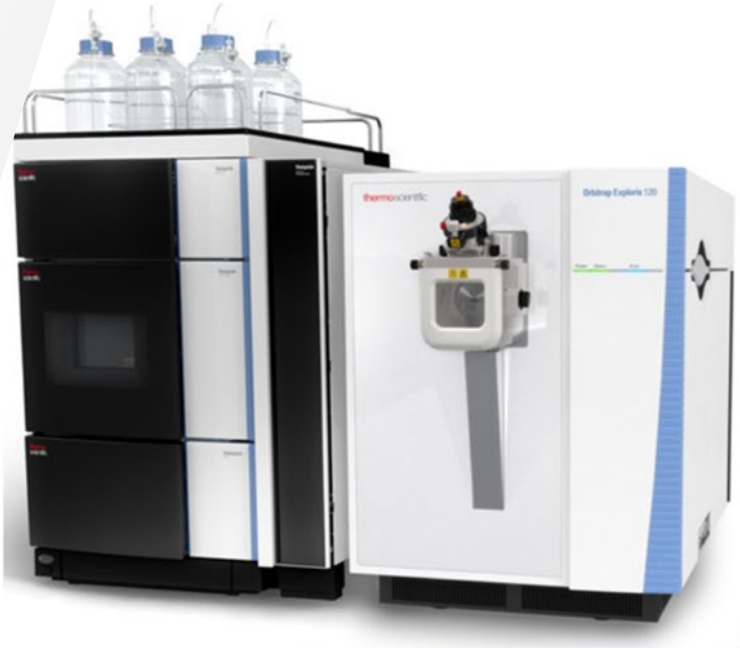
High throughput analysis of synthetic DNA using a compliant LC-MS based workflow



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Abstract

- The advances in high-speed chromatography, mass spectrometry, and software have been used in this study to develop high throughput workflows utilizing quantitation by mass spectrometry detection to match this growing requirement. In this abstract we present the analysis of synthetic oligonucleotides with respect to product identification, purity, and speed of analysis in a compliant environment.

Introduction

Primary Challenge

- Throughput limitations due to LC method overhead times can account for up to 50% of the total analysis time
- Additional software is often required for data analysis

Novel approach

- Rapid analysis of oligonucleotides and impurities using a dual UHPLC system coupled to a HRMS with automated sequence annotation in a compliant ready software.

Overview content Summary

- An oligonucleotide method confirming product identity in a rapid-fire configuration, impurity analysis and desalting using a fast gradient in a tandem column configuration, and high throughput sequencing with MS/MS enabled was developed.

Materials and Methods

Sample prep

- 5pmol of each oligonucleotide sample was analyzed using either a high throughput method or chromatographically separated to perform desalting and impurity analysis, using a tandem column workflow.

