Enhanced for oligonucleotides deconvolution

With the success of the COVID 19 messenger ribonucleic acid (mRNA) vaccines, commercial status of short interfering RNA (siRNA) and antisense oligonucleotides (ASO), and clinical phase of clustered regulatory interspaced short palindromic repeats (CRISPR) therapeutics, research into deoxyribonucleic acid (DNA) and RNA based therapeutics has grown exponentially.

The evolution of the available technology has introduced new therapeutic boundaries but has increased complexity pushing the criticality of establishing safety, efficacy, purity, and stability for these products as a focal point for developers and regulatory agencies worldwide.

Advancements in synthetic chemistry to increase stability and potency for these molecules include intentional base, ribose sugar, and/or phosphodiester linkage modifications. These modifications create a challenge for analytical characterization and monitoring since they can affect the molecular weight, higher order structure, and chemical composition/modifications which impact the approach for assessing quality and determining product specifications.

We at Thermo Fisher Scientific realize it doesn't matter if it's high throughput screening, failure sequence analysis, or molecular weight (MW) confirmation, there's been a growing need for compliance-ready LC-MS assays in the biopharma market, especially for oligonucleotides.

Along with our boundary setting separations and mass spectrometry hardware, the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) has been designed to support targeted and non-targeted analysis of unmodified and modified oligonucleotides with an updated intact deconvolution algorithm and the new application specific default report template. Whether your focus is oligonucleotides, or your portfolio is expanding to include them, Chromeleon CDS can support your compliance-ready oligonucleotides analysis.

Enhanced lab productivity on one software platform

Reduce training, software management overhead, and utilize uniform data tools with a single software solution capable of acquiring, processing, and reporting GC, IC, LC, MS, and CE laboratory data. The operational simplicity provided by Chromeleon CDS reduces the time to on board new team members and allows for easy expansion of the current laboratory analytics portfolio to include oligonucleotides.

Automated intact deconvolution: a key solution

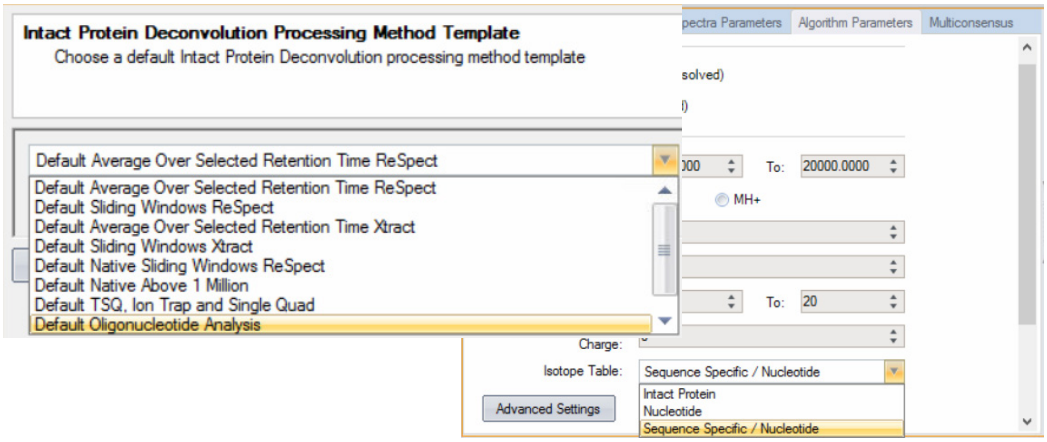


Figure 1. Selecting and setting up the intact deconvolution algorithm for unmodified and modified oligonucleotides

Speed and efficiency of executing analytical methods is crucial for product pipelines in any laboratory. For these products molecular weight confirmation is needed during many steps of the development and manufacturing process. Thus, automating the deconvolution will decrease time to results. With Chromeleon CDS, the “Default Oligonucleotides Analysis” processing method template can streamline method development and execution, utilizing the “Sequence Specific/Nucleotide” selection in the “Isotope Table” setting for analyzing phosphorothioated sequences as shown in Figure 1.

