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Application News

LCMS-2050 Liquid Chromatograph Mass Spectrometer

Improving Oligonucleotide Analysis: Enhancing Mass Spectra Quality with LCMS-2050 Ion-Source Optimization

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User Benefits

- The Shimadzu LCMS-2050 single quadrupole mass spectrometer minimizes mobile phase adducts commonly seen in ion-pairing chromatography through simple optimization of ion source parameters.
- Insight Biologics software offers intuitive data processing for oligonucleotides analysis, including MS deconvolution for sequence confirmation, enhancing both usability and accuracy of single quadrupole data.

■ Introduction

Oligonucleotide analysis has become increasingly important in recent years following the successful introduction of mRNA vaccines and synthetic short interfering RNA (siRNA) therapeutics. The emergence of synthetic DNA and RNA drug products has highlighted the need for advanced analytical techniques capable of accurately confirming the sequences of DNA and RNA after synthesis, which is essential for quality control. The use of LC-MS has gained popularity in recent years in the analysis of therapeutic oligonucleotides due to its high sensitivity and selectivity, fast turnaround time, versatility in analyzing a wide range of oligonucleotides and acceptance in regulatory settings. The linear and highly charged nature of synthetic DNA and RNA presents inherent analytical challenges in mass spectrometry, particularly when trying to deconvolute polyvalent ion spectra. These challenges are further compounded by the presence of amine ion pairs, metal adducts, and mobile phase adducts, which leads to multiple split signals which complicates qualitative analysis.

This application note presents a robust method for analyzing synthetic oligonucleotides, developed using optimized ion-source conditions on a Shimadzu LCMS-2050 single quadrupole mass spectrometer to effectively overcome complex analytical challenges. The optimization of ion-source conditions is outlined, including adjustments to Qarray™ voltage, interface voltage, desolvation temperature, desolvation line temperature, and gas flow rates for nebulizing, drying and heating gasses. The optimization data demonstrate a notable reduction in common mobile phase adducts associated with ion-pairing reverse-phase separation, while simultaneously increasing the total ion intensity of the non-adduct analyte signal.

Further, this application note demonstrates the ability of LabSolutions Insight™ Biologics software to offer efficient data processing for analyzing oligonucleotide characteristics. The software facilitates efficient deconvolution of MS spectra for sequence and adduct confirmation, improving both the usability and accuracy of single quadrupole mass spectrometry data. This streamlined approach supports more reliable oligonucleotide analysis with the LCMS-2050.

Shimadzu LCMS-2050 Single Quadrupole LC/MS

■ Experimental

Sample Preparation

Custom-designed single-stranded 15-mer, 30-mer and 60-mer DNA oligonucleotides, as shown in **Table 1**, were purchased from Integrated DNA Technologies, Inc. (Coralville, IA, USA) with standard desalting purification.

All oligomers were reconstituted to a stock concentration of 100 µM and stored at -20°C when not in use. Injections of 50 pmol mass on column were performed.

Table 1: Analyzed oligonucleotides

Length (nt)	Sequence (DNA, 5'-3')	Average Mass (Da)
15-mer	ACCTGAATACCAATA	4529.0
30-mer	ACACTGAATACCAATCACTGAATACTACGC	9112.0
60-mer	TCAACCTCAATACCAATCACTCACTGAGAATACCAATACACTGAATACCAATAGAATAAT	18293.1

Analytical Conditions

All oligomers were eluted with ion pairing, reversed phase conditions using HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) reagent in the mobile phase. Instrument parameters are shown in **Table 2**. The MS source parameters, including Qarray voltage, interface voltage, desolvation line temperature, desolvation temperature, and gas flows for nebulizing, drying, and heating gasses, were optimized to maximize the peak area for the most abundant charge state while minimizing the abundance of common HFIP adducts.

The variables used for optimizing MS source parameters, including their sequence and optimized values, are shown in **Table 3**.

Table 2: Oligonucleotide analysis instrument parameters.

