

Application Note

## High-throughput Screening of Oligonucleotides for Identity and Purity Assessment Using the ACQUITY QDa Detector and ProMass for MassLynx

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## Abstract

In this study it was demonstrated that the ProMass software can be adopted into an ACQUITY QDa based MassLynx project for confirmation of analyte mass and yield results consistent with the molecular weight of the target sequence within the default mass tolerance settings.

### Benefits

- Addition of mass data to synthetic oligonucleotide assays using MassLynx Software, enabled with MaxEnt deconvolution algorithm
- Increased productivity with automated high-throughput batch processing and analysis of synthetic oligonucleotides
- Readily interpretable results with interactive web-based format for increased confidence in data analysis

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## Introduction

Research into therapeutic oligonucleotides has received steadily increasing attention from the pharmaceutical industry. This is due to potential applications using deoxyribonucleic acid (DNA) sense/antisense oligonucleotides and interfering ribonucleic acid- (RNAi) based therapies.<sup>1,2</sup> The production of oligonucleotides with high yields via automated stepwise synthetic methods is well established. As part of the production process, purification and desalting steps are incorporated to remove byproducts of synthesis, such as failed sequences and production impurities. Characterization of purified synthetic products must be carried out prior to use in therapeutic applications to ensure product identity and purity. Ion Pairing Reversed Phase Liquid Chromatography (IP-RPLC) has become a prevalent technique in the analysis of synthetic oligonucleotides in part due to the selectivity offered by such techniques, as well as its ability to incorporate mass spectrometry-friendly reagents and buffers as first demonstrated by Apffel and colleagues.<sup>3,4</sup>

Mass information afforded by MS detection offers an efficient means of identifying challenging base modifications for improved productivity in synthetic therapeutic oligonucleotide workflows. Pharmaceutical companies engaged in oligonucleotide research are often investigating numerous potential biotherapeutic candidates, which can negatively impact productivity as the characterization process of synthetic oligonucleotides often requires manual processing of LC-MS data. Previous work demonstrated that

incorporation of ProMass (Novatia, LLC) for MassLynx Software enables automated spectrum deconvolution and data analysis for high-throughput screening of UPLC-MS data generated on Waters MS instruments, such as the ACQUITY SQD Detector and SYNAPT.<sup>5</sup> As one of the newest mass detectors from Waters, the ACQUITY QDa has been established as an efficient means for obtaining mass information within existing, optically-based LC workflows in the biopharmaceutical manufacturing environment when used as an orthogonal detection technique.<sup>6-8</sup>

The objective of this application note is to demonstrate that the ACQUITY QDa Detector provides a simple and cost-effective solution in the assessment of identity and purity of synthetic oligonucleotides and that mass spectral data acquired can be readily processed with ProMass for MassLynx Software in an automated fashion.

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## Experimental

### Chemicals and reagents

Triethylamine (99.5% purity) and 1,1,1,3,3,3-hexafluoro-2-propanol (99.8% purity, LC-MS grade) were purchased from Sigma Aldrich. Mass spectrometry grade solvents (Optima series) were purchased from Fisher Scientific. Mobile phase buffers were newly prepared prior to experiments. PolyT oligonucleotide standards were from Waters (P/N 186004135). siRNA upper strand 5'-UCGUCAAGCGAUUACAAGGTT-3' and its complementary lower strand 5' TTCCUUGUAAUCGCUUGACGA-3' were ordered from Integrated DNA Technologies. All samples were prepared at a concentration of 10 pmol/ $\mu$ L. Mass loads on column were kept constant at 50 pmol or 5  $\mu$ L injections.

### LC conditions

LC system:	ACQUITY UPLC H-Class
Detectors:	ACQUITY UPLC TUV w/Ti flow cell, ACQUITY QDa Detector
Absorption wavelength:	260 nm
Column:	ACQUITY UPLC OST BEH C <sub>18</sub> , 1.7 $\mu$ m, 2.1 mm x

50 mm  
(P/N 186003949)

Column temp.: 60 °C

Sample temp.: 10 °C

Injection volume: 5 µL

Mobile phase A: 15 mM TEA, 400 mM HFIP prepared in H<sub>2</sub>O, pH 8.0

Mobile phase B: 15 mM TEA, 400 mM HFIP prepared in MeOH

*\*mobile phases prepared gravimetrically*

#### Gradient table: (polyT standards)

Time	Flow (mL/min)	%A	%B	%C	%D
Initial	0.200	81.0	19.0	0	0
15.00	0.200	73.5	26.5	0	0
16.00	0.200	50.0	50.0	0	0
17.00	0.200	81.0	19.0	0	0
21.00	0.200	81.0	19.0	0	0

