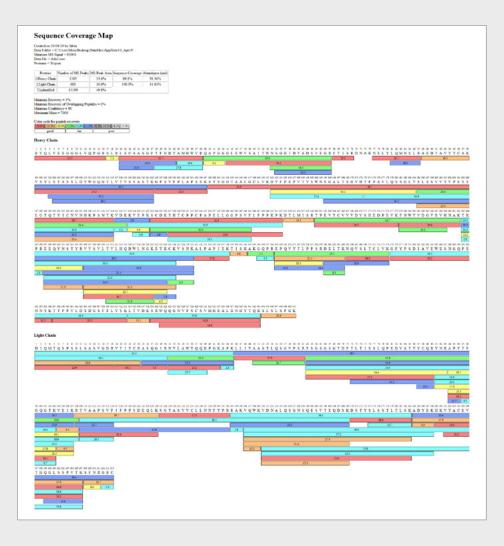
- Fully characterize quality attributes for high-throughput monitoring Confidently discover, identify, quantify, and monitor process and product quality attributes (PQA) with easy-to-understand data visualization. Once identified, PQA components can be monitored in a high-throughput fashion within BioPharma Finder software or exported to Thermo Scientific[™] Chromeleon[™] Chromatography Data System (CDS) software for monitoring within a compliant ready environment.
- Enhance the functionality for workflows with connection to the Ardia Platform

Alleviate the pain of data handling while enabling users to remotely collaborate from anywhere with automated file centralization. Leverage new tools that include the ability to create reports for peptide mapping, oligonucleotide, and intact mass analysis.



Know your peptide sequence

Because small changes in sequence can affect biotherapeutic safety and efficacy, full characterization with complete peptide map coverage is essential. BioPharma Finder software provides unsurpassed capabilities for peptide mapping, including component detection and comparison, predicted MS² spectra for automated peptide identification, and component association across multiple charge states—all in one intuitive interface. Easily identify the primary sequence, sequence variants, and expected and unexpected post-translational modifications (PTMs) using both full scan MS and MS² data for absolute confidence in results. These capabilities, and the ability to rapidly process single or multiple data files, ensure a fast time to results and that you won't miss anything.



Detect all components

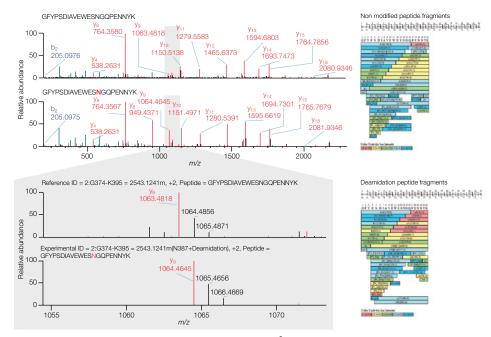
Advanced component detection finds more features, even at low levels, permitting comprehensive identification of all peaks including low-level impurities and sequence variants that might be missed by other software. With built-in comparative analysis capability, the software also streamlines detection of new and unexpected peaks and provides tools to monitor attributes across multiple conditions or treatments.

Increase certainty in sequences

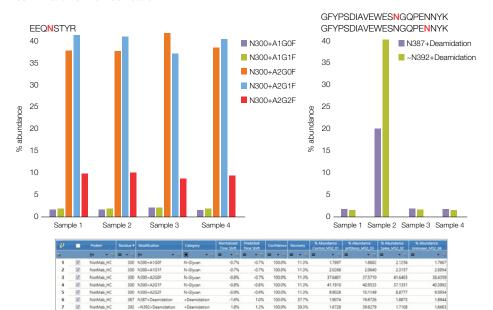
Processing MS and MS² experimental data versus predicted spectra with visualization of fragment coverage maps substantially increases confidence in peptide sequence identifications. A novel identification algorithm uses theoretical MS²-based prediction to automatically identify and confirm sequences and PTMs that could otherwise go undetected. Association of components across multiple charge states adds an additional level of verification and insight.

PTM identification includes expected modifications, like glycosylation, oxidation and deamidation, and unexpected modifications by searching a user-defined mass range. Simply create a peak list and search against modifications, with an expanded search, filling in unidentified gaps and leading to 100% sequence coverage.

Interactive sequence coverage map colored by peptide intensity. Peptide mapping of a Smart Digest using BioPharma Finder software provided 100% sequence confirmation. The ability to perform sequence variant searching, disulfide link analysis, and de novo sequencing assures you won't miss anything.



