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Nota de aplicación

Optimization of LCT Premier XE MS Settings for Oligonucleotide Analysis

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Abstract

This application note illustrates that the LCT Premier XE System is a good choice for sensitive LC-MS analysis of synthetic oligonucleotides and oligonucleotide-based biotherapeutic compounds.

Introduction

The analysis of oligonucleotides via liquid chromatography and mass spectrometry is becoming a common practice. Many applications require the identification of oligonucleotides at low concentrations. For this reason, it is advantageous to utilize highly sensitive mass spectrometers such as Waters LCT Premier XE System.

The exceptional sensitivity of the LCT Premier XE System is in part achieved by greater efficiency of ion transition into the electrospray source. For oligonucleotides that are often analyzed with mobile phase containing triethylammonium and hexafluoroisopropanol aqueous solutions, some degree of TEA and/or HFIP adducts are also often present in the MS spectra. The majority of adducts are observed at low charge states of oligonucleotides, while the high charge states typically have comparatively less adduction.

In this work, we outline the critical parameters that were adjusted to yield significantly lower adduct formation for oligonucleotide analysis at moderate LC flow rates.

Experimental

Sample

For our study, we utilized the Waters Oligonucleotide Separation Technology (OST) standard, which contains oligo deoxythymidine sequences up to 35-mer reconstituted in 500 µL of 0.1 M triethylammonium acetate (TEAA) to yield a solution of 2 pmol/µL per oligonucleotide, and a 25-mer phosphorothioate (5' – CTC TCG CAC CCA TCT CTC TCC TTC T– 3') at 1 µg/µL in TEAA.

LC Conditions

LC system:	Waters ACQUITY UPLC System

Column:	Waters ACQUITY UPLC OST 2.1 x 50 mm, 1.7 µm (P/N 186003949)
Column temp.:	60 °C
Flow rate:	0.2 mL/min
Mobile phase A:	15 mM TEA/400 mM HFIP
Mobile phase B:	50 % MeOH in Mobile A (v/v)

Gradient (OST standard)		
Time	%A	Curve
0 min	31%	
10 min	47%	6

Gradient (Phosphorothioate 25mer)		
Time	%A	Curve
0 min	31%	
5 min	50%	6

Results and Discussion

Figure 1 shows a typical LC-MS chromatogram of Waters OST standard under the specified conditions using the LCT Premier XE System. The OST sample is a mixture of 15-, 20-, 25-, 30-, and 35-mer oligonucleotide standards, and their synthetic N-x impurities. Under normal operating conditions, outlined in the instrument parameters in Table 1, we found evidence for significant adduct formation. The adduct formation is more significant for longer oligonucleotides (ca. 25- and 35-mer, as shown in Figure 2).