Liquid Chromatography (LC) Conditions			
Column	Shimadzu Scepter™-Claris C18-120 (150mm x 2.1mm I.D x 1.9µm)		
Mobile Phase A	1% (95 mM) HFIP, 0.1% (4.3 mM) TEA (triethylamine) in water		
Mobile Phase B	1% (95 mM) HFIP, 0.1% (4.3 mM) TEA in 50% MeOH		
Injection Volume	0.5µL		
Column Temperature	50 °C		
Autosampler Temperature	5 °C		
Gradient (Mass Confirmation)	Time (min)	Flow (ml/min)	B%
	0.0	0.4	1
	0.5	0.4	1
	6.5	0.4	99
	8.0	0.4	99
	8.01	0.4	1
	10.0	0.4	1
MS Conditions			
Mode	SCAN		
Mass Range	550-2000 *m/z*		
Ionization	ESI/APCI (DUIS) (-) mode		
Event Time	0.4 sec (full scan), 0.013 sec (SIM)		
Cycle	0.5 sec		
Detector Voltage	1.3 kV		
PDA Conditions			
Wavelength	260 nm		
Slit Width	1 nm		

Table 3: Optimized MS source parameters and their corresponding optimized conditions used for the optimization process

Order	Source Parameter	Optimization Range	Increments	Optimized Conditions
1	Qarray voltage	-10 to -90 V	10 V	-40 V
2	Interface voltage	-1 to -4 kV	0.5 kV	-3 kV
3	Desolvation line temperature	150 – 250 °C	50 °C	250 °C
4	Desolvation temperature	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

Instrumentation

Samples were analyzed in series using a photodiode array detector (SPD-M30A) and single quadrupole mass spectrometer (LCMS-2050) coupled to a Nexera UHPLC capable of pressures up to 15,000psi. A Shimadzu Scepter Claris column with bioinert surface treatment on the body and frit was used for analysis.

■ Results

The processed data for each ion source parameter were analyzed by comparing the most abundant charge state for each oligomer for the HFIP adduct-free and HFIP adduct signals. The results are displayed in bar graphs that show the relationship between each ion source parameter and the area under the curve for the most abundant charge state of each oligomer.

Qarray Voltage

It was observed that the Qarray voltage had the most substantial impact on increasing spectral intensity of the maximum charge state, increasing spectral quality while minimizing the abundance of HFIP adducts (**Fig. 1**). At the default Qarray voltage of -20 V or lower, the association of HFIP adducts with the oligomer was higher, resulting in lower total ion abundance across all oligomers, regardless of the nucleotide chain length. Gradually increasing the Qarray voltage led to notable improvements in both total ion abundance and the association of non-HFIP adducts with each oligomer. This trend continued up to -40 V for the 15-mer and around -60 V for the 30-mer and 60-mer. However, at Qarray voltages above 70 V, the total ion abundance of both non-HFIP and HFIP adduct-associated signals decreases across all three oligomers. Therefore, the optimized Qarray voltage selected for the 10, 30, and 60-mer was -40 V.