#### Gradient table: (high-throughput screening)

Time	Flow (mL/min)	%A	%B	%C	%D
Initial	0.200	82.0	18.0	0	0
4.00	0.200	80.0	20.0	0	0
4.01	0.200	50.0	50.0	0	0
6.00	0.200	50.0	50.0	0	0
6.01	0.200	82.0	18.0	0	0
10.00	0.200	82.0	18.0	0	0

## Detector settings

Sample rate: 2 points/sec

Mass range: 410–1250 Da

Mode: ESI negative

Collection mode: continuum

Cone voltage: 20 V

Capillary voltage: 0.8 kV

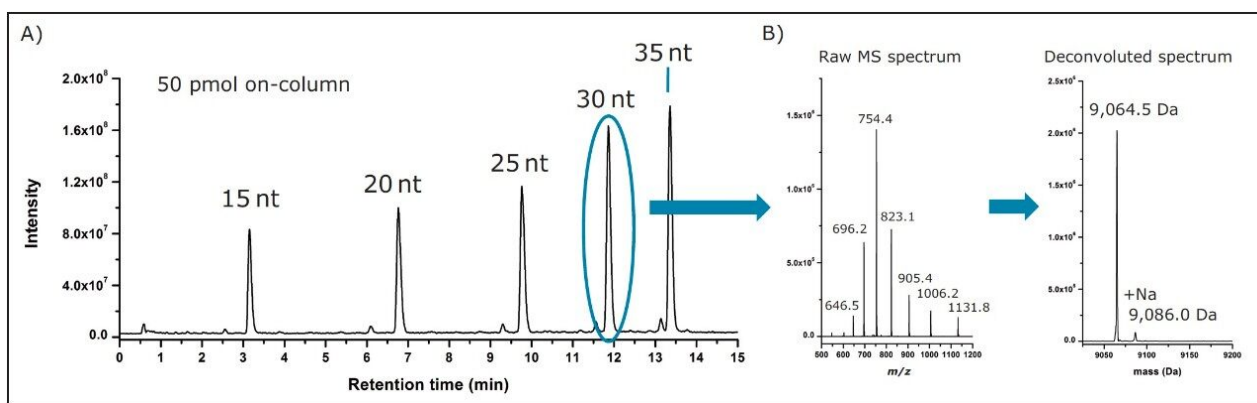
Probe temp.: 600 °C

## Data management

MassLynx SCN 9.25 with MaxEnt1

## Results and Discussion

Recently, it was demonstrated that the ACQUITY QDa Detector is capable of providing mass spectral data using traditional IP-RPLC methods, which incorporate ion pairing agents such as TEA buffered in HFIP, as shown in Figure 1A.<sup>9</sup> In the previous study, it was also shown that the ACQUITY QDa is capable of providing accurate mass information within its operating specifications. In addition, it was shown that MS spectra processed within MassLynx using the MaxEnt1 deconvolution algorithm provided analysts with straightforward data interpretation of complex spectra (Figure 1B).



*Figure 1. Oligonucleotide analysis with the ACQUITY QDa. A) An IP-RPLC separation of five polyT standards with the ACQUITY QDa in a serial configuration post-UV detection. B) Deconvolution of the raw MS spectrum of the 30 nt standard resulted in a parent peak mass of 9,064.5 Da (+0.7 Da) and a minor sodium (Na<sup>+</sup>) adduct peak with a relative intensity less than 6%.*

As aforementioned, pharmaceutical companies engaged in oligonucleotide research often incorporate software such as ProMass that enable automated data analysis in a high-throughput manner for improved productivity. A natural extension of the previous study is to evaluate the compatibility of ProMass with average mass data acquired using the ACQUITY QDa Detector. As with the previous work, a one minute window centered across the peak apex of the polyT standards was used to combine the MS spectrum data acquired by the ACQUITY QDa, as shown in the left panel of Figure 2. ProMass, which uses the ZNova deconvolution algorithm, offers a panel of deconvolution parameters for method flexibility in the deconvolution of components/peaks in the mass chromatograms, as shown in the right panel of Figure 2. For this experiment, the default settings used were: Peak Width=3, Merge Width=0.2, Minimum Score=2, and Normalize Scores=1. Spectrum for each polyT standard was copied to the operating system clipboard and

processed using the manual processing feature of ProMass, as illustrated in Figure 2.