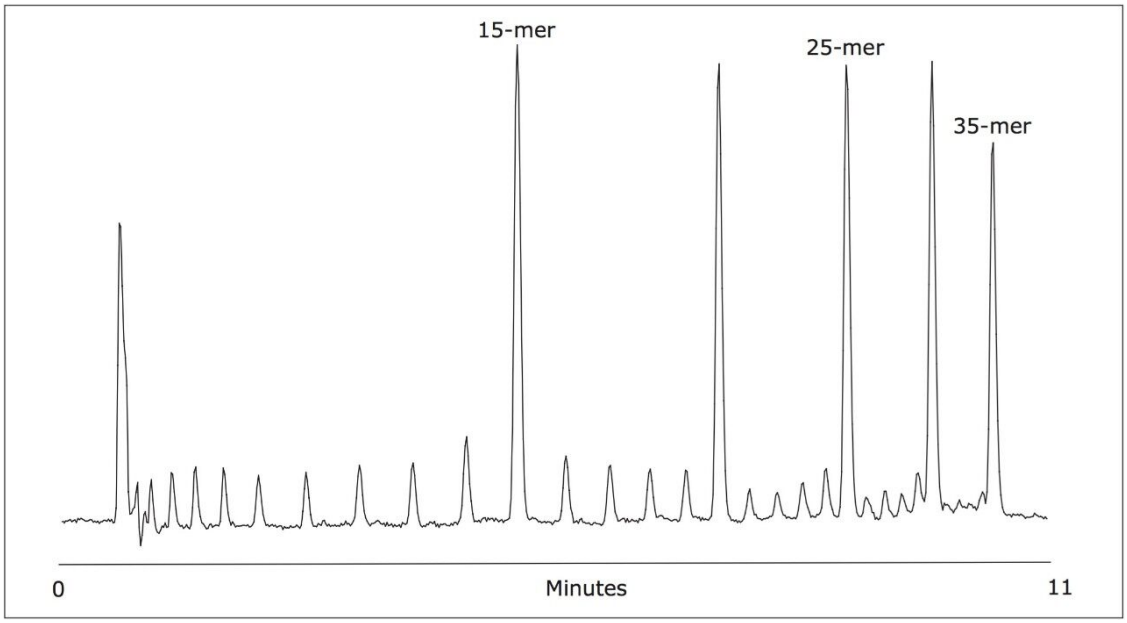

Figure 1. Total Ionic Current (TIC) for the separation of Waters OST standard detected with the LCT Premier XE System.

Polarity	ES-	Puller Offset Voltage	0.00
Analyser	V Mode	MCP Detector (V)	2000.0
Capillary (V)	2600.0	Pusher Cycle Time	Auto (68.0)
Sample Cone (V)	*37.0*	Pusher Frequency	14705.88
Desolvation Temp (C)	**250.0**	Pusher Width	4.00
Source Temp (C)	150.0	Centroid Threshold	1.0
Cone Gas Flow	*50.0*	Min Points	4.0
Desolvation Gas Flow	**500.0**	Np Multiplier	0.70
Syringe Type	Hamilton 250uL	Resolution	6000.0
Ion Guide One	5.0	Lteff	1081.0000
Aperture 1 Voltage	*15.0*	Veff	5681.4629
Ion Energy (V)	105.0	Trigger Threshold (mV)	600.0000
Aperture 2 Voltage	*6.0*	Signal Threshold (mV)	40.0000
Hexapole DC Voltage	6.0	Data Threshold	0.0000
Aperture 3 Voltage	*5.0*	DXC Temperature	25.0
Acceleration (V)	200.0	IonGuide1InitialRF	150.0
Y Focus (V)	0.0	IonGuide1FinalRF	150.0
Steering (V)	0.0	IonGuide2InitialRF	200.0
Tube Lens (V)	192.0	IonGuide2FinalRF	200.0
Attenuated Z Focus (V)	500.0	Fixed Hexapole RF	True
Normal Z Focus (V)	65.0	HexapoleRF	180.0
TOF Flight Tube (V)	5630.0	DRE Mass 0.0000 Setting	5.0000
Reflectron (V)	1780.0	DRE Mass 280.0000 Setting	50.0000
Pusher Voltage	839.0	DRE Mass 1000.0000 Setting	50.0000
Pusher Offset Voltage	-1.47	DRE Mass 2000.0000 Setting	50.0000
Puller Voltage	769.0	DRE Mass 3000.0000 Setting	50.0000

Table 1. Normal LCT Premier XE System operating parameters.