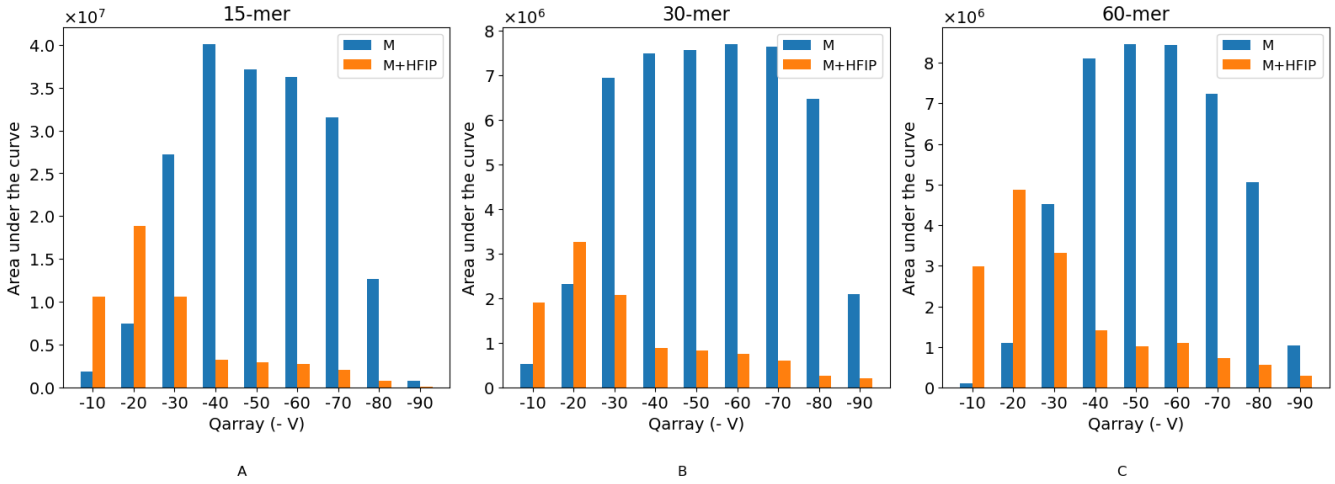

Fig. 1: Comparison between HFIP adducts and the most abundant charge state across different Qarray voltage settings
A) 15-mer, -6 charge state
B) 30-mer, -10 charge state
C) 60-mer, -10 charge state

Interface Voltage

The interface voltage was optimized in 0.5 kV increments from -1.0 kV to -4.0 kV. As shown in **Fig. 2**, the influence of interface voltage on HFIP adduct association with each oligomer is minimal. However, the total ion abundance of both HFIP adduct-free oligomer signals and HFIP adduct oligomer signals gradually increases with increased interface voltage and peaks at -3 kV for 10-mer and 15-mer and -2.5 kV for 60-mer.

Therefore, the ideal interface voltage was determined to be between -2.5 and -3 kV to obtain higher ion signal abundance while lowering the HFIP adduct association.

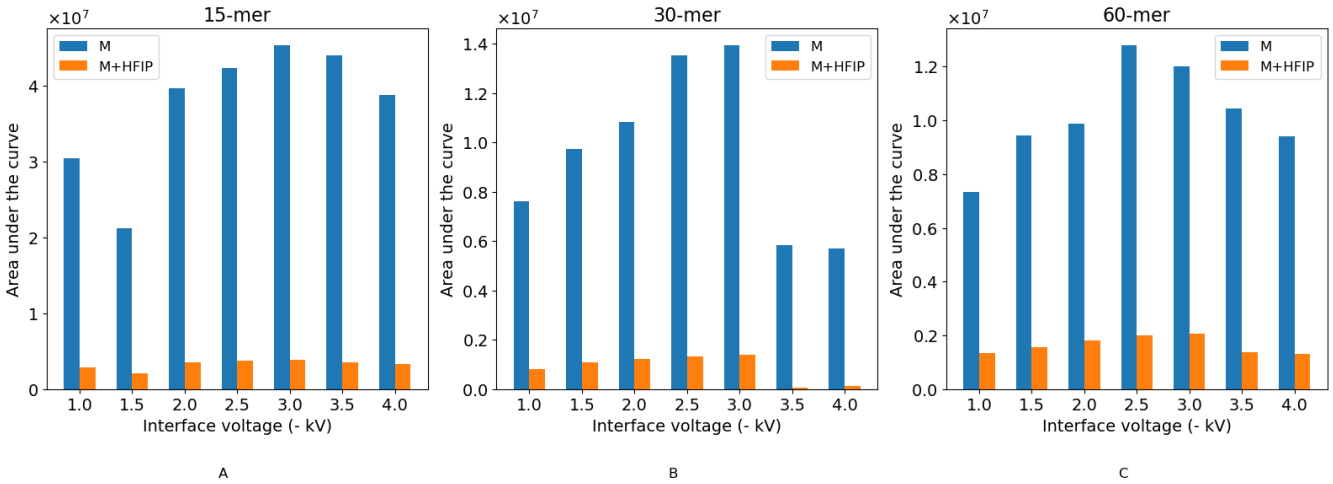

Fig. 2: Comparison between HFIP adducts and the most abundant charge state across different interface voltage settings
A) 15-mer, -6 charge state
B) 30-mer, -10 charge state
C) 60-mer, -10 charge state

Desolvation Line and Desolvation Temperature

The desolvation line temperature was optimized in 50 °C increments from 150 – 250 °C, while the desolvation temperature was optimized in 50 °C increments from 350 – 500 °C. The influence of these ion source temperature parameters on total ion signal abundance and HFIP adduct association are shown in **Fig. 3**.