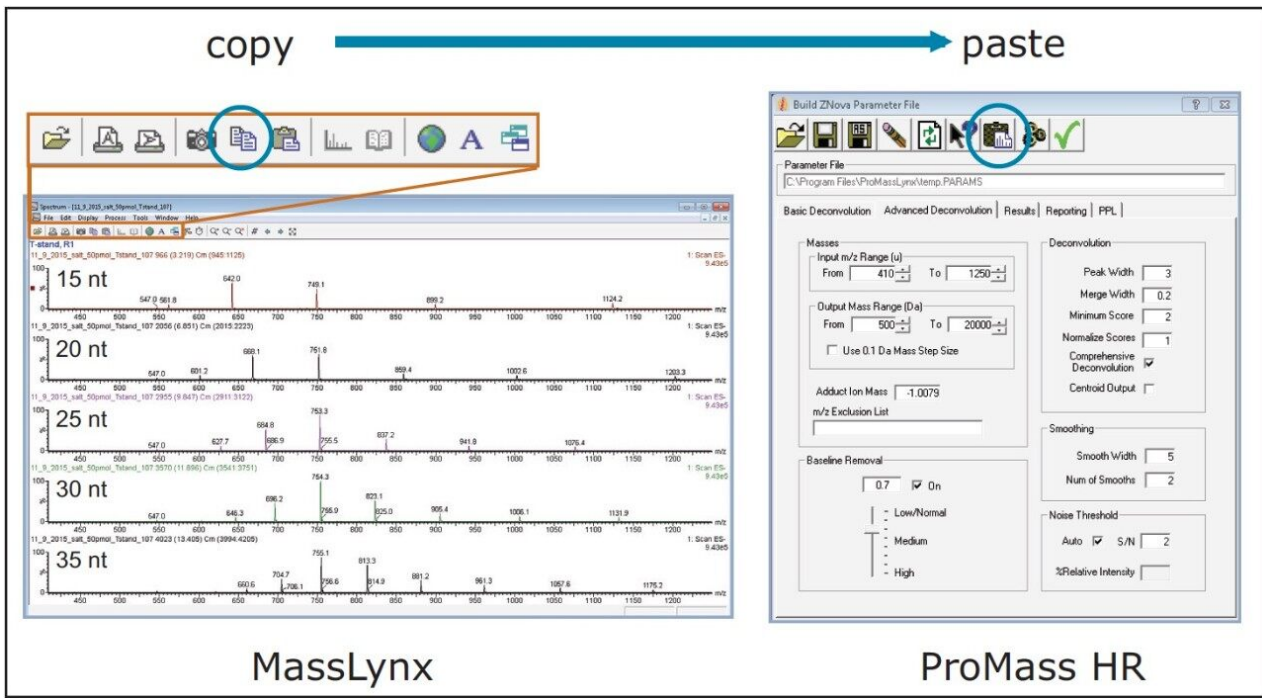
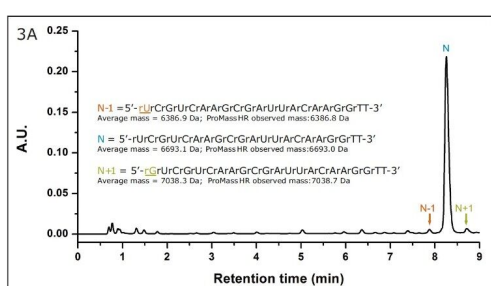


Figure 2. Data compatibility with ProMass HR and ProMass HR compatibility with the ACQUITY QDa. A one minute window of combined spectra acquired with the ACQUITY QDa data was evaluated with the manual processing feature (copy/paste) of ProMass HR using the default deconvolution settings.

The deconvolution results were observed to be highly reproducible across a technical triplicate with mass accuracy observed between -0.1 Da to +0.5 Da across the polyT standards (Table 1). To further evaluate the mass accuracy of ProMass with a sample more representative of one found in a therapeutic setting, a siRNA sample with a sequence length of 21 nucleotides – exhibiting a double thymine overhang – was separated using a high resolution separation gradient (Figure 3A). The pure oligonucleotide (N) was resolved from the base deletion (N-1) and base insertion (N+1) forms in under ten minutes. Using a one minute integration window from 7.7 minutes to 8.8 minutes, ProMass successfully identified the deletion and insertion as shown in the sequence ladder summary report (Figure 3B). Similar to the polyT analysis, spectrum acquired with the ACQUITY QDa was combined from 7.5 to 9.5 minutes for deconvolution. Mass accuracy of the associated spectrum was observed to range between +0.1 Da to +0.4 Da. This demonstrates that ProMass is compatible with mass spectral data acquired with the ACQUITY QDa and is capable of providing adequate mass information for routine identification and purity assessments in the manufacturing process of synthetic oligonucleotides.