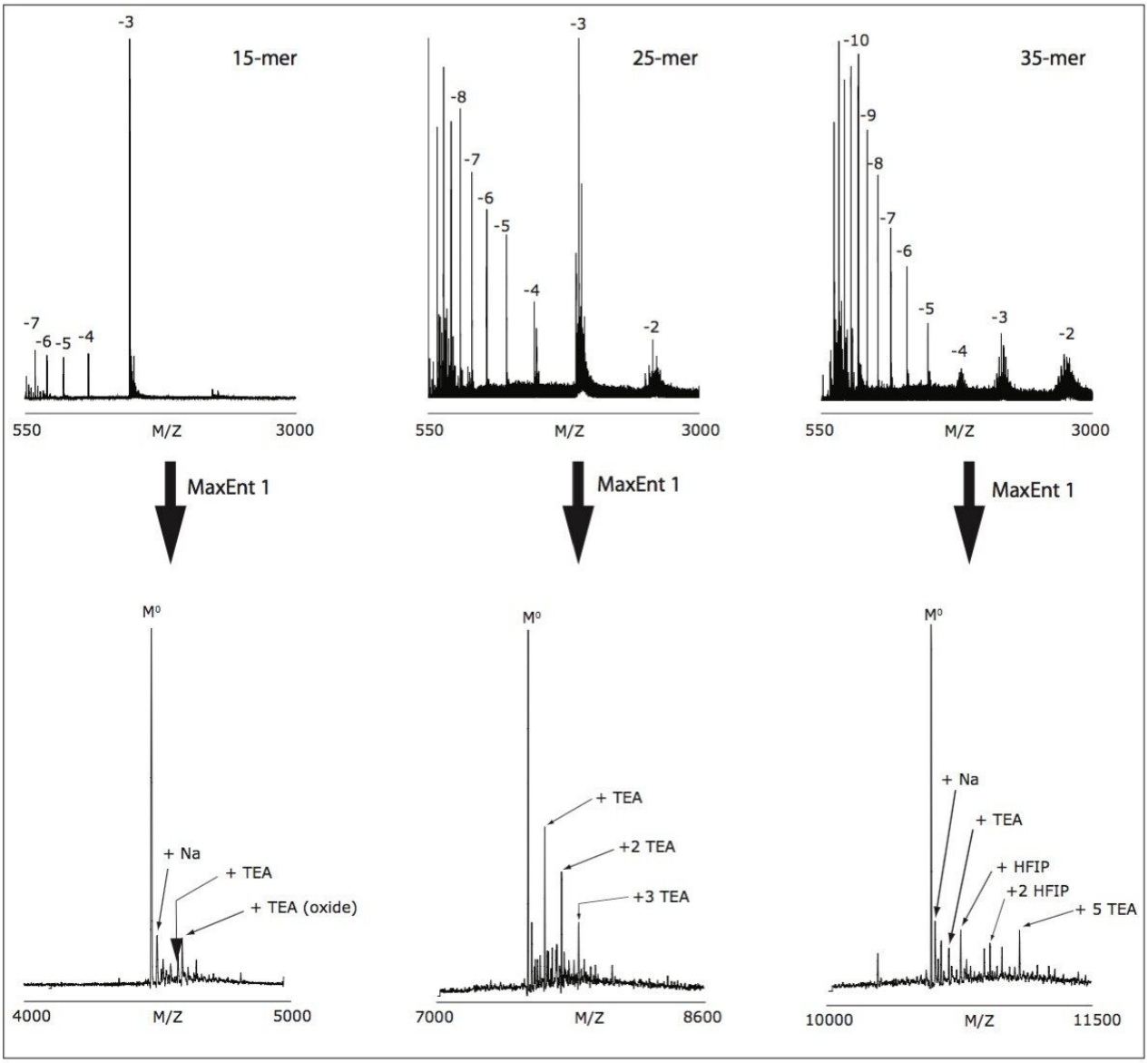

Figure 2. Raw and MaxEnt1 deconvoluted data for 15-, 25-, and 35-mer oligonucleotides of Waters OST standard under normal operating conditions given in Table 1.

Due to the different chemical nature of phosphorothioate oligonucleotides, the adduct formation is more pronounced. Figure 3 shows abundant multiple TEA adducts in both raw and deconvoluted 25-mer phosphorothioate spectra. It can be seen that adduct formation is more pronounced at lower charge states, with -2 and -3 charge states exhibiting a greater extent of adductation than the other charge states.