LC- Parameters

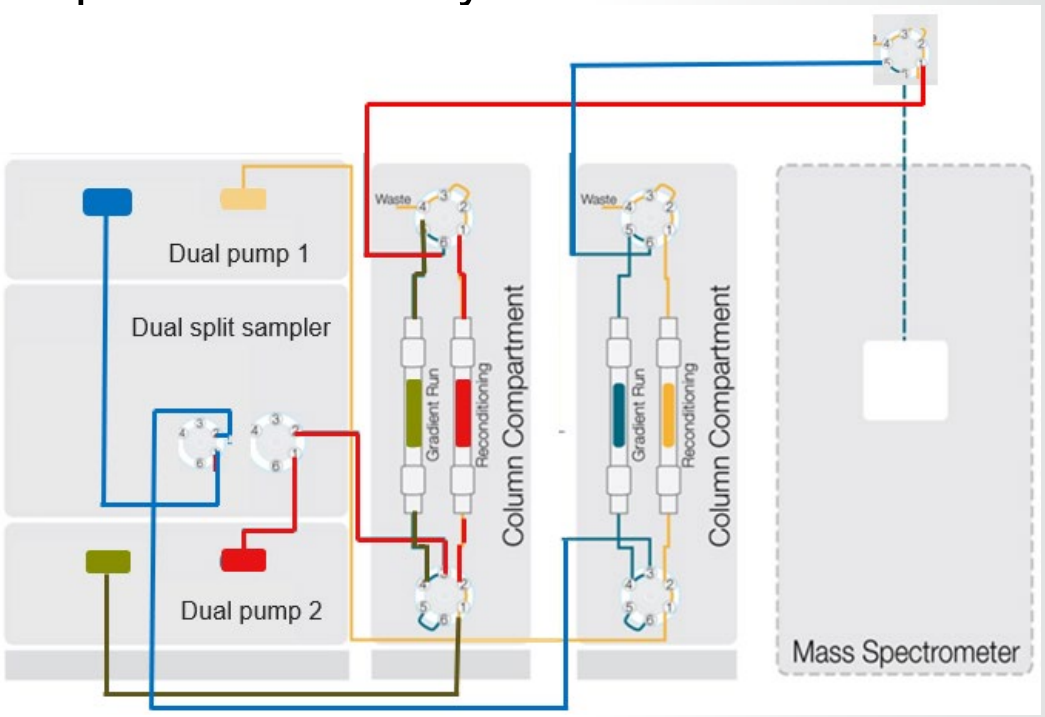
- The Oligonucleotide samples were separated on a Thermo Scientific™ DNAPac RP column using a Thermo Scientific™ Vanquish™ Duo UHPLC system. The columns were maintained at 60° C, with a flow rate of 400 µL/min.

MS and Data Analysis methods

- The eluting analytes were measured on the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer in negative ion mode. Data analyses and system control were performed with Thermo Scientific™ Chromeleon™ 7.2.10 CDS, and any additional sequence data analysis was performed with Thermo Scientific™ BioPharma Finder™ 4.1 software.

Results: standardization and productivity

Rapid fire LC-MS system



- Rapid injections of 30 sec
- Short gradients possible or isocratic rapid injections
- Charger system available for long unattended sequences

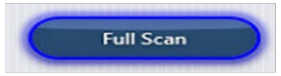
Maximizing productivity

- Dual tandem LC operation to re-equilibrate one set of columns while the other set is running the sample gradient

Results: standard methods and productivity

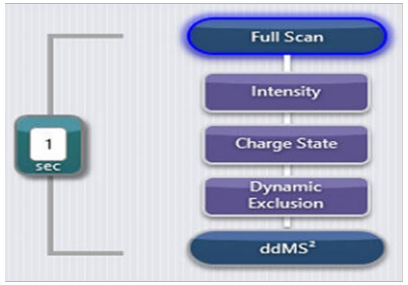
MS system parameters

Full Scan only



- Intact Protein Mode (Standard Pressure)
- Orbitrap Resolution: 120,000 [higher for 100mer]
- Polarity: Negative
- UV: 260 nm
- Microscan 3 to 5

ddMS²



- Peptide Mode
- Orbitrap Resolution (at m/z 200): 120,000 (MS1), 30,000 (MS2)
- Polarity: Negative
- Stepped Normalized Collision Energy: 10-12-14 to 20-22-24 (18-20-22 for impurity analysis)

