ADDING COST EFFECTIVE MASS DETECTION FOR IMPROVED PRODUCTIVITY IN OLIGONUCLEOTIDE SCREENING ASSAYS

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INTRODUCTION

Research into therapeutic oligonucleotides has received steadily increasing attention from the pharmaceutical industry due to potential applications using deoxyribonucleic acid (DNA) sense/antisense oligonucleotides and interfering ribonucleic acid (RNAi) based therapies. 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 MS friendly reagents.

Mass information afforded by MS detection offers an efficient means of identifying challenging base modifications for improved productivity in synthetic therapeutic oligonucleotide workflows. In this study, we evaluate a cost-effective analytical strategy for the simultaneous acquisition of optical and MS based data for enhanced detection in a single workflow using the ACQUITY® QDa mass analyzer.

Figure 1. *The ACQUITY® QDa. The compact footprint of the ACQUITY® QDa allows for convenient integration into laboratories for improved productivity. The straightforward user interface combined with disposable source elements minimizes training and maintenance for daily operation.*

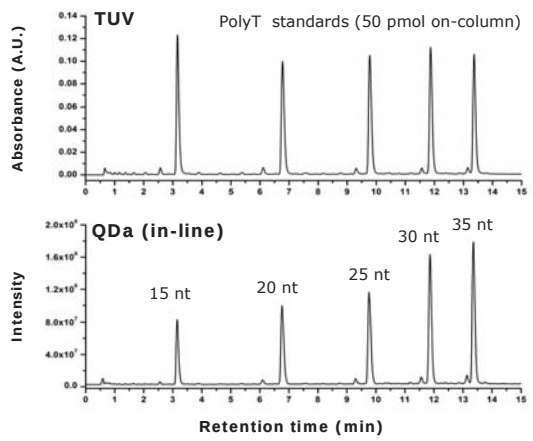


Figure 2. *In-line Orthogonal Detection. The ACQUITY® QDa combines straightforward mass spectral data with optical data for improving productivity and strengthening quality assurance in the analysis of synthetic oligonucleotides.*

METHODS

LC Conditions:
LC System: ACQUITY UPLC® H-Class
Detectors: ACQUITY UPLC® TUV, Ti flow cell
Absorption Wavelength: 260 nm
Column: OST BEH C18 130 Å 1.7, 2.1x50 mm
Column Temperature: 60 °C
Injection Volume: 5 µL (50 pmol mass load)

Mobile phase:
A: H_2O, 15mM TEA, 400 mM HFIP, pH 8.0
B: MeOH, 15mM TEA, 400 mM HFIP

ACQUITY® QDa Settings:
Sample rate: 2 points/sec
Mode: Negative
Mass range: 410 – 1250 Da.
Cone voltage: 20 V
Capillary voltage: 0.8 kV
Probe Temperature: 600 °C

Sample:
ssRNA upper strand (MW 6693.1 Da)
5'-UCGUCAAGCGAUUACAAGGTT-3'
ssRNA lower strand (MW 6607.0 Da)
5'-TTCCUUGUAAUCGCUUGACGA-3'

Gradient table: High Resolution (Poly T)

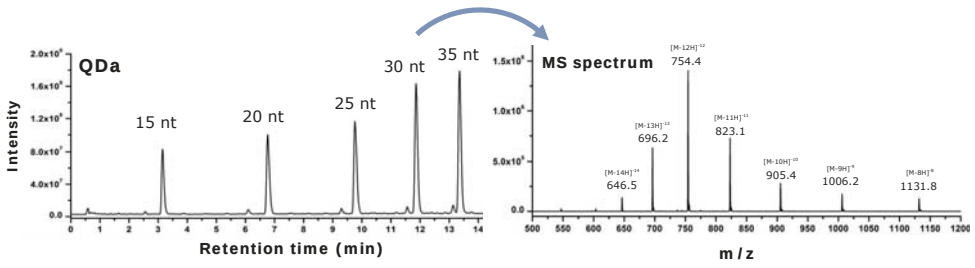
Time	Flow (mL/min)	% A	% B
Initial	0.200	81.0	19.0
15.00	0.200	73.5	26.5
16.00	0.200	50.0	50.0
17.00	0.200	81.0	19.0
21.00	0.200	81.0	19.0