	N=3	15 nt	20 nt	25 nt	30 nt	35 nt
Expected		4500.9	6021.9	7542.9	9063.8	10584.8
Observed average		4500.8	6022.0	7543.3	9063.8	10585.3
$\Delta$ mass		-0.1	0.1	0.4	0.0	0.5

Table 1. ProMass HR deconvolution mass accuracy. Average mass accuracy was observed from -0.1 Da to +0.5 Da for a technical triplicate of the polyT standards based on nominal mass-to-charge ratio data acquired with the ACQUITY QDa Detector.



Target Mass Summary									
RT (min)	Target Mass (Da)	Observed Mass (Da)	Mass Error	Intensity	% Abundance (In Spectrum)	No. Hits (Estimate)	Identity	Search Code	
8.25	6693.1	6693.2	0.1 Da (0.001 %)	2,208+008	97.99	95.07	Target Peak		
Sequence Ladder Summary									
RT (min)	Calculated Mass (Da)	Observed Mass (Da)	Mass Error	Intensity					
7.88	6386.9	6385.2	-1.9 Da (-0.016 %)	3,576+006			6693.1 (Minus A0)		
8.25	6693.1	6693.2	0.2 Da (0.001 %)	2,208+008			6693.1		
8.71	7038.3	7037.5	-0.8 Da (-0.006 %)	2,896+006			6693.1 (Plus C0)		
Chromatogram Summary									
RT (min)	Base Peak Mass (Da)	Intensity	Spectral Quality	LC/MS Peak Area	LC/MS Area Percent				
7.88	6386.9	3,576+006	sk	2,676+005	0.26				
8.25	6693.2	2,208+008	sk	1,786+007	96.97				
8.71	7037.6	2,896+006	sk	4,286+008	2.28				

Figure 3. High-resolution siRNA chromatogram. A) A siRNA sequence 5' UCGUCAAGCGAUUACAAGGTT-3' with a double thymine overhang was separated from the base deletion (N-1) and base insertion (N+1) forms using a ten minute, high resolution separation gradient from 13% B to 21% B and B) analyzed using ProMass.

With the performance confirmed of ProMass utilizing mass spectral data acquired with the ACQUITY QDa, the ability to process data in a high-throughput manner was then evaluated. The software package provides a formatted MassLynx sample list, which is designed to allow UPLC-MS data to be processed immediately after data acquisition while the next sample in the queue is analyzed or batch processed offline post-acquisition in an automated fashion (Figure 4). The user provides the raw data, defines the target sequence, and provides details about the target product search (Target Info) – correct configuration of which is essential for successful data processing. Two columns for defining parameter files required by ProMass for processing the LC-MS chromatograms are also provided in the sample list format and are defined by the



analyst as shown in Figure 4. The ProMassBridge Parameter File, which is part of the ProMassBridge application, facilitates a connection between MassLynx raw files and ProMass, and contains the user defined parameters (e.g. retention time range, smoothing and subtracting settings) for integration of the MS chromatograms. The ProMass ZNova Parameter File contains user-definable settings for MS data analysis such as the input and output masses, ion polarity, deconvolution settings, target mass, impurities tolerance thresholds, and options for the data report.

Sequence	Target Info	ProMassBridge Parameter File	ZNova Parameter File	Process
UrCrGrUICrArGrCrGrArUrUrArCrArArGrGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe