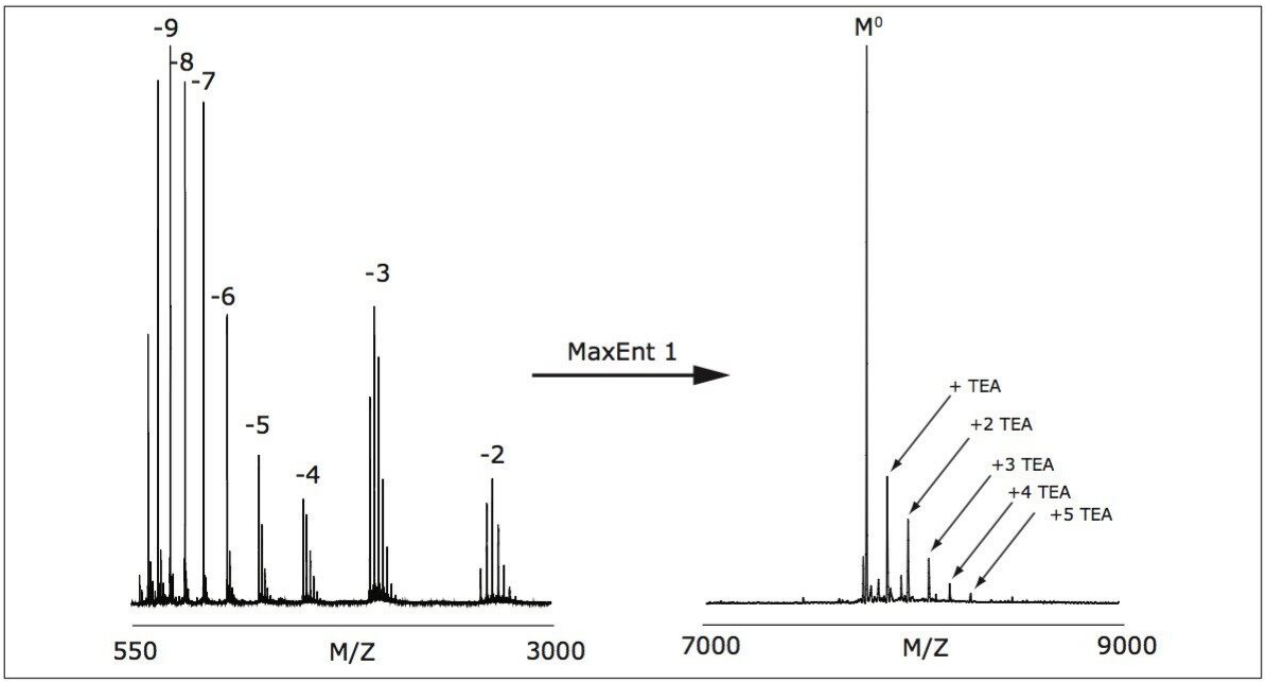

Figure 3. Raw and MaxEnt1 deconvoluted data for 25-mer phosphorothioate under normal operating conditions given in Table 1.

In an attempt to decrease adduct formation, we adjusted various LCT Premier XE System parameters including aperture 1, aperture 2, aperture 3, cone voltage, desolvation temperature, desolvation gas flow, and cone gas flow. Of these parameters, we found the largest benefit from the adjustment of desolvation temperature and desolvation gas flow.

While we did find evidence that adjustment of aperture 1, ca. from 15 to 30, yielded modest improvement in adduct formation, the benefits were not sufficient to justify a change from normal conditions.

The parameters providing the best LC-MS results and efficient desolvation for oligonucleotides are listed in Table 2. As shown in Figures 4 and 5 for OST and phosphorothioate analysis respectively, the optimal parameter settings yield significantly less adduct formation as compared to Figures 2 and 3. This benefit was particularly evident for the phosphorothioate, with virtually all TEA adducts eliminated, as shown in Figure 5.

Polarity	ES-	Puller Offset Voltage	0.00
Analyser	V Mode	MCP Detector (V)	2000.0
Capillary (V)	2600.0	Pusher Cycle Time	Auto (68.0)
Sample Cone (V)	*37.0*	Pusher Frequency	14705.88
Desolvation Temp (C)	**500.0**	Pusher Width	4.00
Source Temp (C)	150.0	Centroid Threshold	1.0
Cone Gas Flow	*50.0*	Min Points	4.0
Desolvation Gas Flow	**800.0**	Np Multiplier	0.70
Syringe Type	Hamilton 250uL	Resolution	6000.0
Ion Guide One	5.0	Lteff	1081.0000
Aperture 1 Voltage	*15.0*	Veff	5681.4629
Ion Energy (V)	105.0	Trigger Threshold (mV)	600.0000
Aperture 2 Voltage	*6.0*	Signal Threshold (mV)	40.0000
Hexapole DC Voltage	6.0	Data Threshold	0.0000
Aperture 3 Voltage	*5.0*	DXC Temperature	25.0
Acceleration (V)	200.0	IonGuide1InitialRF	150.0
Y Focus (V)	0.0	IonGuide1FinalRF	150.0
Steering (V)	0.0	IonGuide2InitialRF	200.0
Tube Lens (V)	192.0	IonGuide2FinalRF	200.0
Attenuated Z Focus (V)	500.0	Fixed Hexapole RF	True
Normal Z Focus (V)	65.0	HexapoleRF	180.0
TOF Flight Tube (V)	5630.0	DRE Mass 0.0000 Setting	5.0000
Reflectron (V)	1780.0	DRE Mass 280.0000 Setting	50.0000
Pusher Voltage	839.0	DRE Mass 1000.0000 Setting	50.0000
Pusher Offset Voltage	-1.47	DRE Mass 2000.0000 Setting	50.0000
Puller Voltage	769.0	DRE Mass 3000.0000 Setting	50.0000

Table 2. Optimal LCT Premier XE System operating parameters for oligonucleotide analysis.