Automated site-specific modification confirmation uses MS² fragmentation data for increased certainty and allows for direct comparison of the modified peptide to the non-modified peptide, enabling confirmation of the modification.



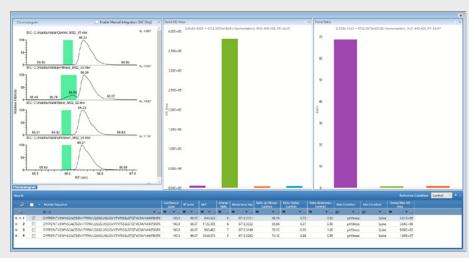
Post translation modifications are easily visualized in customizable modification plots. In the above figure, identified modifications are plotted and compared across four samples. Modifications are also conveniently listed in table format, displaying the modification type, retention time shift, confidence, and percent recovery (bottom).

Batch process and visualize data

Workflow-driven, the software enables users of all experience levels to easily and confidently obtain complete peptide coverage. Batch processing with an interactive wizard for editing methods allows data analysis with maximum throughput. Compare extracted ion chromatograms to evaluate a batch of samples and use ratio information to quickly find changes allowing rapid monitoring of PTMs due to stress conditions. Rapidly visualize data to see low-level impurities and sequence variants, and to profile relative abundances, including site specific PTMs and O- and N-glycans.

New peak detection

In addition to identifying peptides and PTMs, the peptide mapping workflow can also identify new peaks that appear in one sample but not the others. BioPharma Finder software can detect changes in component abundance across multiple samples and provides graphical visualization tools for investigation. Users can leverage their results to search for changes that exceed the defined acceptance criteria using columns in the results table including ratio, min condition, max condition, and overall MS area.



Identification of new peaks has never been easier using the BioPharma Finder software superior component detection algorithm and graphical interface for fast verification of differences that may be present from sample to sample.



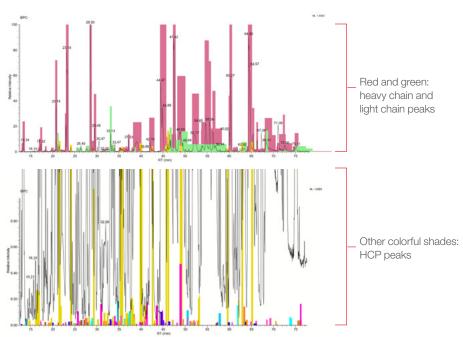
Save time from the start: complete peptide mapping workflows

It can take weeks to evaluate sequencing data without powerfully efficient software. BioPharma Finder software includes complete data processing workflows that eliminate the need to spend hours fine-tuning parameters. From the beginning, users can process data for all peptide-based biotherapeutic characterization assays, including host-cell protein (HCP) analysis, peptide and disulfide bond mapping, and hydrogen deuterium exchange (HDX).

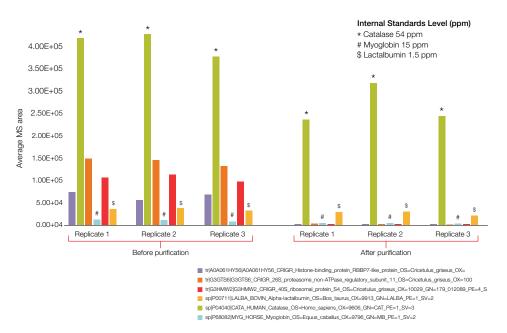
Detect and quantify host cell proteins

Detection and quantification of residual HCP as process-related impurities is critical per regulatory guidelines. BioPharma Finder software provides a complete HCP workflow that identifies product-specific peptides and their modifications, and then searches the unidentified MS² spectra against user-selected protein databases (i.e., Cricetulus griseus (CHO), Homo sapiens (Human), *Mus musculus* (Mouse)) and merges the information together into one simple report.

The software automatically selects the top three most abundant peptides from each HCP for relative quantitation. The user can adjust the peptides used for quantitation and generate quantitation profiles of HCPs across multiple conditions to demonstrate clearing. The integrated workflow allows for identification and quantification of target molecules, PTMs (including n-linked glycopeptides) and HCPs in the same sample and in one comprehensive analysis.



The chromatographic coloring and shading of the components present within a sample provides an easy-to-use visual tool to simplify chromatographic review. In the above figure, components from the light chain (green) and heavy chain (red) regions of the antibody drug product are shaded throughout the chromatogram (top), while HCP components identified within the chromatogram are shaded with a varying range of colors for easy identification (bottom).



HCPs identified within the sample can be plotted to easily visualize and compare protein quantitation from sample to sample. In the above figure HCPs identified within the sample search, in addition to internal standard proteins, are plotted before (left) and after (right) sample purification.