It was observed that the highest total ion signal abundance occurred at the highest desolvation line and desolvation temperatures, which are 500 °C and 250 °C, respectively. Simultaneously, the association of HFIP adducts decreased as the temperatures increased.

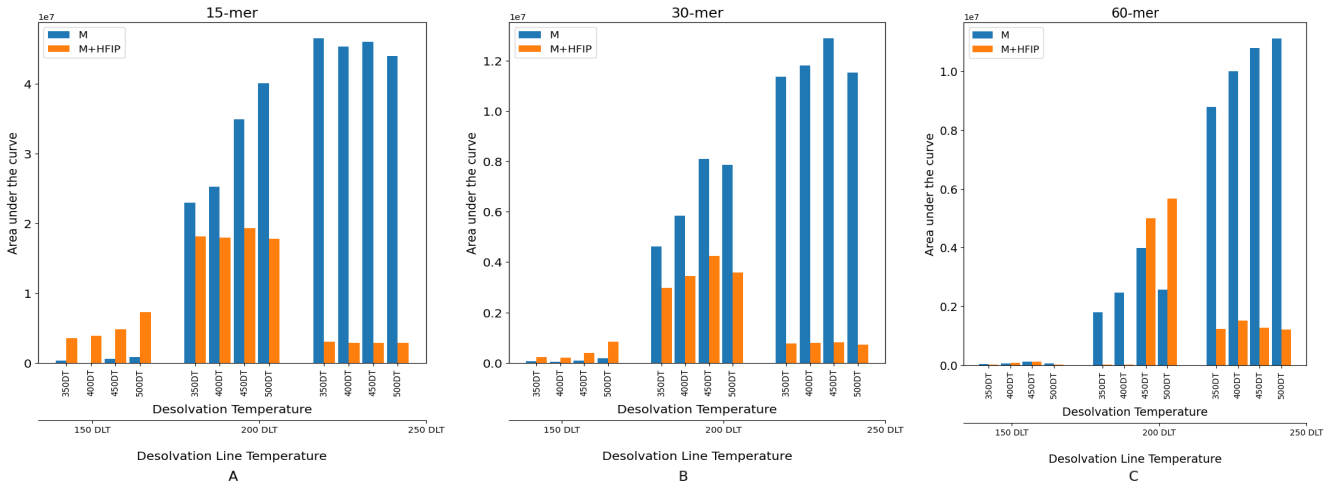

Fig. 3: Comparison between HFIP adducts and the most abundant charge state across different Desolvation Line Temperature settings
A) 15-mer, -6 charge state
B) 30-mer, -10 charge state
C) 60-mer, -10 charge state

Nebulizing, Drying and Heating Gas Flow

The gas flows that were analyzed included nebulizing, drying, and heating gases. Among these, nebulizing gas flow had a major impact on total ion abundance as shown in **Fig. 4**. At lower nebulizing gas flows, ion signal levels were insufficient, but when the flow exceeded 2 L/min flow, there was a marked increase in the ion signal levels. The influence of drying gas flow is minimal for total ion abundance for all three oligomers (**Fig. 5**).

The influence of heating gas flow remained consistent for 10-mer and 30-mer oligonucleotides. However, the longer 60-mer oligonucleotide exhibited lower ion abundance at reduced heating gas flows, with a notable increase only when the flow reached 6 L/min, as shown in **Fig. 6**.

