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# Improving Oligonucleotide MS Data Quality with Ion Source Conditions in a Single Quadrupole Mass Spectrometer

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## 1. Introduction

Nucleic acid-based therapeutics have gained attention as innovative treatments. The rise of synthetic DNA and RNA highlights the need for advanced techniques to confirm sequences and quantitatively measure concentration, both crucial for quality control. Liquid chromatography-mass spectrometry (LC-MS) has become popular for oligonucleotide analysis due to its sensitivity, versatility, and regulatory acceptance.

**Fig.1.** LCMS-2050

This poster highlights the Shimadzu LCMS-2050 single quadrupole mass spectrometer (**Fig.1**) for oligonucleotide quantitation. A robust method optimized ion-source conditions to resolve challenges from mobile phase adducts that can complicate qualitative analysis. The utilization of photo diode array detection with MS enhances quantitation accuracy through strong nucleic acid absorption at 260nm

Insight Biologics software was utilized for efficient data processing for analyzing oligonucleotide characteristics. The software facilitates deconvolution of MS spectra for sequence and adduct confirmation, improving both the usability and accuracy of single quadrupole mass spectrometry data.

## 2. Sample Preparation and Analytical Methods

Custom designed single-stranded DNA oligomers (**Table 1)** were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). All oligomers were diluted in nucleus free water for analysis.

**Table 1.** Analyzed oligonucleotides.

| Length (nt) | Sequence | Avg. mass (Da) |
|---|---|---|
| 15-mer | ACCTGAATACCAATA | 4529.0 |
| 30-mer | ACACTGAATACCAATCACTGAATACTACGC | 9112.0 |
| 60-mer | TCAACCTCAATACCAATCACTCACTGAGAATACCAATAC ACTGAATACCAATAGAATAAT | 18293.1 |

Oligomers were eluted under ion-pairing reversed-phase conditions with HFIP in the mobile phase. Data was processed with LabSolutions and Insight Biologics software (**Fig. 2**). Analytical conditions are listed in **Table 2/3**.

**Table 2**. Nexera UHPLC parameters (15,000psi capability)

| | |
|---|---|
| Column | Shimadzu Scepter-Claris C18 120 (150mm, 2.1mmI.D x 1.9µm) |
| Mobile Phases | A) 1% HFIP, 0.1% TEA in $H_20$ B) 1% HFIP, 0.1% TEA in MeOH |
| Gradient (Quant.) | B 10% (0-0.4min); B 24% (0.4-4.65min); B 24% (4.65-4.90min); B 99% (4.91-6.40min); B 10% (6.41-9.0min) |
| Gradient (opt.) | B 1% (0-0.5min); B 99% (0.5-6.5min); B 99% (6.5-8.0min); B 1% (8.10-10.0 min) |
| Flow Rate | 0.4 ml/min |
| Col. Temp. | 50°C |
| Inj. Vol. | 0.5µL (mass confirmation), 1 µL (calibration) |

**Table 3**. MS and PDA parameters

| | |
|---|---|
| Ionization | ESI/APCI (DUIS) (-) mode |
| Mode | SCAN (Mass Confirmation) SIM (Quantitation) |
| Mass Range | 550-2000 m/z |
| Event Time | 0.4 sec (SCAN), 0.013 (SIM) |
| Cycle Time | 0.5 sec |
| Det. Voltage | 1.3 kV |
| Wavelength | 260 nm |
| Slit Width | 1 nm |

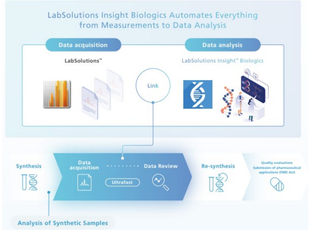


**Fig. 2**. LabSolutions LCMS and Insight Biologics s/w

MS source parameters, (**Table 4**) were optimized to maximize the peak area for the most abundant charge state while minimizing the abundance of common HFIP adducts.