Identify product quality attributes with high-throughput analytics

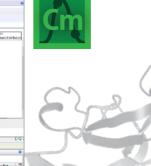
BioPharma Finder software can be used to identify and evaluate relevant PQA with high-throughput analytics. Using the built-in peptide workbook, information can be easily transferred and then monitored across multiple samples throughout drug development. Alternatively, for compliant monitoring of critical quality attributes (CQA), the PQAs can be exported to Chromeleon CDS as part of a multi-attribute method (MAM).

Regardless of the destination, high-confidence peptide identification and sequence mapping make the software ideally suited to identifying and monitoring PQAs and CQAs. Acquired spectra are compared to predicted spectra based on FASTA sequence and accurate mass, retention time shifts, charge state, and MS² fragmentation spectra to produce confident peptide identifications. Relative quantitation of targeted peaks and fast screening of large datasets streamlines PQA/CQA identification.



Identification	Peptide Sequence	Modificat	tion
= (NonBlanks) • 🔻	<u>A</u> a ▼ V _x		▼ Y.
2:E296-R304 = 1188.5047m(N300+A2G1F)	EEQYNSTYR	A2G1F	Filters Export All Components
2:E296-R304 = 1188.5047m(N300+A3G1F)	EEQYNSTYR	A3G1F	Export Checked Components *
2:E296-R304 = 1188.5047m(N300+M5)	EEQYNSTYR	M5	Create .mgf File
2:E296-R304 = 1188.5047m(N300+A2G0F)	EEQYNSTYR	A2G0F	Run De Novo Processing
2:E296-R304 = 1188.5047m(N300+A2G0F)	EEQYNSTYR	A2G0F	Show Component Information
			Save As Peptide Workbook *

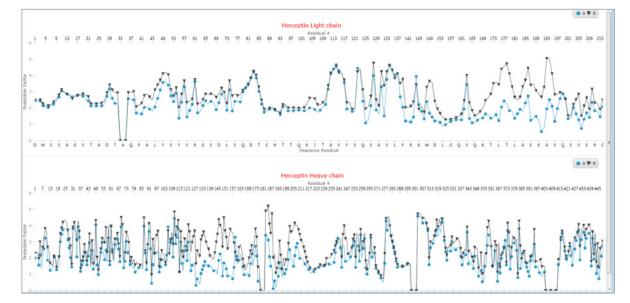




Easily use BioPharma Finder software to create PQA Peptide Workbooks and transfer this information into Chromeleon CDS for monitoring and compliant workflows.

Streamline Hydrogen Deuterium Exchange workflows

BioPharma Finder software automates HDX data processing. The HDX workflow generates a protection factor plot that can be used to quickly assess and compare protein folding across batches of biopharmaceuticals.



HDX protection factor plot for the light and heavy chains of the antibody Herceptin.

Enhance data management with connected features from the Ardia Platform

Featured with the connection of BioPharma Finder 5.3 software with the Ardia Platform, users can now track, manage, and improve data transparency with versioning



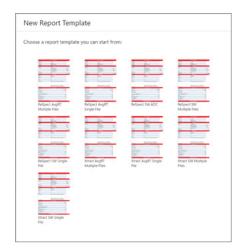
From the Ardia Platform File Explorer, view and sort results based on version number, date and time they were saved, what user saved them, and which method was used to generate the results. Selecting a previous version allows users to revert to that result file.



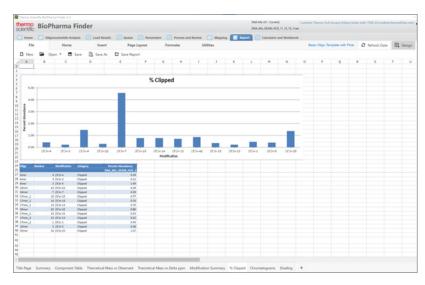
Select two result versions from the menu and open them in the comparison window to visually compare and understand the differences in how they were generated.

Along with data versioning, the Ardia Platform connected to BioPharma Finder 5.3 software introduces the ability to create, edit, and save summary reports so users can:

- Easily visualize data and results for oligonucleotide, peptide mapping and intact mass applications.
- Save individual reports that can be reviewed later without having to open BioPharma Finder software.
- Utilize default reporting templates to speed development of customized reporting templates.
- Effectively modify templates to achieve desired result summary with the help of the customizable report designer.



Choose from the available templates.



Edit reports in the report designer tab.