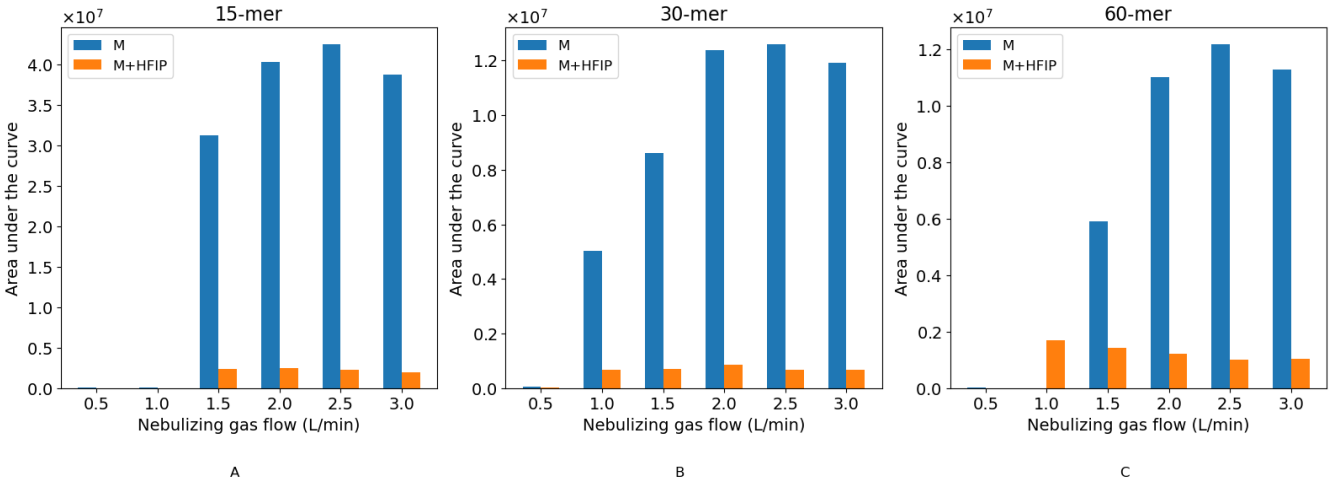

Fig. 4: Comparison between HFIP adducts and the most abundant charge state across different Nebulizing Gas Flow
A) 15-mer, -6 charge state
B) 30-mer, -10 charge state
C) 60-mer, -10 charge state

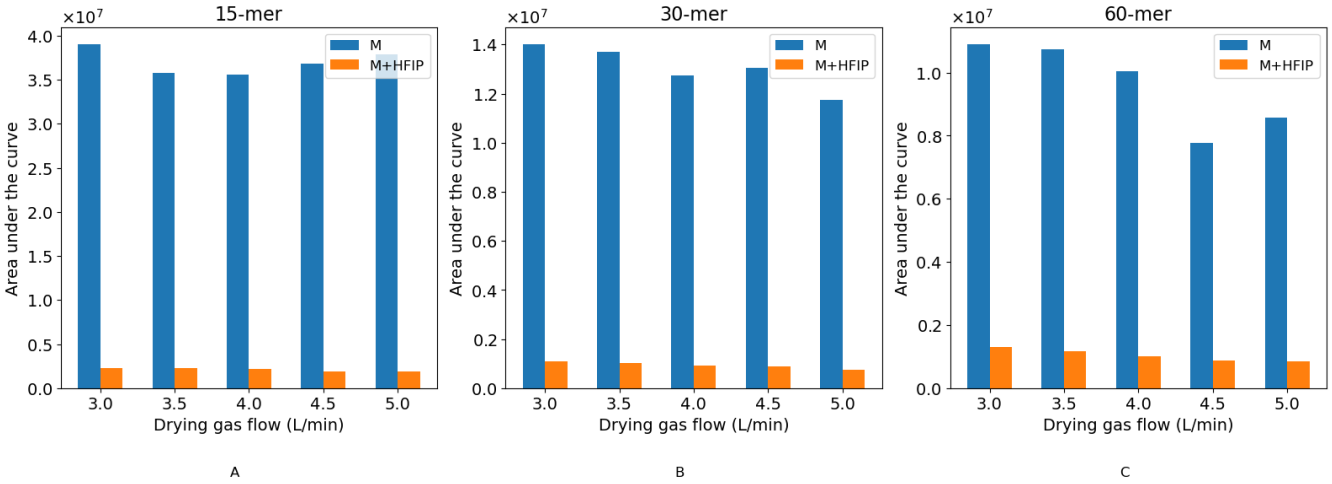

Fig. 5: Comparison between HFIP adducts and the most abundant charge state across different Drying Gas Flow
A) 15-mer, -6 charge state
B) 30-mer, -10 charge state
C) 60-mer, -10 charge state