Gradient table: High throughput (ssRNA)

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

RESULTS AND DISCUSSION

Improving Productivity of Oligonucleotide Screening Assays Using MS



green = observable charge state

nt	Avg. MW	[M-4H]$^{-4}$	[M-5H]$^{-5}$	[M-6H]$^{-6}$	[M-7H]$^{-7}$	[M-8H]$^{-8}$	[M-9H]$^{-9}$	[M-10H]$^{-10}$	[M-11H]$^{-11}$	[M-12H]$^{-12}$	[M-13H]$^{-13}$	[M-14H]$^{-14}$	[M-15H]$^{-15}$	[M-16H]$^{-16}$	[M-17H]$^{-17}$
15	4500.9	1124.2	899.2	749.1	642.0	561.6	499.1	449.1	408.2	374.1	345.2	320.5	299.1	280.3	263.8
20	6021.9	1504.5	1203.4	1002.6	859.3	751.7	668.1	601.2	546.4	500.8	462.2	429.1	400.5	375.4	353.2
25	7542.9	1884.7	1507.6	1256.1	1076.5	941.9	837.1	753.3	684.7	627.6	579.2	537.8	501.9	470.4	442.7
30	9063.8	2265.0	1811.8	1509.6	1293.8	1132.0	1006.1	905.4	823.0	754.3	696.2	646.4	603.2	565.5	532.2
35	10584.8	2645.2	2116.0	1763.1	1511.1	1322.1	1175.1	1057.5	961.2	881.1	813.2	755.0	704.6	660.5	621.6

Figure 3. *Oligonucleotide Detection with MS. The ACQUITY® QDa readily detects multiple charge states within its scan range when operating in a negative mode. Up to nine charge states* were observed for each of the oligonucleotide standards as shown in the table *affording analyst significant flexibility in method development of screening assays using the ACQUITY® QDa.*

Straightforward Data Interpretation

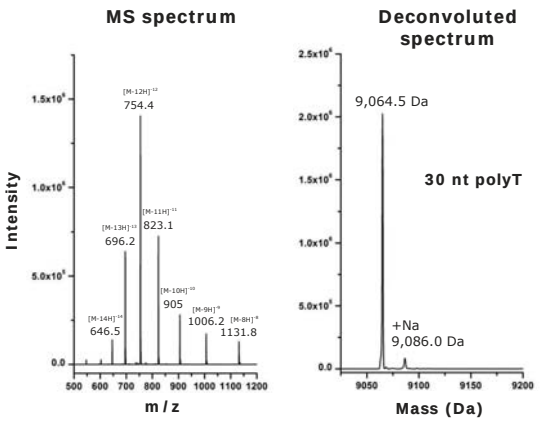


Figure 4. *Deconvolution with MassLynx. The chromatography data system MassLynx when used in conjunction with the ACQUITY® QDa provides the means to readily interpret mass spectra of increasing complexity. Oligonucleotide identity confirmation via zero charge state mass data is achieved in an efficient manner using the MaxEnt1 deconvolution algorithm for improved workflow productivity.*

ACQUITY® QDa Evaluation

charge state reproducibility

30 nt N=3	[M-8H]$^{-8}$	[M-9H]$^{-9}$	[M-10H]$^{-10}$	[M-11H]$^{-11}$	[M-12H]$^{-12}$	[M-13H]$^{-13}$	[M-14H]$^{-14}$	[M-15H]$^{-15}$
Expected	1132.0	1006.1	905.4	823.0	754.3	696.2	646.4	603.2
Observed average	1132.1	1006.1	905.4	823.1	754.3	696.2	646.5	603.4
S.D.	0.07	0.12	0.04	0.00	0.04	0.04	0.14	0.08
R.S.D	0.01	0.01	0.00	0.00	0.01	0.01	0.02	0.01