- High resolution MS and MS/MS

Maximizing productivity

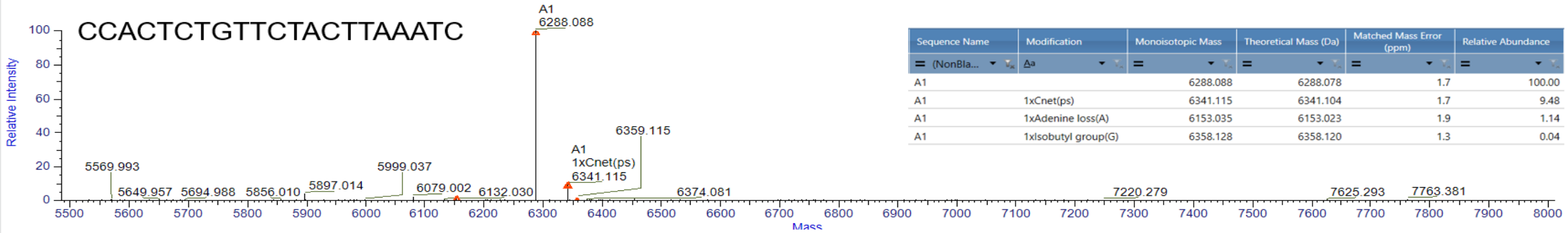
- The MS only method was used for the rapid analysis of oligonucleotides with intact deconvolution.
- The ddMS2 method was used for sequence analysis of oligonucleotides and was used on QE Plus, orbitrap Exploris 120 and 240
- Enables the identification and relative quantification of oligonucleotides and their impurities, even those present at very low levels, in a single experiment.
- Easy to do with Chromeleon software for compliant intact analysis and BioPharma Finder for automated annotation and identification with sequence analysis

Results: precision with accurate identification in BioPharma Finder software

ThermoFisher SCIENTIFIC

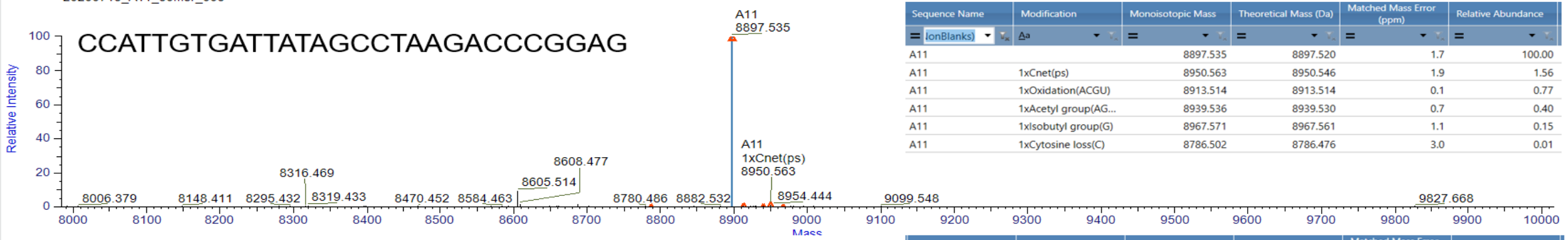
Quality of results at 120,000 resolution while maintaining sensitivity to see low abundant species

20200713_A1_21mer_0_1µL_LowP



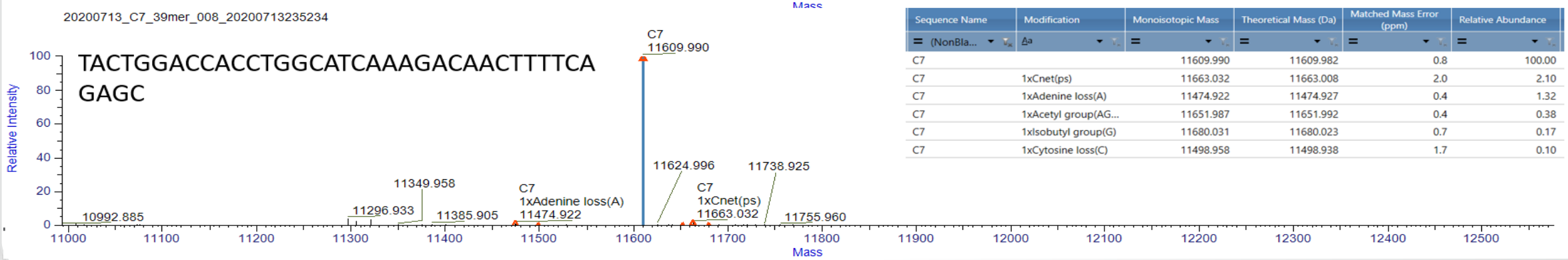
Sequence Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Relative Abundance
A1		6288.088	6288.078	1.7	100.00
A1	1xCnet(ps)	6341.115	6341.104	1.7	9.48
A1	1xAdenine loss(A)	6153.035	6153.023	1.9	1.14
A1	1xIsobutyl group(G)	6358.128	6358.120	1.3	0.04

