Comprehensive oligonucleotide analysis

Oligonucleotide analysis presents multiple analytical and data processing challenges. Thermo Scientific™ BioPharma Finder™ software helps overcome these hurdles to provide confidence with comprehensive capabilities.

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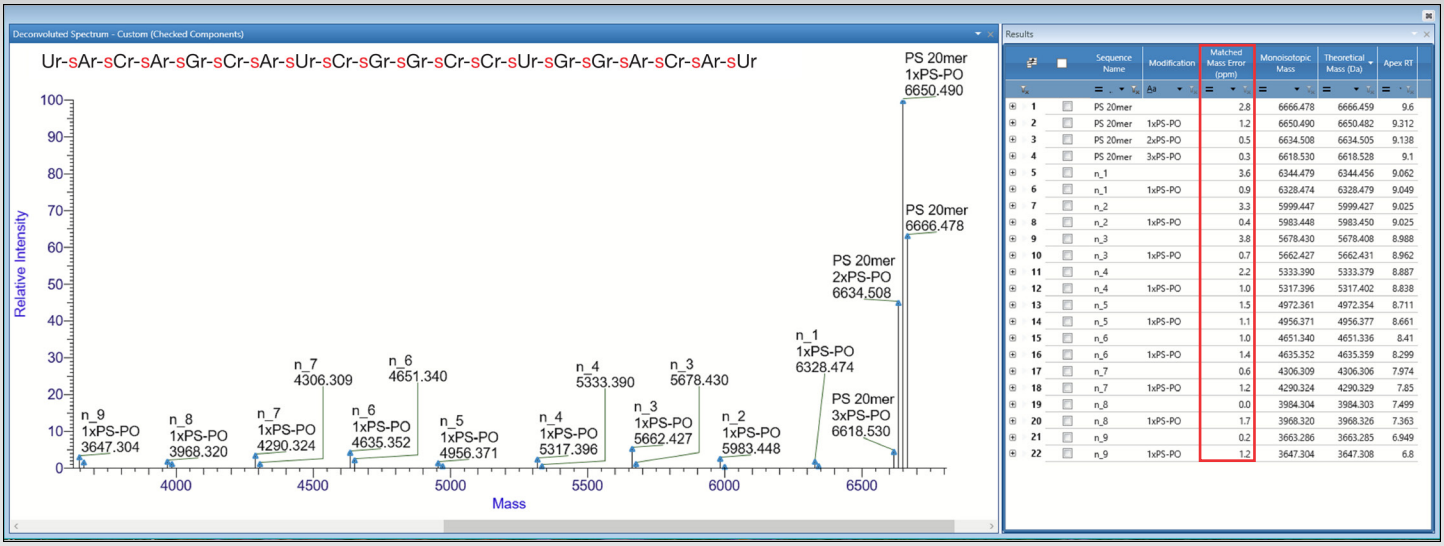
Intact mass analysis of heavily modified oligonucleotides

