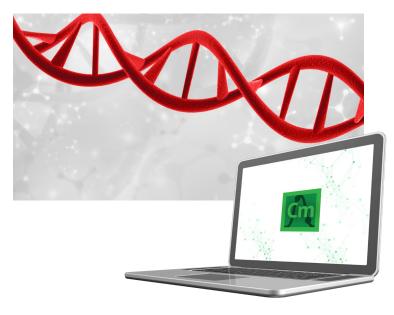
Enhanced for oligonucleotides deconvolution

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.

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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

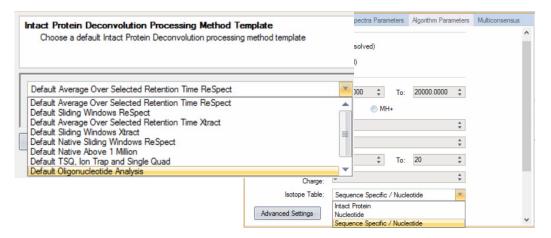


Figure 1. Selecting and setting up the intact deconvolution algorithm for unmodified and modified oligonucleotides

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 Ma | tch The Exp | ected 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 | | B:D2 | 10.0 | 6227.054 | 6227.042 | Pass |
| 3 | Example | 3 | B:D3 | 10.0 | 9371.559 | 9371.552 | Pass |
| | | | | | | | |
| Deconvoluted S | | | | | | | |
| 10mer_12 | 0K | | | | | | Deconvoluted Spectrum |
| 100 | | | | | | 3082.5435 | |
| 60 75 10 10 10 10 10 10 10 10 10 10 10 10 10 | | | | | | | |
| 75 50 | | | | | 2753,4906 | 3082_5435 3104_5232 | |
| 6 75 50 50 50 0 25 0 0 0 0 0 0 0 0 0 0 0 0 | | | | | 2753,4805 | | Da |
| 50 25 | 2,125 | 2,250 2 | 375 2,500 | 2,625 | 2,750 2,875 | | Da 3,250 3,4 |
| 25 | 2.125 | 2,250 2, | 375 2,500 | 2,625 Mass II | 2,750 2,875 | 3 04,5232 | |
| 28 0 -20 2,000 | Results | | | Mass [] | 2,750 2,875 al | 3 04 5232 | |
| 2 75- 0 -20 | Results Int RT | | 375 2.500 c Mass Delta Mass Da | Mass (Intensity Fraction | 2,750 2,875 al | 3 (04.5232 3,000 3,125 Janco Number of | |
| 25 0 200 2,000 | Results ont RT min t 1 0.84 | Monoisotopi Da 3(| c Mass Delta Mass Da 082.544 0.00 | Intensity Fraction counts 12854871 8 | 2,750 2,875 al I Abundance Rel. Abun 0,5593 100.0 | 3 04 5232 3,000 3,125 Janco Number of Charge States | 3,250 3, Identification full length product |
| 25 0 2,000 Perconvolution result Componen tesult Componen | Results mt RT min t t 1 0.84 t 2 0.84 | Monoisotopi Da 3(31 | c Mass Delta Mass Da 082.544 0.00 104.523 21.98 | Intensity Fraction counts 12854871 8 682990 | 2,750 2,875 al I Abundance Rel. Abun ounts % 1,5593 100.01 4927 5,311 | 3 04 5232 3,000 3,125 Janco Number of Charge States | 3,250 3, Identification full length product Na adduct |
| 225 0 225 0 2,000 2000 2000 2000 2000 20 | Results min RT min 0.84 t 2 0.84 t 3 0.79 | Monoisotopi Da 3(3 ¹ 2) | c Mass Delta Mass Da 082.544 0.00 104.523 21.98 753.491 -329.05 | Mass II Intensity Fraction counts 12854871 8 662990 3 358437 | 2,750 2,875 al I Abundance Rel. Abun Units % 1,5593 100.0 4927 5,31 3578 2,79 | 3 04 5232 3,000 3,125 Janco Number of Charge States | 3,250 3,4 Identification full length product Na adduct M-G |
| 20 20 20 20 20 20 20 20 20 20 | Results min RT min t t 0.84 t 0.79 t 0.72 | Monoisotopi Da 30 31 21 22 | c Mass Delta Mass Da D82.544 0.00 104.523 21.96 753.491 -329.05 349.506 -133.03 | Mass II Intensity Fraction counts 12854871 8 662990 9 3358437 3 229036 | 2,750 2,875 al I Abundance Rel. Abund ounts % 1,5593 100,0 .4927 5,31 3,578 2,79 5,066 1,78 | 3 04 5232 3,000 3,125 Janco Number of Charge States | 3,250 3, Identification full length product Na adduct M-G G Deputination |