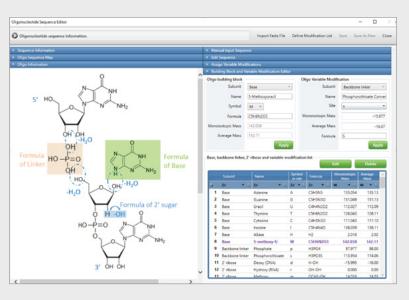
Advancing oligonucleotide sequence confirmation

The oligonucleotide building blocks of DNA and RNA consist of their own unique chemical makeup and unit nomenclature, especially when compared to peptides and proteins. These unique characteristics require specialized software features and capabilities. BioPharma Finder software provides a wide array of specialized tools to meet the specific needs of oligonucleotide analysis, including custom sequence modification, *in-silico* digestion, predicted sequence fragmentation, and automated fragment matching and spectrum annotation. The powerful suite of tools provided within the oligonucleotide workflow enables streamlined experiment setup and in-depth sample analysis to facilitate processing data from simple to complex.

Import, modify, and edit oligonucleotide sequences

The flexibility to incorporate custom chemical modifications into an oligonucleotide sequence is the first step in building a robust sequence confirmation workflow. BioPharma Finder software provides an intuitive set of tools to support sequence modifications at each subunit of the oligonucleotide, including the nucleoside base, backbone-linker, and 2' ribose positions. Custom building blocks can easily be inserted into existing sequences or utilized for future sequence creation. Chemical modifications can also be created and searched to identify unexpected or variable modifications of the target sequence.

- Enter or edit sequences in the Sequence Manager with the help of text file import or direct editing.
- Create custom building blocks and generate a structural visualization for the sequence to aid in editing and modification.
- Search for unexpected modifications in the sequence with the variable modification searching functions.
- Automatically create randomized oligonucleotide sequences matching the exact base content of existing chains to ensure precise and dependable mapping results with the Randomized Sequence function.
- Generate simulated spectrum and predictive MS² from entered oligonucleotide sequences with the Calculator and Workbook Editor function.



An example structure is provided to visualize each region of the nucleotide sequence and aid the creation of custom building blocks and modifications (left). Custom modifications are easily added for both building blocks and variable modifications. Predefined building blocks are listed in black and user-defined modifications are listed in purple to aid visualizations (right).



Variable modification searching can be added to an oligonucleotide sequence and by selecting from a list of predefined or custom modifications.



Select the number of randomized sequences to generate.

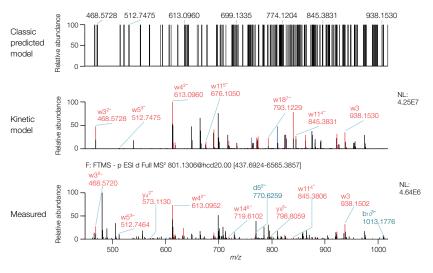
Harness the power of MS² fragmentation for oligonucleotides

LC-MS fragmentation data provides a powerful tool for structural and sequence confirmation in many application areas. BioPharma Finder software MS² fragment identification can be seamlessly implemented to enhance oligonucleotide sequence confirmation. With the implementation of a novel kinetic model for oligonucleotide fragmentation prediction that incorporates predicted fragment intensity, spectral matching is performed with greater confidence, significantly improving fragment identification.

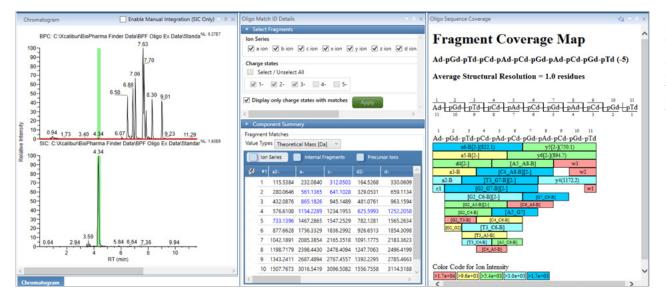
Identified fragments are automatically annotated and displayed in the MS² spectrum window for easy data review and fragment coverage maps provide a visual tool for rapid fragmentation evaluation. Predicted fragmentation *m/z* values are also available in a table format that include dynamic filters linked to both the fragment coverage map and MS² spectrum window to simplify the review process.

- Easily review annotated MS² fragmentation and compare with predicted MS² spectrum.
- Access color-coded fragmentation maps for easy fragmentation visualization.
- Display predicted fragment tables to evaluate fragment mass accuracy.
- Implement dynamic filters to customize data display across multiple windows.
- Customize window docking for simplified data review.