Table 2. *Charge State Reproducibility. The average m/z for the observed charge states for the 30nt sequence are within the instrument specification of ± 0.2 Da with a high degree of method repeatability for each charge state demonstrating the ACQUITY® QDa is capable of providing accurate results over multiple injections for oligonucleotide analyses.*

deconvolution mass accuracy

N=3	15 nt	20 nt	25nt	30 nt	35 nt
Expected	4500.9	6021.9	7542.9	9063.8	10584.8
Observed average	4500.9	6022.5	7543.5	9064.5	10585.5
Δ mass	0.0	0.6	0.6	0.7	0.7

Table 3. *Deconvolution Mass Accuracy. Mass accuracy was observed from +0.0 Da to +0.7 Da across the polyT standards demonstrating the ACQUITY® QDa is capable of providing adequate mass information in an efficient manner for screening assays in the assessment of synthetic oligonucleotides.*

Compatibility with ProMass HR

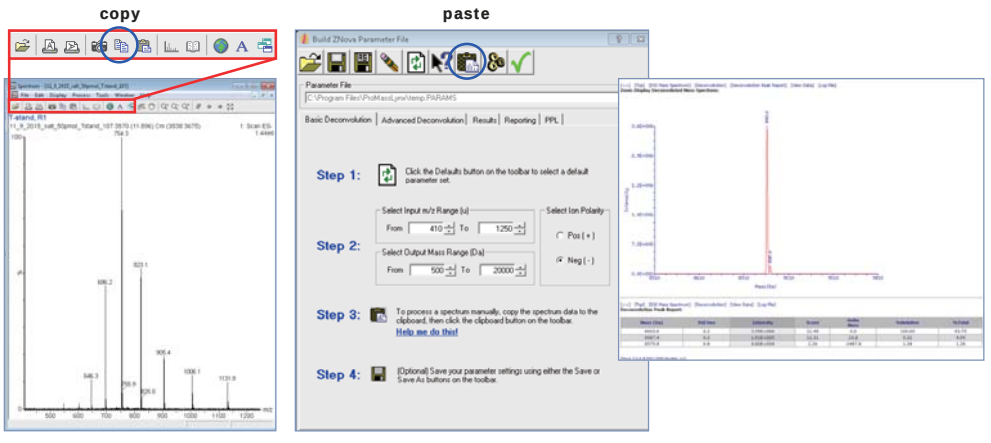
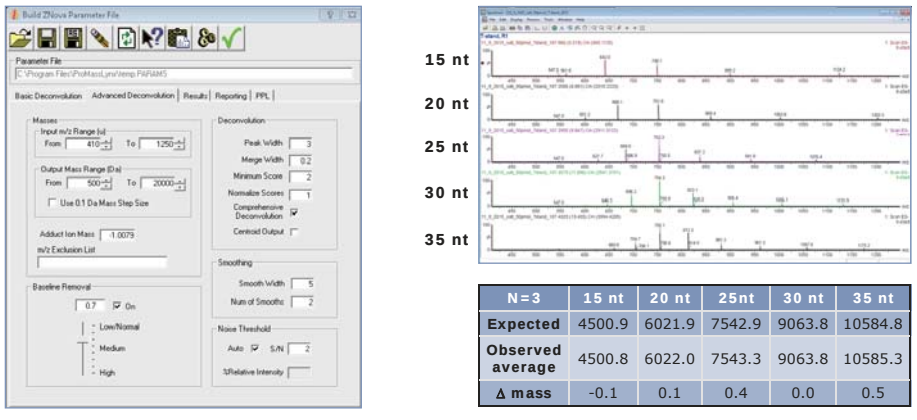