20200713_A11_30mer_005



Sequence Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Relative Abundance
A11		8897.535	8897.520	1.7	100.00
A11	1xCnet(ps)	8950.563	8950.546	1.9	1.56
A11	1xOxidation(ACGU)	8913.514	8913.514	0.1	0.77
A11	1xAcetyl group(AG...	8939.536	8939.530	0.7	0.40
A11	1xIsobutyl group(G)	8967.571	8967.561	1.1	0.15
A11	1xCytosine loss(C)	8786.502	8786.476	3.0	0.01

20200713_C7_39mer_008_20200713235234

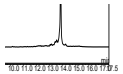


Sequence Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Relative Abundance
C7		11609.990	11609.982	0.8	100.00
C7	1xCnet(ps)	11663.032	11663.008	2.0	2.10
C7	1xAdenine loss(A)	11474.922	11474.927	0.4	1.32
C7	1xAcetyl group(AG...	11651.987	11651.992	0.4	0.38
C7	1xIsobutyl group(G)	11680.031	11680.023	0.7	0.17
C7	1xCytosine loss(C)	11498.958	11498.938	1.7	0.10

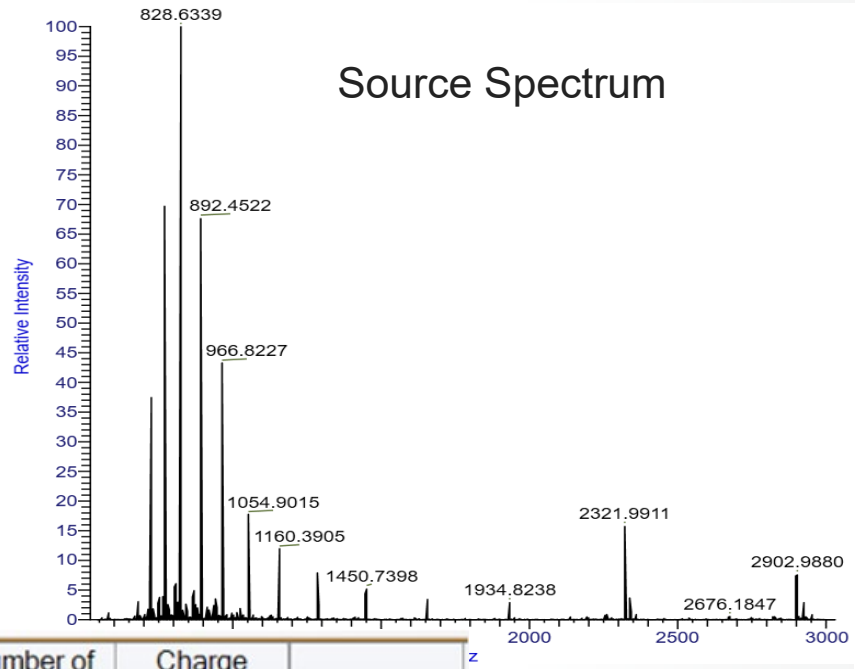
Results: precision with isotopic resolution in Chromeleon