The combination of confident fragmentation prediction and matching MS^2 fragmentation prediction displaying a standard fragmentation m/z model (top) and a kinetic model with m/z and predicted fragment intensities (middle) versus experimental fragmentation results (bottom).



User-definable displays with floatable/dockable windows enable multiple data displays for a customized oligonucleotide data review layout.

Chromatograms displaying the base peak chromatogram and selected ion chromatogram (left), the predicted fragment mass table with ion series filters (middle top), color coded fragment coverage map (middle bottom), interactive MS² spectrum window with annotated fragment identification (right).

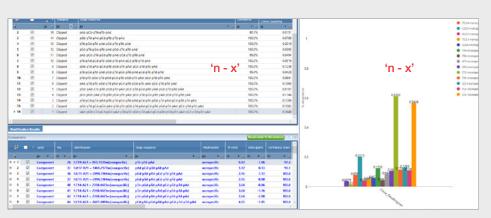
Visualize high-quality oligonucleotide data with ease

Arranging and interpreting data results for complex samples is essential for producing informed decisions. The capability to visualize key components within a sample, view all components in the sample, and plot trends between different samples dramatically simplifies the data review process. BioPharma Finder software provides a comprehensive set of integrated data review tools to visualize key components and modifications within a single sample as well as trends across multiple sample sets.

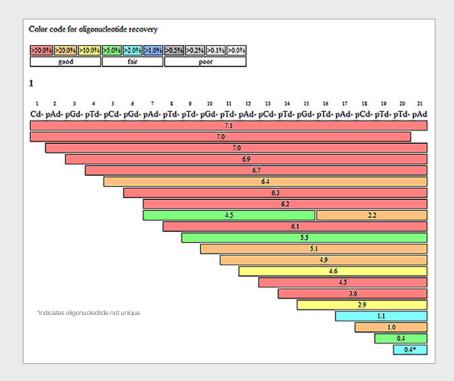
Identify and display oligonucleotide target components and sample impurities

The identification and confirmation of the full length oligonucleotide sequence, as well as the identification of sample impurities, are the primary goals for oligonucleotide sequence confirmation. BioPharma Finder software oligonucleotide workflows automatically generate modification and component tables, as well as graphical representations of target sequences for both expected and unexpected sample impurities.

- Generate customizable color-coded mapping table displays.
- Display, filter, and sort modification summary tables.
- Plot and compare modification intensities and compare across samples.
- Calculate percent abundance for target components and modifications.
- Display predicted sequence site location for identified modifications.



Modification summary table of the identified modifications within the sample (left). Relative abundance plot of the selected modifications (right).



Sequence coverage map of identified components within the sample, including the full length oligonucleotide product and a wide range of impurities. Chromatograhic retention time is displayed for each identified component and each component is color coded based on relative abundance within the sample.

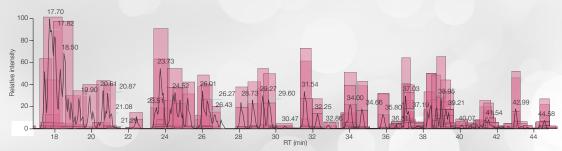
Leverage advanced in silico digestion tools to perform mRNA sequence mapping

The size and complexity of mRNA samples present analytical challenges for analysis at the intact level. mRNA digestion utilizing restriction endonucleases provides a suitable approach for the generation of predictable mRNA fragments that can be further analyzed to generate a complete mRNA sequence map. BioPharma Finder software provides advanced *in silico* digestion tools designed to support the identification of mRNA digestion fragments and fragment modifications to enable mRNA mapping workflows.

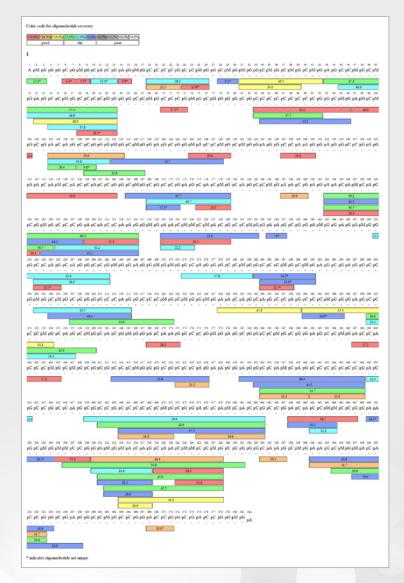
- Perform mRNA mapping using in silico mRNA digestion tools.
- Select from predefined RNase options or specify user-defined cleavage locations.
- Assess overall mRNA sequence coverage using interactive coverage map tools.
- Identify mRNA sequence fragments as well as expected and unexpected modifications.
- Visualize sequence map coverage for unique and non-unique mRNA sequence fragments.
- Search against user-defined and randomized sequencests

RNase	RNase T1	~	Custom Specificity	G-
Phosphate Location	3'-linear	~		
Specificity Level	High	~		

The ribonuclease selection parameter provides a predefined selection of RNase options as well as the capability to specify custom cleavages.