Figure 5. *Data Compatibility with ProMass HR. Pharmaceutical companies engaged in oligonucleotide research often investigate numerous potential biotherapeutic candidates at any given moment, requiring the use of high throughput processing for improved productivity. ProMass HR by Novatia for MassLynx Software offers the ability to process MS data acquired with the ACQUITY® QDa in individual (above) or batched workflows.*

ProMass HR Evaluation Using ACQUITY® QDa Data



N=3	15 nt	20 nt	25nt	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
Δ mass	-0.1	0.1	0.4	0.0	0.5

Figure 6. *ProMass Mass Accuracy using ACQUITY QDa Data. ProMass HR offers flexible method parameters in the deconvolution of components/peaks in the mass chromatograms using the ZNova deconvolution algorithm. A 1 min window of combined spectra was used to evaluate ProMass HR compatibility with ACQUITY® QDa data. Mass accuracy was observed from -0.1 Da to +0.5 Da across the polyT standards demonstrating ProMass HR is compatible with the ACQUITY® QDa and is capable of providing relatively accurate mass information based on nominal mass in the assessment of synthetic oligonucleotides.*

High Throughput Screening with ProMass HR

Interactive Results

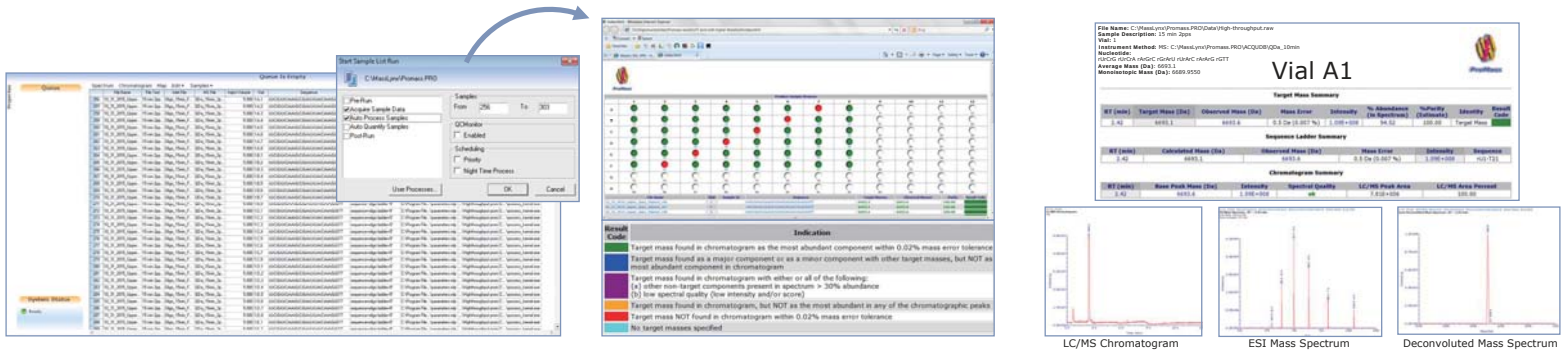


Figure 7. *High Throughput Screening with ProMass HR. The addition of complementary mass information in a single workflow afforded by the ACQUITY® QDa provides analysts an efficient means in the identification and assessment of purity in synthetic therapeutic oligonucleotide screening assays for improved productivity. When coupled to programs such as Promass HR by Novatia, mass information from the ACQUITY® QDa can be batch processed in a automated high-throughput manner for increased productivity and confidence in routine identification and purity assessments of synthetic oligonucleotides.* The interactively viewed color-coded results are user-friendly and offer straightforward data interpretation.

CONCLUSION

- Detect and monitor analytes over a wide molecular weight range
- Increase productivity and confidence in data analysis within existing assays
- Straightforward data interpretation with MaxEnt Deconvolution algorithm
- Compatibility with ProMass HR by Novatia
- Enable high throughput identity and purity screening with MS functionality

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