**Table 4.** Optimization sequence, parameters, and optimized value

| Order | Source parameter | Opt. range | Increments | Opt. value |
|---|---|---|---|---|
| 1 | Qarray voltage | -10 to -90 V | 10 V | -40 V |
| 2 | ESI needle voltage | 1 to 4 kV | 0.5 kV | -3 kV |
| 3 | Desolvation line temp. | 150 - 250°C | 50°C | 250°C |
| 4 | Desolvation temp. | 350 - 500°C | 50°C | 500°C |
| 5 | Nebulizing gas flow | 0.5 - 3 L/min | 0.5 L/min | 2 L/min |
| 6 | Drying gas flow | 3 - 5 L/min | 0.5 L/min | 5 L/min |
| 7 | Heating gas flow | 3 - 7 L/min | 1.0 L/min | 7 L/min |

## 3. Results and Discussion

A linear relationship with an $r^2$ value of 0.998 was observed for the 30mer oligonucleotide from 0.1-50 µM with absorbance at 260 nm (**Fig. 2**, left). The MS based calibration curve, generated using the peak area of a SIM event for the average mass of the -10-charge state of the intact oligonucleotide, also exhibits a strong fit with an $r^2$ value of 0.999 (**Fig. 2**, right). Both the UV and MS calibration curves demonstrated accuracy levels within 30%.

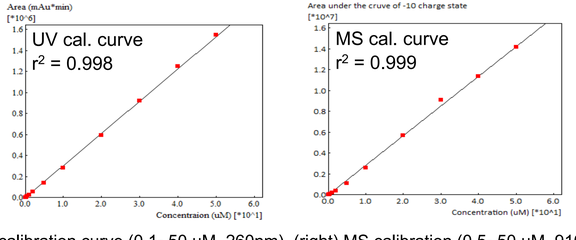


**Fig. 2**. (left). UV calibration curve (0.1- 50 µM, 260nm). (right) MS calibration (0.5- 50 µM, 910.2 *m/z*

Prior to ion source optimization (**Fig. 3**, top), the MS spectra for the 15-mer, 30-mer, and 60-mer were populated with HFIP adducts and exhibited lower ion signal counts, complicating the deconvolution process. After implementing the optimized ion source conditions, the quality of the MS spectra improved, as HFIP adducts associated with each charge state were removed (**Fig. 3**, bottom).

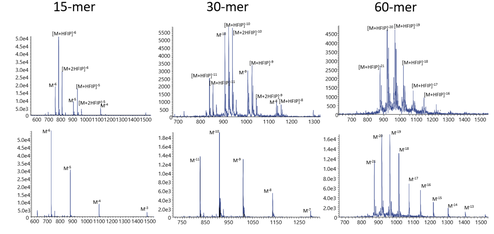


**Fig. 3.** Mass spectra of 15-mer, 30-mer and 60-mer oligonucleotides before (top) and after (bottom) ion source optimization

The analyte peak in the total ion chromatogram (TIC) acquired under optimized conditions was deconvoluted using Insight Biologic software. Deconvolution of the mass spectra (**Fig. 4**) for all three oligomers confirmed their identities, with mass deviation of less than 1.0 Da for the 15-mer and 30-mer, and 1.8 Da for the longer 60-mer (**Table 5**)

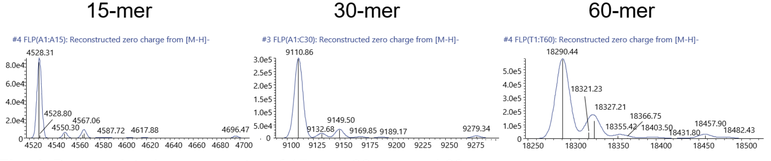


**Fig. 4.** Deconvoluted mass spectra of 15-mer, 30-mer and 60-mer

| Oligo | Mass type | Theor. mass (Da) | Obs. mass (Da) | Mass error (Da) |
|---|---|---|---|---|
| 15-mer | Most Abundant | 4528.8 | 4528.3 | 0.5 |
| 30-mer | Most Abundant | 9111.6 | 9110.9 | 0.7 |
| 60-mer | Most Abundant | 18292.2 | 18290.4 | 1.8 |

## 4.Conclusion

The Shimadzu LCMS-2050 and PDA detector offers a simple and reliable MS and UV based quality control analysis for oligonucleotides, achieving . excellent quantitation accuracy in both methods. The source parameters of the LCMS-2050 were optimized to maximize signal intensity for the highest charge state of each oligomer while minimizing the relative abundance of HFIP adducts. The straightforward use of Insight Biologics with unit resolution data acquired from a single quadrupole mass spectrometer provides accurate mass confirmation results without knowing the analyte mass before data analysis. This streamlined approach supports more reliable oligonucleotide analysis for LCMS-2050.



Conflicts of interest statement : The authors are affiliated and funded by Shimadzu Corporation.