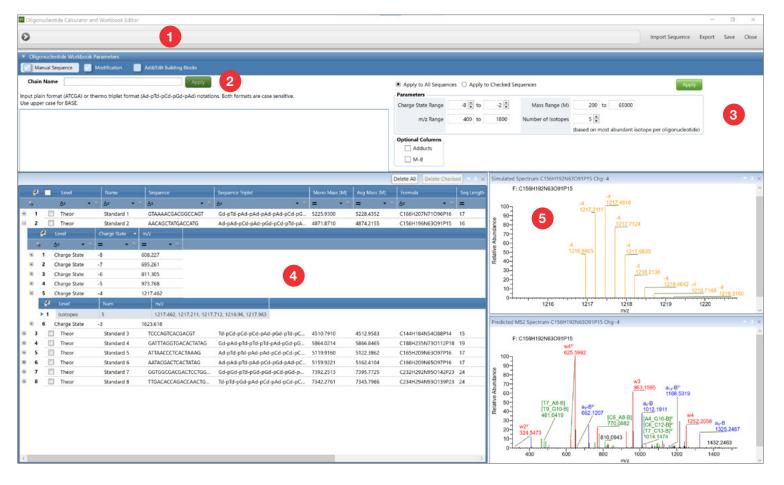
Chromatographic shading is used to quickly and easily identify mRNA digestion fragments throughout the chromatogram. In the above figure, identified mRNA digestion fragments are shaded red and pink across the chromatogram for each of the identified mRNA components.



Interactive sequence coverage map colored by mRNA fragment intensity and labeled by retention time. Non-unique sequence fragments are labeled with an asterisk.

Quickly perform advanced calculations and summarize oligonucleotide sequence information

To support characterization of oligonucleotide sequences, the predictive Calculator and Workbook Editor will produce a comprehensive table of average and monoisotopic mass, expected m/z charge states and theoretical fragmentation spectra based on the input sequence. The resulting tabulated information can be imported or exported.



- 1. Open Calculator and Workbook Editor.
- 2. Provide chain name and import or enter chain information.
- 3. Adjust parameters.
- 4. Review the predicted sequences, mass information and more.
- 5. Review the simulated spectra and predicted MS².

Confidently and easily characterize intact proteins and subunits

Subunit, intact, and native protein MS characterization is the preeminent approach to determining the molecular weights of protein and oligonucleotide sequences. These methods have numerous advantages, including reduced sample pretreatment and minimization of potential artifacts introduced during sample handling. However, the huge amount of data generated from the analysis of hundreds of samples presents a formidable challenge. BioPharma Finder software processes subunit, intact, and native MS data with increased throughput, ease, flexibility, and confidence.

Increase data processing throughput

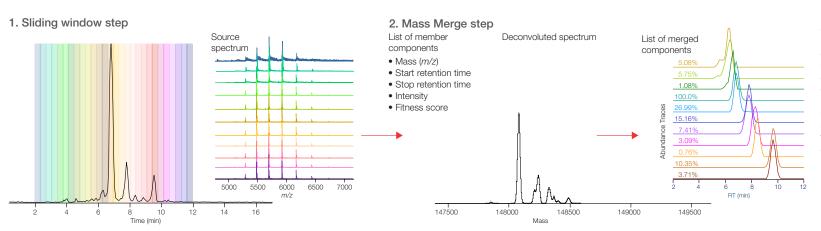
BioPharma Finder software facilitates automated batch processing and reporting, coupled with the ability to compare results across multiple samples to eliminate data processing bottlenecks so laboratories can process more samples per study with less user intervention. Batch processing methods also leverage two different algorithms to generate the most accurate mass measurements for components in simple to very complex mixtures. These capabilities speed up time to results and provide deeper insights into samples and batches.

Accurately determine molecular weights

Two complementary deconvolution algorithms optimized for isotopically resolved (Thermo Scientific™ Xtract™ deconvolution algorithm) and unresolved data (ReSpect™ algorithm) ensure accurate molecular weight determination for small oligonucleotides to intact viruses weighing more than nine MDa, in both denaturing and native conditions, and at low abundances.

