Impurity Profiling and Characterization of Therapeutic Oligonucleotides using Nominal Mass Spectrometry on a Single Quadrupole LC-UV-MS system

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Overview

- LabSolutions Insight Biologics is an oligonucleotide characterization software that now supports nominal mass LC-MS data analysis.
- Nominal mass single quadrupole LC-MS data acquisition and Insight Biologics data analysis delivers a simple data processing pathway for reporting the full-length product and impurity content in oligonucleotide characterization studies.
- The work also highlights the application of flow injection analysis of oligonucleotides using nominal mass single quadrupole LC-MS in a compliance-ready platform.

1. Introduction

Oligonucleotide therapeutics have opened up new therapies for unmet clinical needs by suppressing or interfering with mRNA translation, immune stimulation, protein binding, or through induction of exon skipping. Developing new oligonucleotide therapeutics poses new challenges to the pharmaceutical manufacturing process with methodologies that can address product and product-related impurities characterization. In this work, methods were developed on an LC-IP-UV-MS system using both UV and nominal mass spectrometry data processing to rapidly evaluate sequence confirmation of chemically modified oligonucleotides and product-related impurity profiling.

2. Materials and Methods

2.1 LC-MS/MS method

A phosphorothioate-modified oligonucleotide purified was used as a model sample (20 mer anti-sense oligo; C229 H316 N69 O121 P19 S19; monoisotopic mass 7164.04032, Te-smCe-sTe-sTe-sGe-sGd-sTd-sTd-sAd-sCd-sAd-sTd-sGd-sAd-sAd-sAe-sTe-smCe-smCe-smCe). A mixture sample that was spiked with 10% impurities (N-1(5') and N-3(5') shortmers) to the full-length product was analyzed by ion-pair reversed-phase chromatography mode using HFIP and DBA .

LC Separation. Components were separated using the Shimadzu Nexera XS™ inert LC and Shim-pack Scepter™ Claris column using ion pair additives.

Mass Spectrometry Detection. Shimadzu Single Quadrupole LCMS™-2050, Data acquired in profile mode. MS mass scan *m/z* 600-2000; 1 secs; negative ion mode.

Nexera XS inert and **LCMS-2050**

×

Shim-pack Scepter™ **Claris**

×

LabSolutions Insight™ **Biologics**

3. Results

3.1 Optimal separation of the full-length product and impurities; calculating product purity and impurity ratio

With an optimized LC separation, 3 components were chromatographically resolved and detected as single components by LC/PDA. The sample was spiked with 2 impurities at a 10% level, N-1(C2:C20) and N-3(T4:C20).

Following the user input for the target sequence including selected target modifications, ion additions, sequence deletions [shortmers] and sequence additions [longmers] and a dictionary of predictable impurities. For data with LC, peak integration is applied to the selected target chromatogram (PDA, TIC or BPC) and spectra are charge-state-deconvoluted for each detected chromatographic peak.

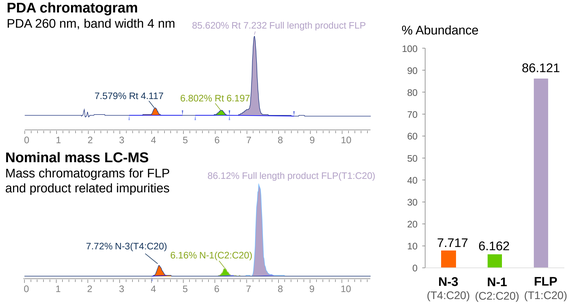

Figure 1. PDA and nominal mass chromatograms for the analysis of 3 resolved components (full length product and 2 product related impurities). The comparison of abundance is also available as a data visualization tool in Insight Biologics to quickly view a comparison of abundance.

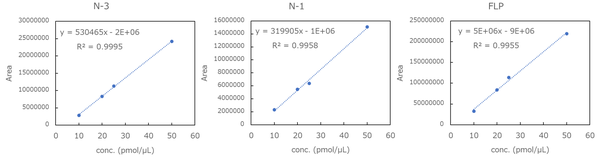

Figure 2. Calibration curves for each detected component. The response was linear for all components over the calibration range studied (calibration standards at 10, 20, 25 and 50 pmol/µL).