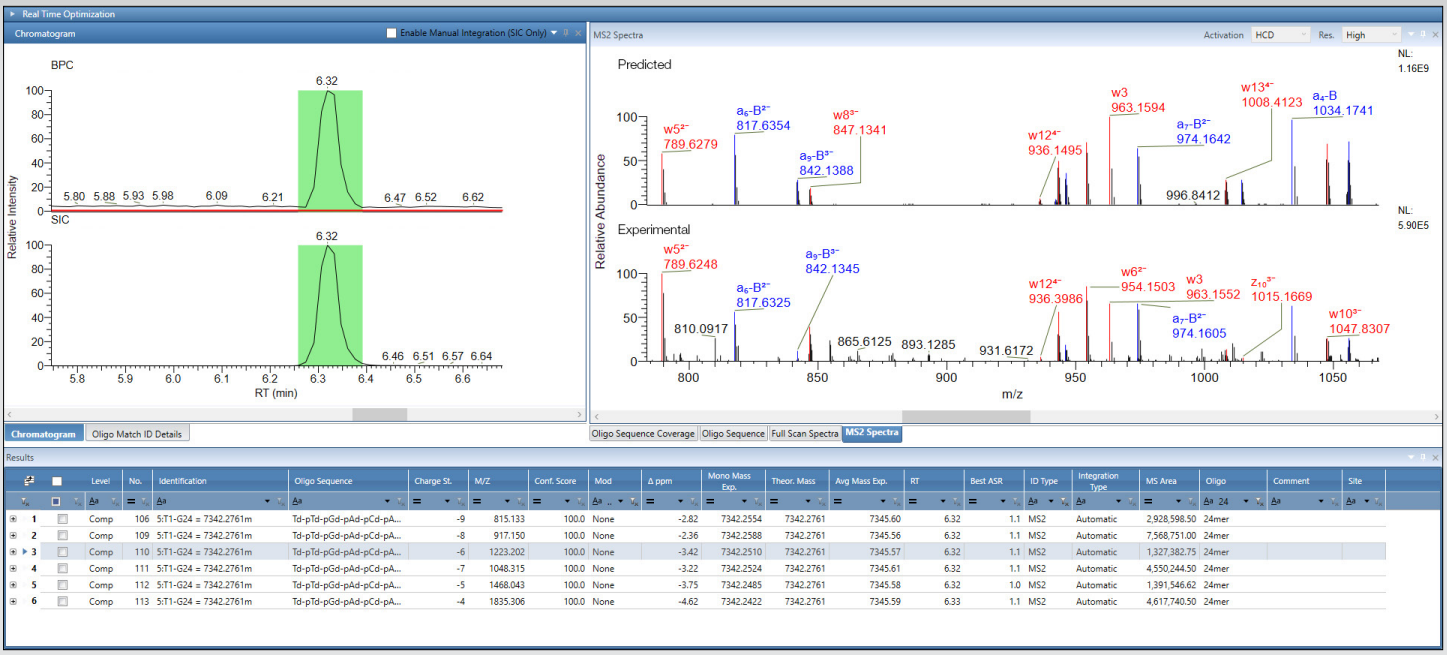
Oligonucleotide biotherapeutics require multiple modifications to increase stability and efficacy. BioPharma Finder software utilizes an easy-to-use interface to quickly build out and visualize customized oligonucleotide sequences. Traditional data acquisition and data processing algorithms often struggle with the analysis of heavily modified oligonucleotides due to the inclusion of phosphorothioates (PS), a common modification replacing one non-bridging oxygen atom in the phosphodiester linkage of DNA or RNA. However, the high-resolution accurate mass measurements of Thermo Scientific™ Orbitrap™ mass spectrometers coupled with BioPharma Finder software leverage the unique abundance distribution of sulfur isotopes to generate a sequence-specific isotopic model and obtain accurate deconvolution results. Pairing this powerful model with the advanced Sliding Window algorithm allows for sensitive and accurate deconvolution of simple and complex oligonucleotide LC-MS data.



Annotation of a deconvoluted spectrum of an analyzed sample containing a fully phosphorotiolated RNA sequence as well as several impurities resulting from phosphorothioate conversion. The automated detection of the full-length product as well the additional impurities demonstrates both the exceptional mass accuracy provided by high-resolution accurate mass Orbitrap analysis and the confident assignment of identified sample components through sequence-specific isotopic modelling with BioPharma Finder software.

Confident identification and relative quantitation of oligonucleotides

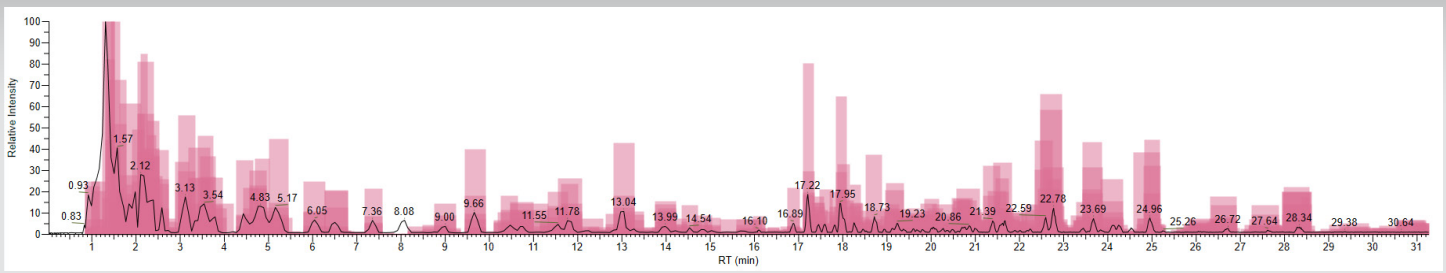
Relying solely on the intact mass of oligonucleotides falls short of the confidence needed to fully analyze biotherapeutics, due to the presence and likelihood of isomeric species given the conservation of the 4 bases in oligonucleotides. Confident sequence order confirmation, identification and relative quantitation of expected and unexpected modifications, and the identification of impurities and metabolites requires the inclusion of fragmentation information in oligonucleotide data acquisition and processing. BioPharma Finder software addresses this requirement by utilizing an advanced kinetic model algorithm for oligonucleotide fragmentation prediction that generates not only the predicted masses of oligonucleotide fragment ions, but also their intensity. This capability provides increased confidence for sequence identification and confirmation, including the order of bases, which is not possible with Full MS data alone when isomeric species are present. In addition, by automatically generating selected ion chromatograms (SICs) for all relevant ions across the chromatographic time range, BioPharma Finder software can dig deeper into components matched through monoisotopic mass within the full scan data to provide low level component identification and confirmation by combining these results with MS^2 based matching.