An automated parameter optimizer simplifies deconvolution, making it easy to obtain accurate results. Consequently, it's possible to gain detailed information about heterogeneity, intact protein and mRNA masses, mass shifts of just a few Da, PTMs like glycosylation and oxidation, and higher order structure.

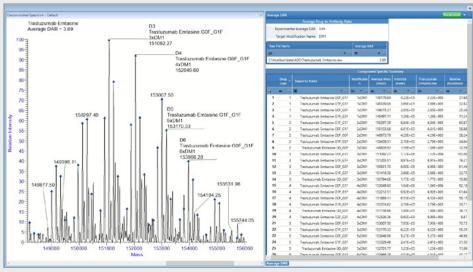
Sequence annotations include modifications for a complete picture. Data from ion trap, single or triple quadrupole, or Orbitrap mass analyzers can all be deconvoluted, providing extensive workflow flexibility.



Sliding Window deconvolution provides a key benefit of low abundant variant detection in a single analysis and the ability to accurately detect co-eluting components with different relative abundances. It averages spectra over a succession of sliding windows across retention time, deconvolutes each of these averaged spectra and then merges similar masses to identify components.

Easily deconvolute unresolved peaks and when retention times shift

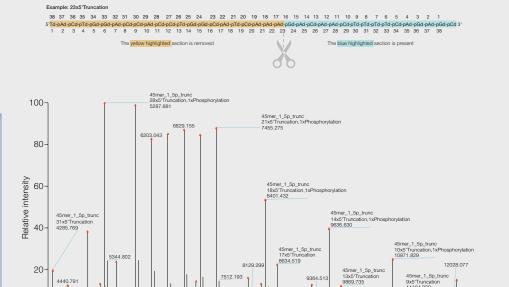
The Sliding Window algorithm is a robust and powerful deconvolution tool that can be used for comprehensive characterization of charge variant analysis data from monoclonal antibodies (mAbs). Poorly defined peaks or retention time shifts can be encountered during LC-MS intact analysis of biotherapeutics. Sliding Window uniquely overcomes these challenges to enable deconvolution, identification, and visualization of coeluting components across the chromatographic time scale. These capabilities make it easy to identify failed sequence and process-related impurities as low as 0.05%, accurately measure drug to antibody ratios (DARs), and detect antibody-drug conjugates (ADCs).



The Thermo Scientific[™] Q Exactive[™] UHMR Hybrid Quadrupole mass spectrometer in the BioPharma Finder software for the glycoslyated molecule.

Confirm intact oligonucleotide sequences

Expanded sequence matching for oligonucleotides is provided by the intact mass analysis workflow, including Terminal Truncation Searching capabilities from the 3' or 5' terminus, allowing for easier identification of impurities.



Terminal Truncation Search, showing the annotated deconvolution spectrum produced by Sliding Window deconvolution (Xtract). The oligonucleotide sequence was searched for both the 5' and 3' removal and several different variable modifications.

8000

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Streamline data processing

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Built-in default methods and automation are available to guide users through intact mass analysis workflows, including impurity analysis in complex samples, determination of ADCs, and automated DAR calculations. Multi-consensus reporting for multiple samples provides for understanding differences between batches and trends over time.

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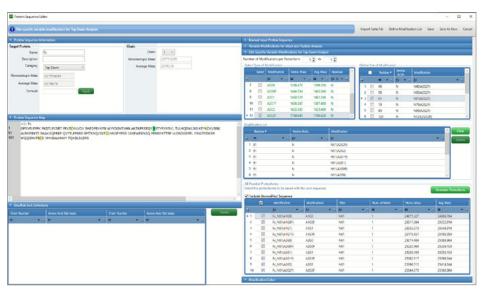
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Gain more insights with rapid top-down analysis

Peptide mapping is the gold standard for in-depth characterization of biotherapeutics; however, sample preparation, LC runtime and data analysis can be labor intensive and time consuming. Top- or middle-down mass spectrometry experiments show promise as an alternate technique for primary order structure confirmation. However, historically top- or middle-down characterization has been limited by bottlenecks in data processing and lower sequence coverage. Top-down workflows in BioPharma Finder software remove this bottleneck.