| 2.000 | Results mt RT min t1 t1 0.84 t2 0.84 t3 0.79 t4 0.72 t5 0.87 | Monoisotopi Da 3(3 ⁻ 21 22 3 ⁻ | c Mass Delta Mass Da 082.544 0.00 104.523 21.98 753.491 -329.05 349.506 -133.03 126.506 43.96 | Mass (Intensity Fraction counts 0 12854871 8 662990 3 328437 3 229036 3 199124 | 2,759 2,875 al I Abundance Rel. Abun ounts % 4927 5,31 3576 2,79 5066 1,78 3098 1,55 | 3 04 5232 3,000 3,125 Janco Number of Charge States | 3.250 3. Identification full length product Na adduct M-G G Depurination na |
| 25 25 25 25 20 20 20 20 20 20 20 20 20 20 | Results min min t1 0.84 t2 0.84 t3 0.79 t4 0.72 t5 0.87 t6 0.67 | Monoisotopi Da 30 31 21 25 31 21 21 21 21 21 21 21 21 21 21 21 21 21 | C Mass Delta Mass Da Da 082.544 0.00 044.523 21.98 753.491 -329.05 349.506 -133.03 126.506 43.96 778.499 -304.04 | Mass II Intensity counts Fraction 12854871 8 0 682990 3 358437 229036 199124 1 188167 | 2,750 2,875 al 1 Abundance Rel. Abun ounts \$553 100 4927 5,31 3576 2,19 5666 1,78 3098 1,55 2378 1,46 | 3 04 5232 3,000 3,125 Janco Number of Charge States | 3.250 3. Identification full length product Na adduct M-G G Depuniation na M-T |
| 2000 200 2000 2 | Results min RI t1 0.84 t2 0.84 t3 0.79 t4 0.72 t5 0.87 t6 0.67 t7 0.64 | Monoisotopi Da 30 21 22 31 22 31 22 24 22 | c Mass Delta Mass Da 082.544 0.00 104.523 21.99 753.491 -329.05 49.506 -133.03 126.506 4.396 -778.499 -304.04 140.438 -642.10 | Mass II Intensity Fraction counts 12854871 0 682990 3 358437 2 229036 199124 188167 10 1439 | 2,750 2,475 1 Abundance Rel. Abun 00015 % 15593 1000.0 4527 5.31 3578 2.79 5065 1.78 3098 1.55 2,278 1.46 6850 0.81 | 3 04 5232 3,000 3,125 Janco Number of Charge States | 3.250 3. Identification full length product M-G G Depurination na M-T na |
| 75 50 25 25 25 25 25 25 25 25 25 25 | Results min min t1 0.84 t2 t4 0.79 t4 0.72 t5 0.87 t6 0.67 t7 t8 0.83 | Monoisotopi Da 33 22 23 34 21 22 34 34 23 34 23 34 34 34 34 34 34 34 34 34 34 34 34 34 | C Mass Delta Mass Da Da 082.544 0.00 044.523 21.98 753.491 -329.05 349.506 -133.03 126.506 43.96 778.499 -304.04 | Intensity Fraction counts Fraction 12854871 8 682990 358437 3284437 8 12829036 919124 188167 148197 9 93988 | 2,750 2,875 al 1 Abundance Rel. Abun ounts \$553 100 4927 5,31 3576 2,19 5666 1,78 3098 1,55 2378 1,46 | 3 04 5232 3,000 3,125 Janco Number of Charge States | 3.250 3. Identification full length product Na adduct M-G G Deputination na M-T |

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



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