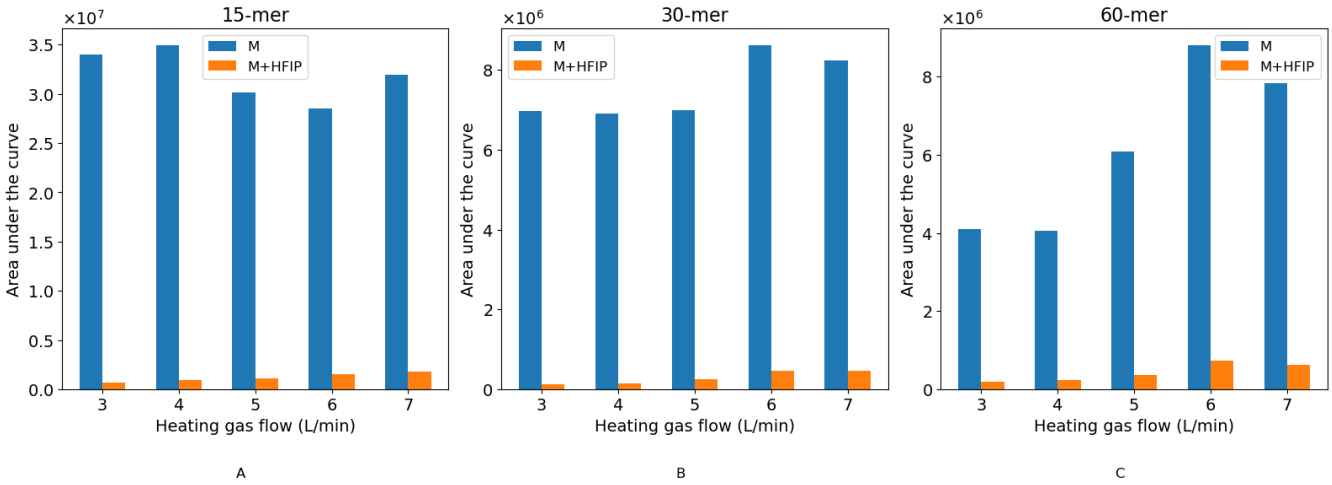

Fig. 6: Comparison between HFIP adducts and the most abundant charge state across different Heating Gas Flow
A) 15-mer, -6 charge state
B) 30-mer, -10 charge state
C) 60-mer, -10 charge state

MS Spectra Comparison Between Before and After Ion Source Optimization

As shown in **Fig. 7**, Panel A, prior to ion source optimization, the MS spectra for the 15, 30, and 60-mer were populated with HFIP adducts and exhibited lower ion signal counts, particularly for 30-mer and 60-mer, complicating the deconvolution process.

However, after implementing the optimized ion source conditions, the quality of the MS spectra improved, as HFIP adducts associated with each charge state were removed, leading to an increase in the total ion count for the 30-mer and 60-mer (**Fig. 7**, Panel B).