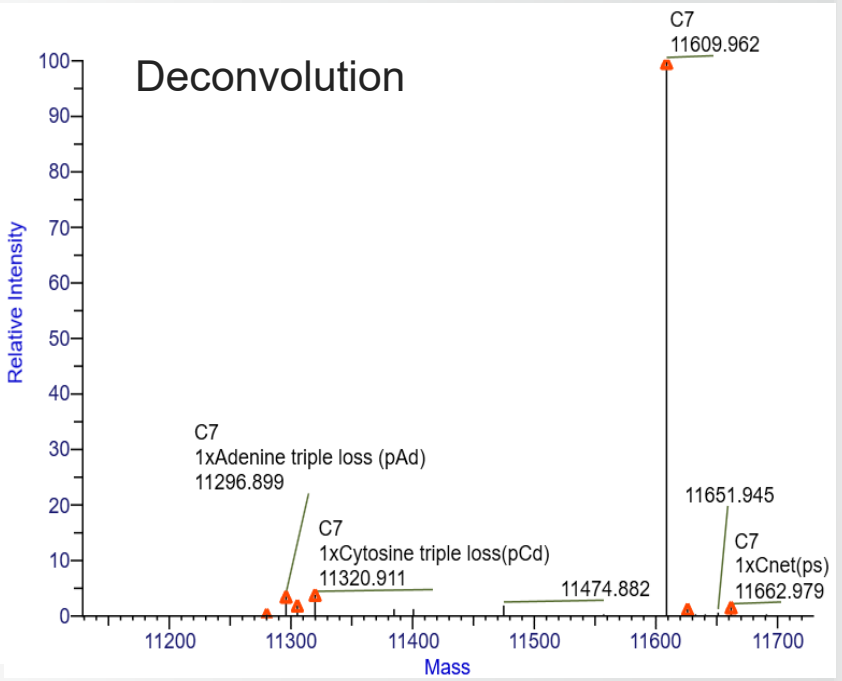
Quality of results at 120,000 resolution with UV and HRMS data collection in Chromeleon