Define proteoforms for searching

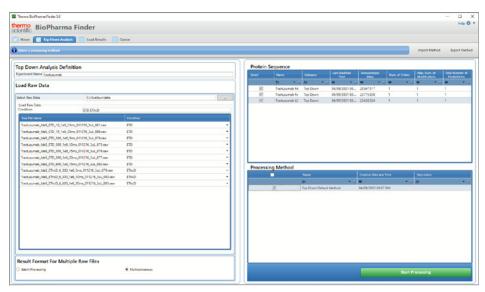
The protein sequence manager allows the user to define the category as top down, which enables a site specific variable modification. Here the user can add modifications including, but not limited to, oxidation, deamidation, and N-glycans. Multiple proteoforms are saved into the single protein sequence and multiple sequences are selected during processing.



Protein sequence editor provides easy-to-use and flexible interface for the creation and customization of protein proteoforms used in the top-down workflow.

Process multiple files in tandem

The top-down workflow enables rapid data processing and comparison of up to ten data files for faster time to results. Multiple protein sequences can be selected for processing and each assigned to a specific peak. Each protein sequence can contain many different proteoforms through user-defined custom modifications for increased workflow flexibility.

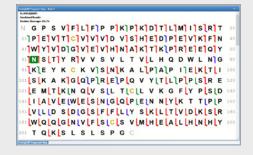


Multi-consensus processing of up to ten raw files enables direct comparison of sequences with user-defined modifications. Protein sequence editor allows site specific variable modification across multiple proteoforms.

Visualize results your way

Powerful data displays permit users to display results in the manner that provides the most insight and value. Results can be reviewed by peak, proteoform, raw data file, and more.

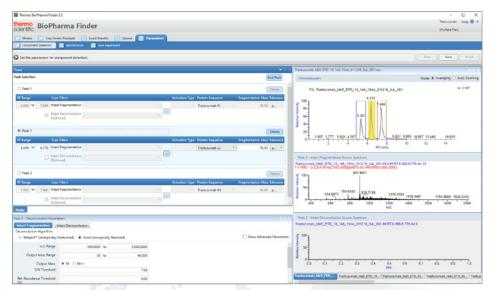
freighte freprint Mer. Rock 3 FAL Gentleme Brown		
Roider (Assegor (N) 76		
N [V T L] R] E] S] G] P] A] L] V] K] P] T] Q] T	וןדןנ	20
21 TCTFSGFSLSTAG MSV	WIR	40
4]QPP GKALEWL AD INWIND	KKH	60
41 YNP S LKDRILTI S KDT ST	NQ V	80
IN LIKIVITINIM DIP A D T AIT YIY	A R)D	100
101 M I F]N]F]Y]F]D]V]W[G Q G T[T V[1	r v sls	120
ALSTIKO P SEV FOP LEALPES SEKES	TLSEG	1.40
41 G T A A L G C L V K D Y F P E P L	/ TLVLS	160
161 WINES GLALL T S G VLH TEFENLALVE	Lalsts	180
SEE GLEYES LESIS EVEVET EVER ESTATE	LTLQLT	200
201 YLI CINIV NIHIKIPISINITIKIVIDIKI	RLVLELP	220
221 KLS CLDKIT H T CLPIPICIP ALP E I	LGC	



Visualize results in the ways that meet your research goals.

Dig deeper into proteoforms with flexible processing

The software makes it easy to dig deeper into your top-down data to view proteoforms containing both static and variable sites of modifications with user-selected peaks and scan filters. Interactive chromatograms assist with peak definition and each peak can be assigned its own deconvolution parameters for accurate and adaptable results.



The Parameter Window can be used to quickly define peaks, select scan filters, and protein sequences. Interactive chromatograms make it easy to define peaks.

Automatically process data with multiple fragmentation modes

BioPharma Finder software uniquely processes data obtained using multiple modes of fragmentation, including CID, HCD, ETD, EThcD, and UVPD, and generates a combined fragment map. Characterization of subunits such as light and heavy chains is facilitated with deconvolution followed by MS² searches using powerful Thermo Scientific™ ProSightPC™ software algorithms to make identifications.



The combined search of ETD and UVPD data can be used to produce a combined coverage map displaying the dissociation type for each of the generated fragments identified.



Offering a complete biotherapeutic solution: explore the applications

Simplify and streamline your analysis using the BioPharma Finder software solution that includes everything from batch processing to in-depth data analysis with workflows designed to guide experts and non-experts through a range of applications.

thermofisher.com/biopharmafinder



Connecting your laboratory: discover new functionality

Connecting instruments and software on the Ardia Platform powers enhanced functionality for Thermo Scientific Chromatography and Mass Spectrometry Software. Discover the functional enhancements provided by the Ardia Platform.

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Offering in depth support: visit the help portal

Connect with support and explore the operation manuals, release notes, quick start guides and more all from one location.

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