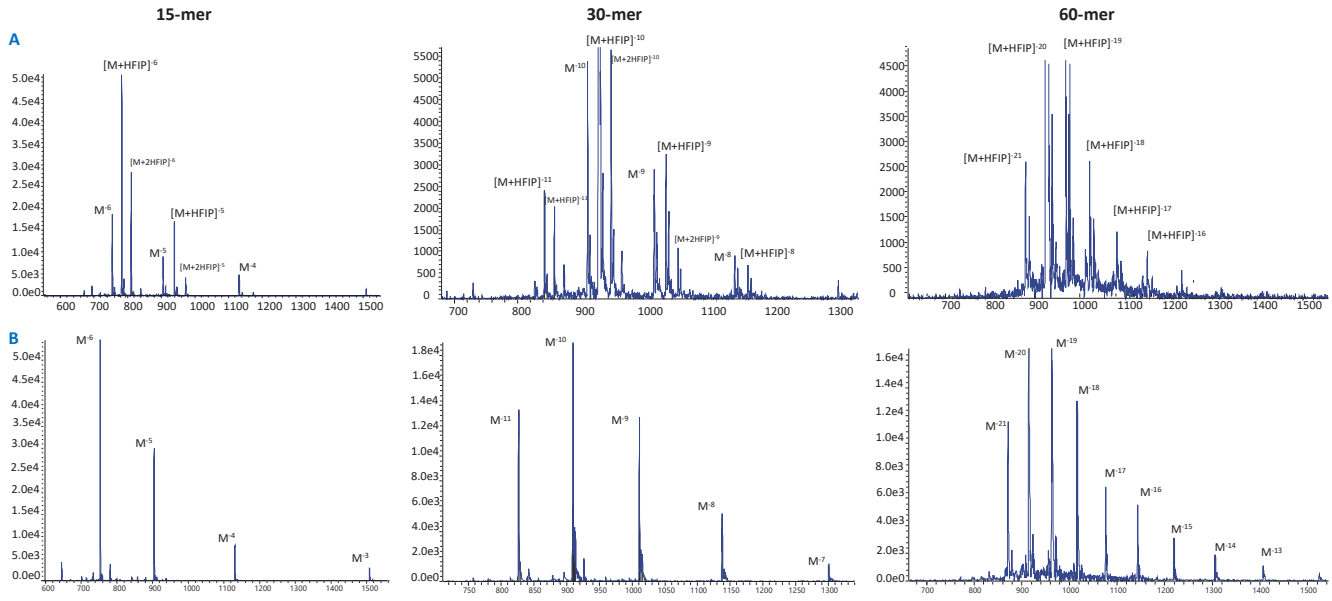

Fig. 7: Mass spectra of 15,30 and 60-mer oligonucleotides before and after ion source optimization.
Panel A; Before the ion source optimization, Panel B; After the ion source optimization.

Deconvolution

The analyte peak in the total ion chromatogram (TIC) acquired under optimized conditions was deconvoluted using Insight Biologics software. Deconvolution of the mass spectra (**Fig. 8**) from all three oligomers confirmed the identity of each oligomer with less than 1.0 Da mass deviation for 15-mer and 30-mer, and 1.8 Da for the longer oligomer, the 60-mer.

Results from Insight Biologics are shown in **Table 4**, indicating the mass error for each oligomer.

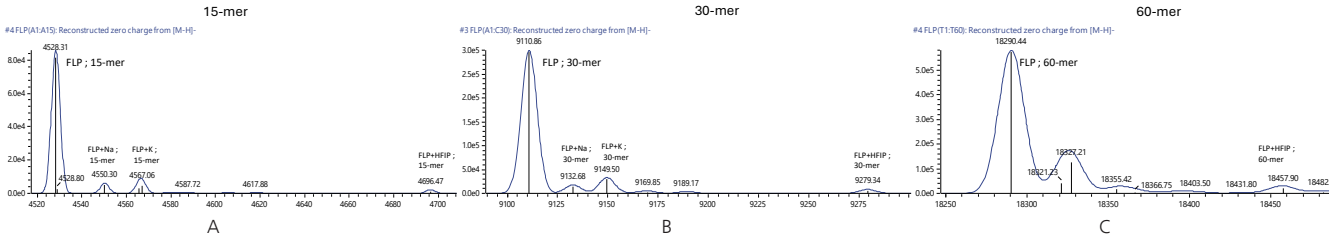

Fig. 8: Deconvoluted mass spectra of 15-mer (A), 30-mer (B), and 60-mer (C) after processing data from Insight Biologics

Table 4: Deconvolution results extracted from Insight Biologics

Compound	Mass Type	Theoretical Mass (Da)	Observed 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

■ Conclusion

The LCMS-2050 single quadrupole mass spectrometer source parameters were optimized to provide the highest signal intensity of the maximum charge state for each oligomer while maintaining the lowest relative abundance of the 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.

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