Queue	Spectrum	Chromatogram	Map	Edit	Sample	MS File	Inject Volume	Wt%	Sequence	Target Info	ProMassBridge Parameter File	ZNova Parameter File	Process
256	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.1	5.000	1.8.1	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
257	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.2	5.000	1.8.2	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
258	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.3	5.000	1.8.3	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
259	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.4	5.000	1.8.4	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
260	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.5	5.000	1.8.5	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
261	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.6	5.000	1.8.6	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
262	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.7	5.000	1.8.7	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
263	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.8	5.000	1.8.8	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
264	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.1	5.000	1.8.1	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
265	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.2	5.000	1.8.2	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
266	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.3	5.000	1.8.3	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
267	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.4	5.000	1.8.4	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
268	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.5	5.000	1.8.5	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
269	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.6	5.000	1.8.6	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
270	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.7	5.000	1.8.7	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
271	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.8	5.000	1.8.8	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
272	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.1	5.000	1.8.1	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
273	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.2	5.000	1.8.2	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
274	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.3	5.000	1.8.3	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
275	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.4	5.000	1.8.4	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
276	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.5	5.000	1.8.5	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
277	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.6	5.000	1.8.6	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
278	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.7	5.000	1.8.7	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
279	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.8	5.000	1.8.8	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
280	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.1	5.000	1.8.1	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
281	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.2	5.000	1.8.2	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
282	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.3	5.000	1.8.3	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
283	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.4	5.000	1.8.4	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
284	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.5	5.000	1.8.5	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
285	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.6	5.000	1.8.6	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
286	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.7	5.000	1.8.7	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
287	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.8	5.000	1.8.8	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
288	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.1	5.000	1.8.1	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe
289	10_31_2015_Upper	15 min Zpp	Olgo_15min_F	0D_u_15min_Zp	5.000	1.8.2	5.000	1.8.2	AMCGJCAAGGCGGALUACAAAGGTT	sequence=oligo ladder=5'	C:\Program File...\parameters.mlp	...\Highthroughput prom	C:\process_kernel.exe

**Start Sample List Run**

C:\MassLynx\ProMass.PRO

Pre-Run

Acquire Sample Data

Auto Process Samples

Auto Quantity Samples

Post-Run

Samples: From 256 To 303

QC Monitor:  Enabled

Scheduling:  Priority,  Night Time Process

User Processes... OK Cancel

Figure 4. MassLynx sample list format. A unique, formatted MassLynx sample list designed for UPLC-MS data to be processed following data acquisition or batch processed offline post-acquisition in an automated fashion.

The siRNA sample previously used (upper strand) and its complementary sequence (lower strand) were used to evaluate the batch processing capability of ProMass. A ten minute high-throughput separation method was used for the analysis (Figure 5). The ProMassBridge Parameter File was configured to integrate MS chromatograms between 2.0 and 3.5 minutes with lockspray functionality disabled (this function is used for HRMS data; e.g., QToF MS). All other parameters were left at their default settings. For high-throughput analysis mode, ProMass software has the ability to display the data summary in an interactive sample plate format or Excel sheet.

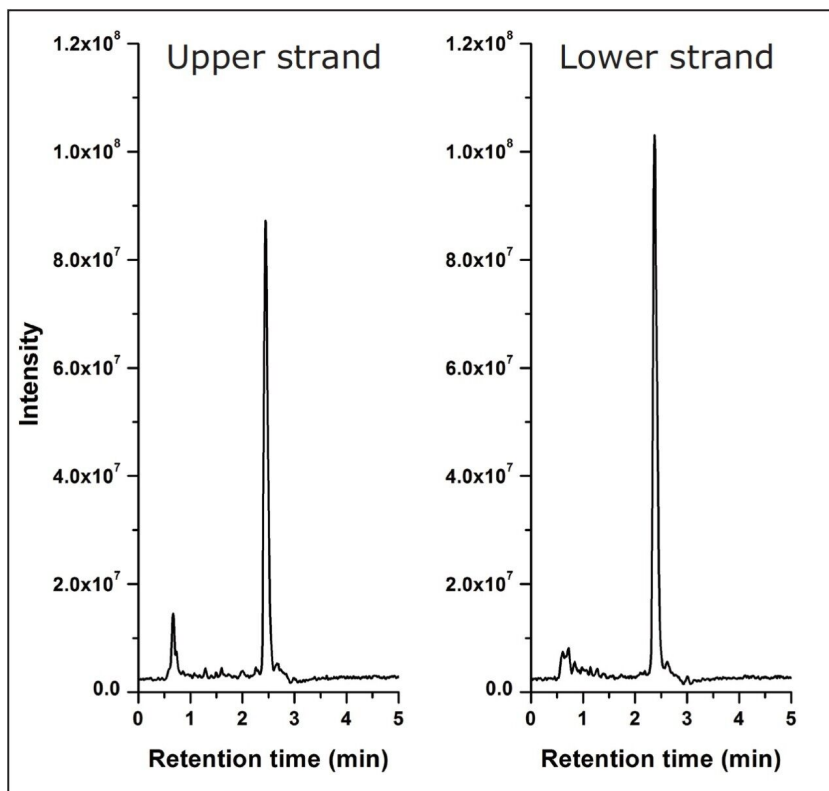


Figure 5. siRNA chromatogram. A siRNA sequence 5' UCGUCAAGCGAUUACAAGGTT-3' (upper strand) with a double thymine overhang and its complementary sequence 5'-TTCCUUGUAAUCGCUUGACGA 3' (lower strand) were separated using a ten minute high-throughput screening gradient.