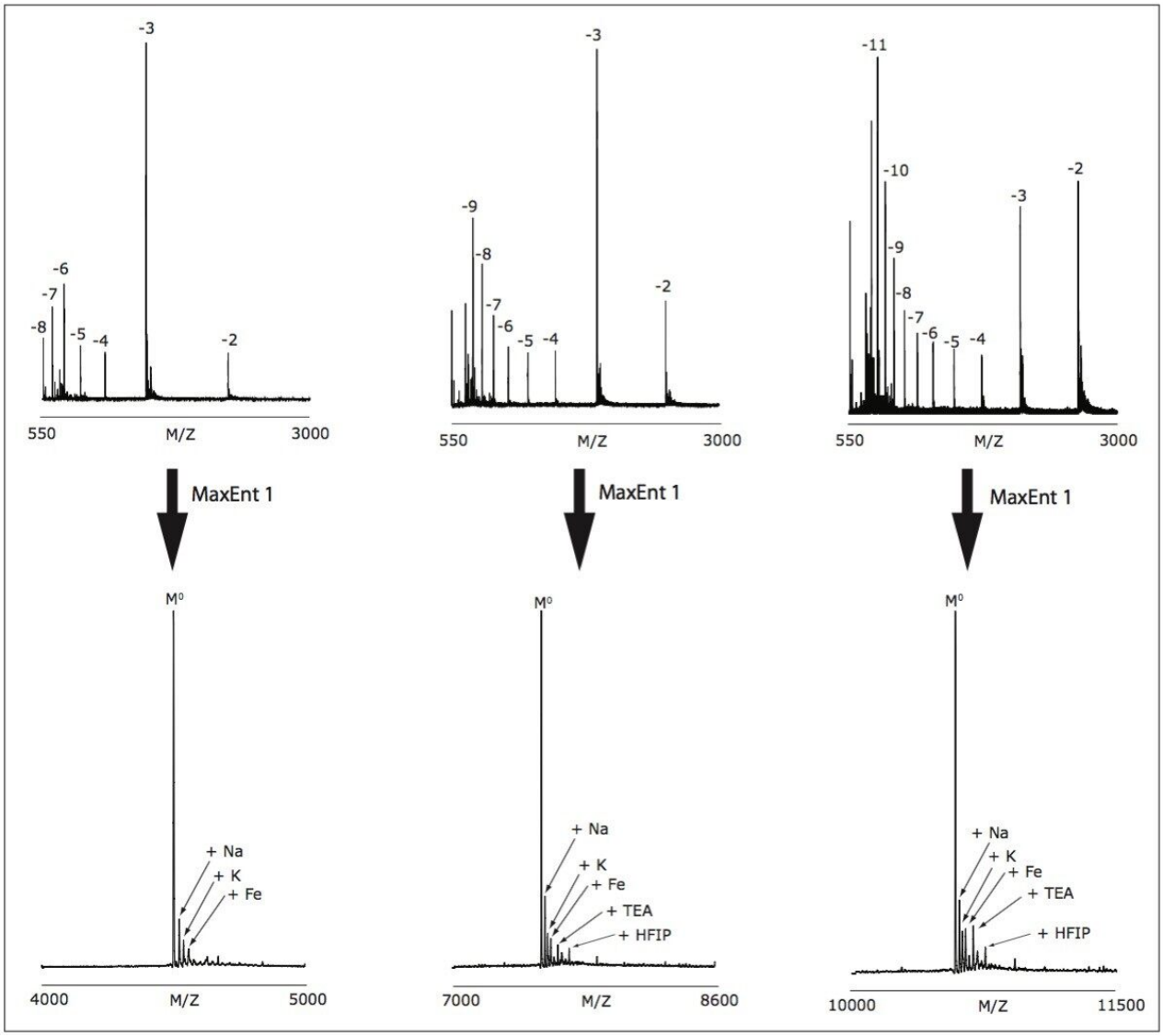

Figure 4. Raw and MaxEnt1 deconvoluted data for 15e, 25e, and 35-mer oligonucleotides of Waters OST standard under optimal operating conditions given in Table 2.