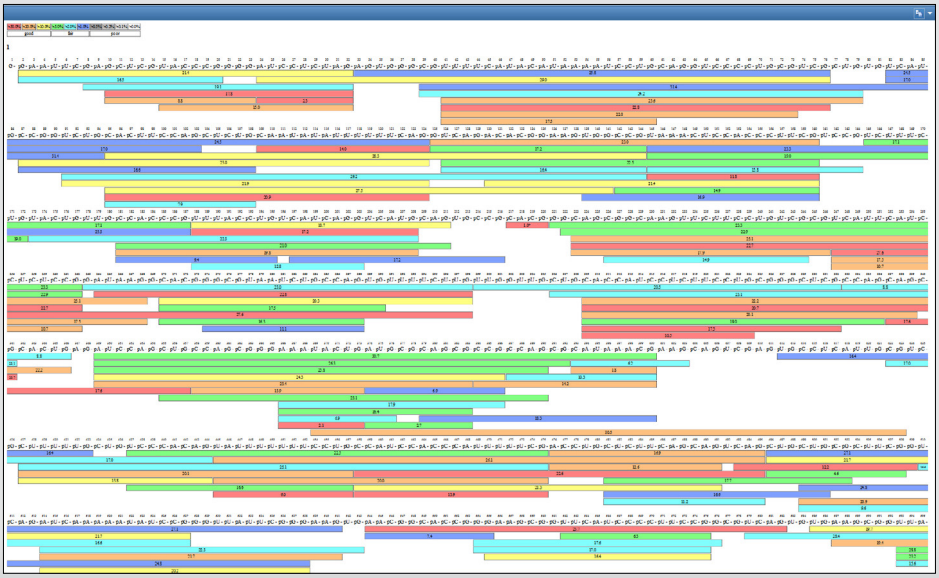


Result view of predicted and experimental spectra for an oligonucleotide sequence showing the excellent predictive capabilities of the Kinetic algorithm for both the mass of fragment ions and their intensities, including those with very low abundance.

Comprehensive mRNA mapping

mRNA mapping involves the digestion of mRNA with RNase enzymes followed by digestion fragment identification to then map the results back onto a coverage map. For long mRNA sequences this can often produce exceedingly complex results that need to be managed and filtered based on a number of evaluation parameters. BioPharma Finder software provides an easy-to-use workflow to fully enable confident mRNA mapping. Predefined RNase options can be selected to perform *in silico* ribonuclease digestion for a given mRNA sequence, or alternatively customizable RNase digestion options may be applied. Fragment coverage maps are then generated by matching experimental MS^2 spectra to predicted *in silico* sequences matched via predicted MS^2 spectra generated from the kinetic algorithm. These matches can be quickly reviewed in the results through interactive visualization tools, such as the customizable labeling of 5' and 3' ion series as well as internal fragment identifications with the experimental MS^2 spectra. Ultimately, the comparison between the predicted and experimental MS^2 spectra is utilized to generate a numeric confidence score value which can be evaluated to qualify and add confidence to each digestion fragment match. Results tables can be refined through building, saving, and applying custom filters based on any column to achieve the desired acceptance criteria in the resulting sequence coverage maps. Further confidence can be gained using the randomized sequence generator, which provides a randomized mRNA sequence containing the same numbers of each base as the target sequence but in random order along with a list of *in silico* digestion fragments corresponding to the randomized sequence. This additional built-in layer of analysis helps to enable statistical analysis of false positive identifications within the mRNA mapping workflow.





Oligonuclotide	Number of MS peaks	MS peak area	Sequence coverage	Abundance (mol)
1:1	4,251	59.9%	95.8%	100.00%
1:1*	4,251	59.9%	95.8%	100.00%
Unidentified	15,218	40.0%		

Minimum recovery = 1%
Minumum recovery of overlpping oligonucleotides = 0%
Minimum confidence = 0.8
Maximum mass = 40,000

Visualization of mRNA mapping result showing identified mRNA fragments matched for mapping. Fragments are then mapped with coloring corresponding to confidence in identification.

Learn more at **thermofisher.com/BioPharmaFinder**