3.2 Co-elution of the full-length product and impurities; calculating product purity and impurity ratio

To simulate a scenario of co-elution the separation conditions were changed so that N-3(T4:C20) impurity was chromatographically resolved from the full-length product and N-1(C2:C20) co-eluted with full-length product. In the figure highlighted below N-3(T4:C20) and N-1(C2:C20) are both spiked into the sample at a 10% impurity level.

Using a nominal mass LC-MS detector and Insight Biologics data processing software, each component was mass resolved resulting in a linear response over the concentration range studied (10-50 pmol/µL). Insight Biologics software processing parameters included searching for the expected target oligonucleotide sequence and related shortmers. Charge-state-deconvolution parameters were optimized for nominal mass tolerances.

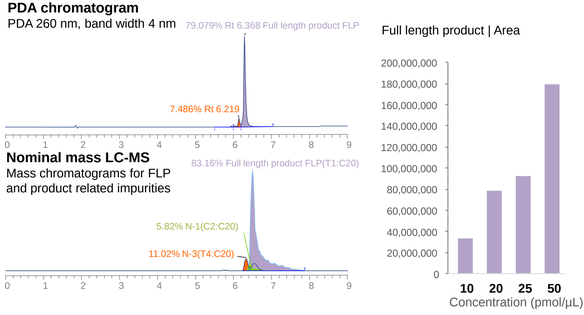

Figure 3. Using a nominal mass LC-MS detector components were mass resolved despite chromatographic coelution with the impurity N-1(C2:C20). The bar chart in Insight was configured to show the response to the full-length product over the concentration range studied.

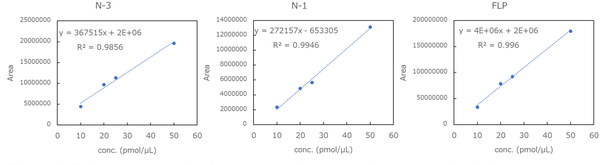

Figure 4. Calibration curves for 3 components in a co-elution scenario for the impurity N-1(C2:C20) with the full-length product.

Compared with N-3 under the separation condition, N-1 coeluted with FLP had a smaller area value due to suppression. However, the results indicated that each component was detectable in a sample concentration-dependent manner even under co-elution conditions. Insight biologics software can compare the abundance of each component across data making it easy to profile and compare the abundance ratio of impurities due to differences in methods when optimizing synthesis and purification methods.

3.3 Flow injection analysis of the full-length product and impurities: calculating product purity and impurity ratio

As an alternative data scenario, the same samples were analyzed using a flow injection method. All components co-elute using flow injection analysis and can only be mass resolved and identified using Insight Biologics processing software. The result highlights a close agreement in % abundance compared to a chromatographically resolved analysis. All components were detected in this analysis.

This approach may provide a rapid solution for confirming the molecular weight confirmation of the target full-length product sequence and help to quickly assess product related impurity levels.

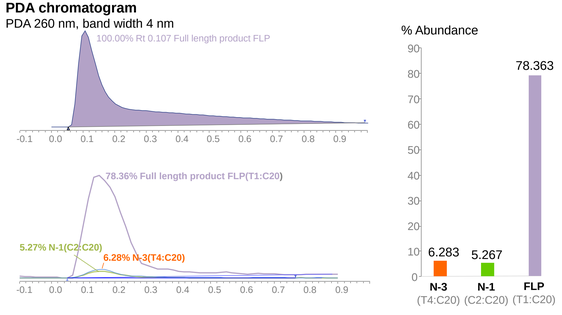

Figure 5. Flow injection analysis of the 3-component mixture highlighting co-elution of all components. Full length product and 2 spiked product related impurities were detected and confirmed by Insight Biologics resulting in a % abundance in close agreement to a chromatographically resolved separation.

4. Conclusions

- Nominal mass LC-MS on a single quadrupole mass analyser was used in characterizing oligonucleotide product and product related impurities.
- Different separation scenario's highlighted the power of mass resolution to confirm the target sequence and related product impurities even using a flow injection analysis method with co-elution of all components.