TACTGGACCACCTGGCATCAAAGACAACTTTTCAGAGC 38mer



UV



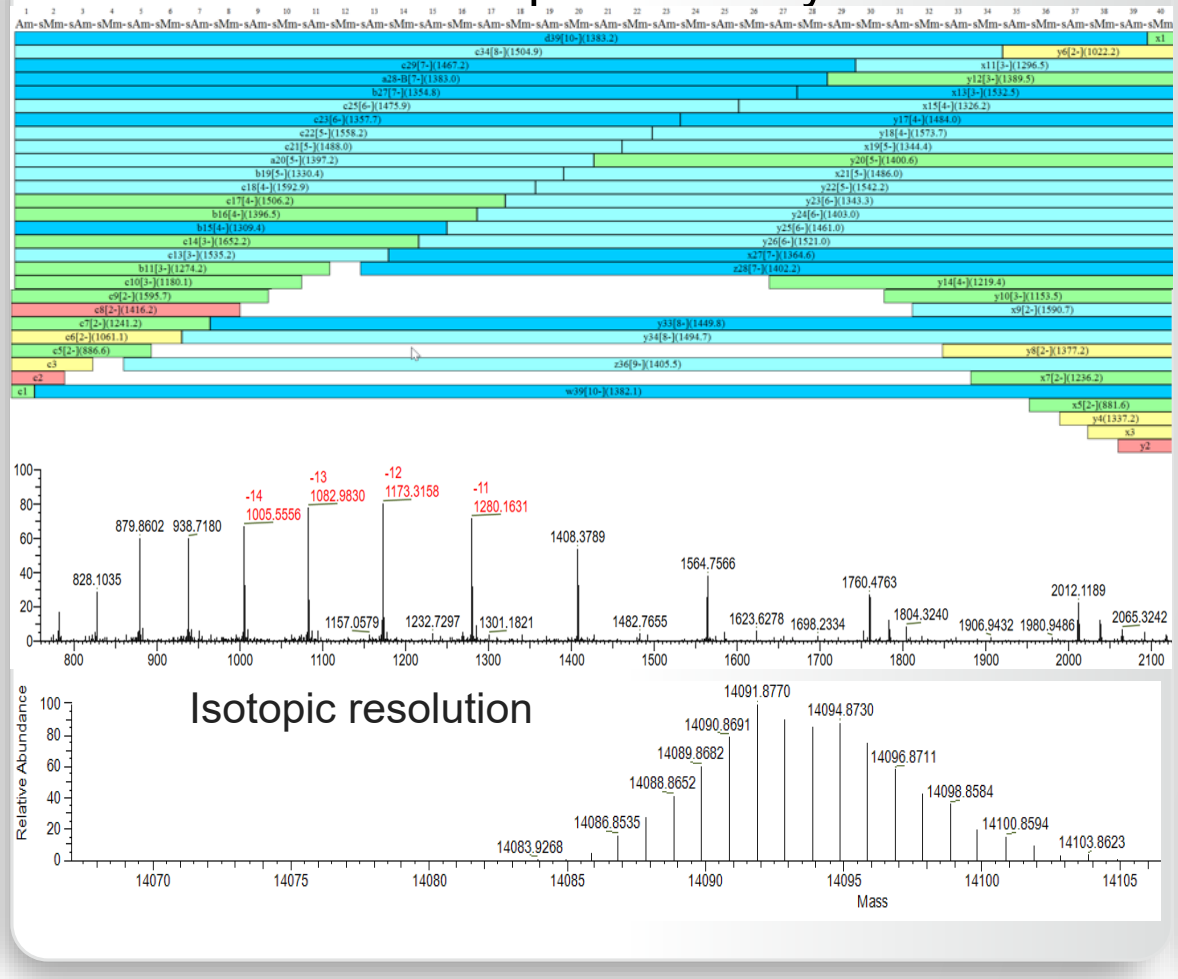


Result Component	Monoisotopic Mass	Relative Abundance	Number of Charge States	Charge State Distribution	Delta Mass
FLP	11610	100.00	14	4 - 17	0
Cytosine loss	11321	3.70	8	5 - 16	-289
Adenine loss	11297	3.41	8	4 - 16	-313
Cnet	11663	2.07	10	4 - 16	53
Thymine loss	11306	1.71	6	5 - 16	-304
Oxidation	11626	1.07	5	12 - 16	16
Guanine loss	11281	0.26	3	13 - 15	-329

- HRMS and UV data from Chromeleon software
- Isotopic accurate mass with abundance at high sensitivity

Results: sequence analysis

40mer siRNA full sequence analysis



Reporting

- Target mass is compared with experimental mass to give a “pass/Fail” with Chromeleon in a compliant environment
- Can include fractional abundance
- Component identification
- UV and HRMS signal reported
- Oligonucleotide sequencing with automated annotation within BioPharma Finder software

Injection Details

No.	Injection Name	Expected mass	Target Mass is most abundant component	TargetAccuracy	Fractional Abundance	Result Component Count	Calculated Mass
		Da			%		Da
1	Sample D11	10802.0	Pass	5	73	5	10805.0
2	Sample F04	13839.9	Pass	5	52	9	13843.9
3	Sample H07	18394.9	Fail	5	28	19	18400.4

Conclusions

We developed high-throughput methods using the Vanquish Duo UHPLC system with DNAPac RP columns coupled with Orbitrap Exploris mass spectrometers. Rapid fire methods of 30 seconds are possible or tandem gradients for desalting and impurity separations. Using high resolution Orbitrap mass spectrometry methods, isotopic resolution can be achieved while maintaining the sensitivity required to confidently identify low abundant species.

MS/MS fragmentation methods allow sequence confirmation of oligonucleotides up to 100nt with automated annotation within BioPharma Finder software.

Rapid oligonucleotide primer QC testing is possible in a compliant environment using Chromeleon software for instrument control of the dual UHPLC and HRMS Orbitraps. Oligonucleotide deconvolution and “Pass/Fail” reporting within the same compliant software package.

TRADEMARKS/LICENSING