To test the interactive sample plate format display option, upper siRNA samples were loaded in the standard 48-well plate format with every 7th vial position loaded with the lower siRNA complementary sequence as a negative control. As shown in Figure 4, samples from line 256 to line 303 were selected for batch processing with the upper siRNA sequence targeted for identification. After batch processing, the data is displayed in a color-coded, internet browser-based format (Figure 6). The colors, which represent the result of the targeted mass search, are defined in the legend of Figure 6. The plate format display is interactive; the analysis results can be displayed by selecting the actual vial position. Information such as a summary report of the analysis with target peak identification, spectral abundance, and peak purity can be accessed in this manner along with the corresponding spectrum and chromatogram. Additional information includes deconvolution results, identified impurities, and details of associated spectra.

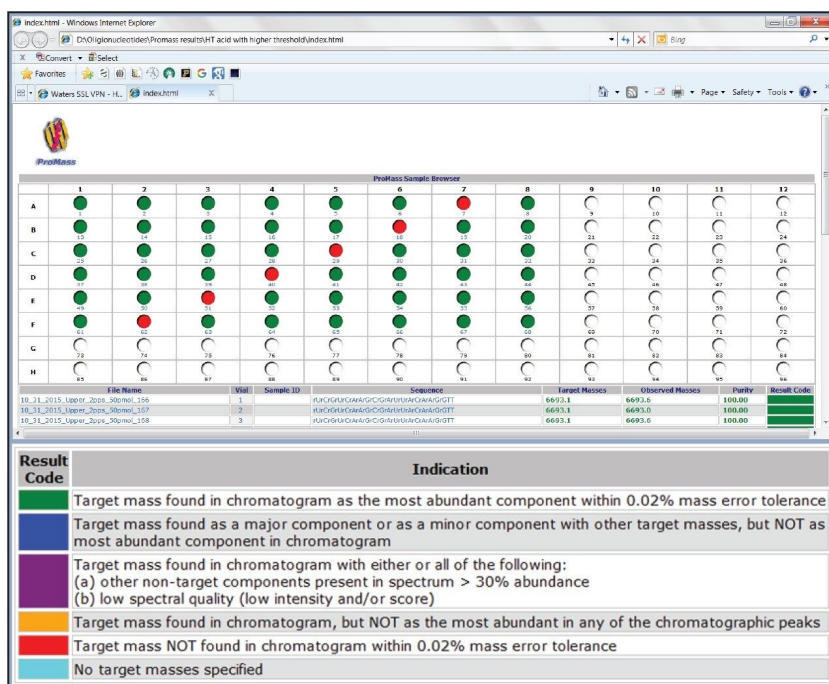


Figure 6. Interactive data summary. ProMass HR provides the ability to display data results in a color-coded, internet browser-based format for efficient analysis review.

## Conclusion

Mass information afforded by MS detection offers an efficient means of identifying challenging base modifications for improved productivity in synthetic therapeutic oligonucleotide workflows. Methods that can be rapidly deployed and automated in the assessment of product identity and purity in the production of synthetic oligonucleotides are highly desirable. In this study it was demonstrated the ProMass software can be adopted into an ACQUITY QDa based MassLynx project for confirmation of analyte mass and yield results consistent with the molecular weight of the target sequence within the default mass tolerance settings. Through the use of ProMass, assignment of target peaks, base modifications, and process impurities can be performed in a high-throughput automated fashion with significantly reduced analysis time for improved productivity. The interactive color-coded results in web browser-based format are user-friendly and enable straightforward data interpretation. This work demonstrates the ACQUITY QDa is an ideal addition to an analyst's lab for increased productivity and confidence of data analysis for routine identification and purity

assessments in the manufacturing process of synthetic oligonucleotides.

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## References

1. Agrawal S., Zhao Q. Antisense therapeutics. *Current Opinion in Chemical Biology*. 1998. 2:519–28.
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