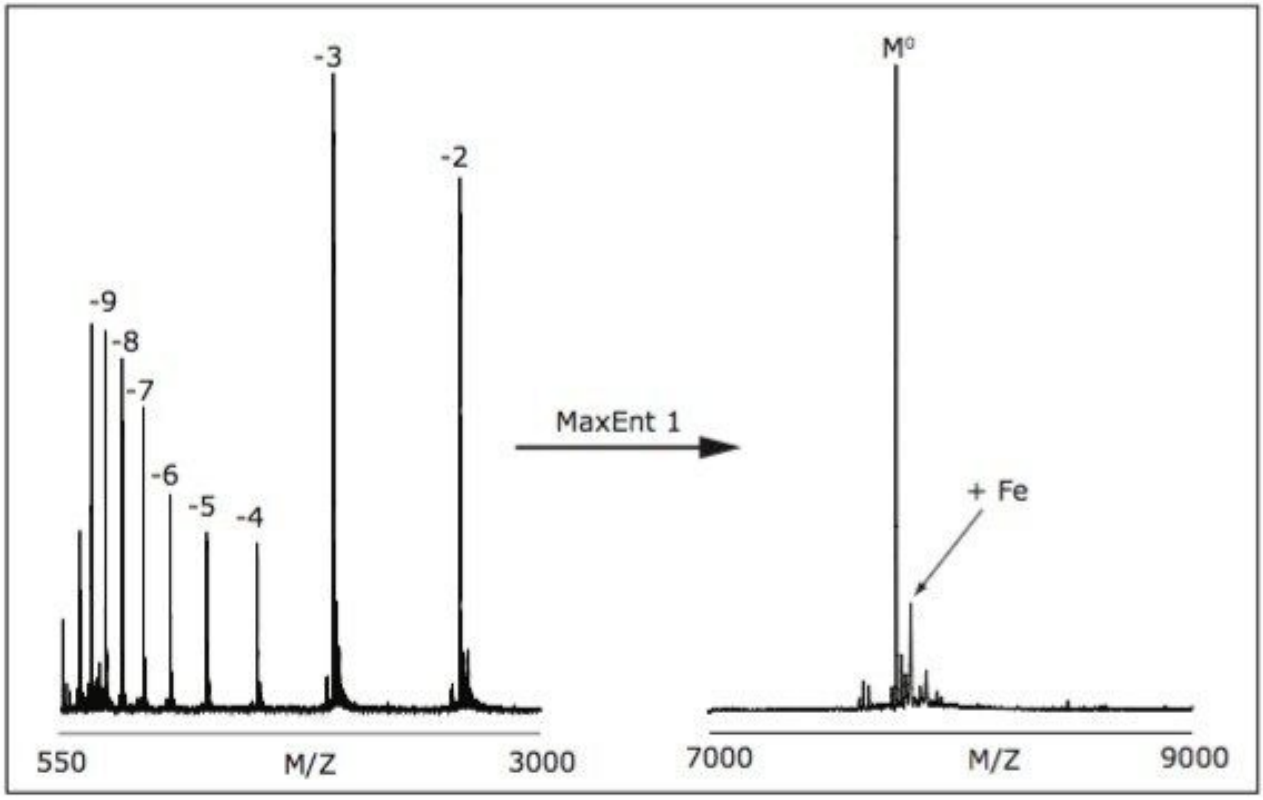

Figure 5. Raw and MaxEnt1 deconvoluted data for 25-mer phosphorothioate under normal operating conditions given in Table 2.

Additionally, increasing the desolvation temperature and gas flow yielded a significant improvement in signal-to-noise (S/N), which is particularly evident when compared to 25- and 35-mer OST oligonucleotides under normal and optimal conditions. This was likely due to increased population of the parent ions from a decrease in adduct formation, further highlighting the benefits of this change.

Conclusion

This application note illustrates that the LCT Premier XE System is a good choice for sensitive LC-MS analysis of synthetic oligonucleotides and oligonucleotide-based biotherapeutic compounds.

By adjusting the desolvation parameters, one can achieve efficient desolvation with the LCT Premier XE System at typical LC flow rates of ~0.2 mL/min. The desolvation at lower flow rates is more efficient.

The data presented here illustrates that by modifying the normal operating conditions to our recommended setup, one can significantly reduce adduct formation, resulting in improvements in S/N.

Sensitive and adduct free LC-MS analysis of oligonucleotides are very important for the identification of structurally related components and degradation products in synthetic and therapeuticoligonucleotide compounds.

The LCT Premier XE System, coupled with the separation efficiency offered by the ACQUITY UPLC System and OST Column Chemistry, offers biopharmaceutical laboratories a complete system solution for achieving their research goals. With a fast development of oligonucleotide therapies, such tools become more desired by the biopharmaceutical industry.

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