Versatile molecular weight confirmation

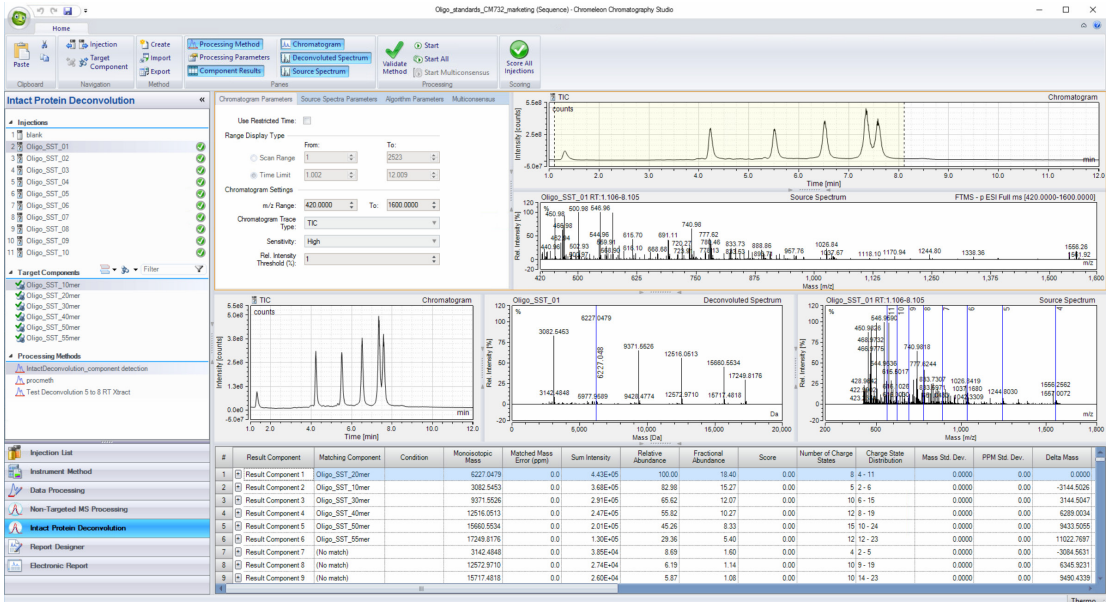


Figure 2. Chromeleon CDS supports targeted molecular weight confirmation for unmodified and modified oligonucleotides and their known impurities and failure sequences.

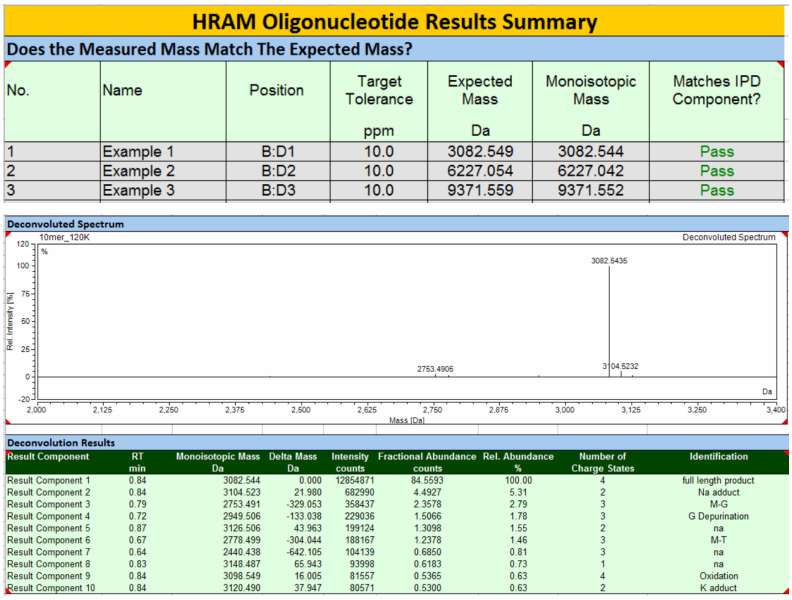
For contract testing laboratories given minimal sample information (such as molecular weight or chemical formula), Chromeleon CDS can successfully deconvolute the negative mode mass spectrometry data of oligonucleotides.

Targeted molecular weight confirmation as shown in Figure 2 for the full length product (FLP) and known impurities, such as adducts and failure sequences, as well as detection of unknown components can be analyzed and reported. These targeted components can be added manually or by importing a csv file, or Thermo Scientific™ BioPharma Finder™ software workbook.

Quick and efficient results reporting

Support automation with straightforward customizable report building, as seen in the example in Figure 3, from the included default Oligonucleotide report template, to fast track report development and facilitate quick and easy review.

With electronic reports and signatures, every step of analysis is captured, documented, and secured to seamlessly ensure data integrity.



HRAM Oligonucleotide Results Summary

Does the Measured Mass Match The Expected Mass?

No.	Name	Position	Target Tolerance ppm	Expected Mass Da	Monoisotopic Mass Da	Matches IPD Component?
1	Example 1	B:D1	10.0	3082.549	3082.544	Pass
2	Example 2	B:D2	10.0	6227.054	6227.042	Pass
3	Example 3	B:D3	10.0	9371.559	9371.552	Pass

Deconvolution Results

Result Component	RT min	Monoisotopic Mass Da	Delta Mass Da	Intensity counts	Fractional Abundance counts	Rel. Abundance %	Number of Charge States	Identification
Result Component 1	0.84	3082.544	0.000	12854871	84.5593	100.00	4	full length product
Result Component 2	0.84	3104.523	21.980	682990	4.4927	5.31	2	Na adduct
Result Component 3	0.79	2753.491	-329.053	358437	2.3578	2.79	3	M-G
Result Component 4	0.72	2949.506	-133.038	229036	1.5066	1.78	3	G Depurination
Result Component 5	0.87	3126.506	43.963	199124	1.3098	1.55	2	na
Result Component 6	0.67	2778.499	-304.044	188167	1.2378	1.46	3	M-T
Result Component 7	0.64	2440.438	-642.105	104139	0.6850	0.81	3	na
Result Component 8	0.83	3148.487	65.943	93998	0.6183	0.73	1	na
Result Component 9	0.84	3098.549	16.005	81557	0.5365	0.63	4	Oxidation
Result Component 10	0.84	3120.490	37.947	80571	0.5300	0.63	2	K adduct

Figure 3. Chromeleon CDS Oligonucleotides report template with the deconvolution spectrum, results list, and tables for evaluating acceptance criteria

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