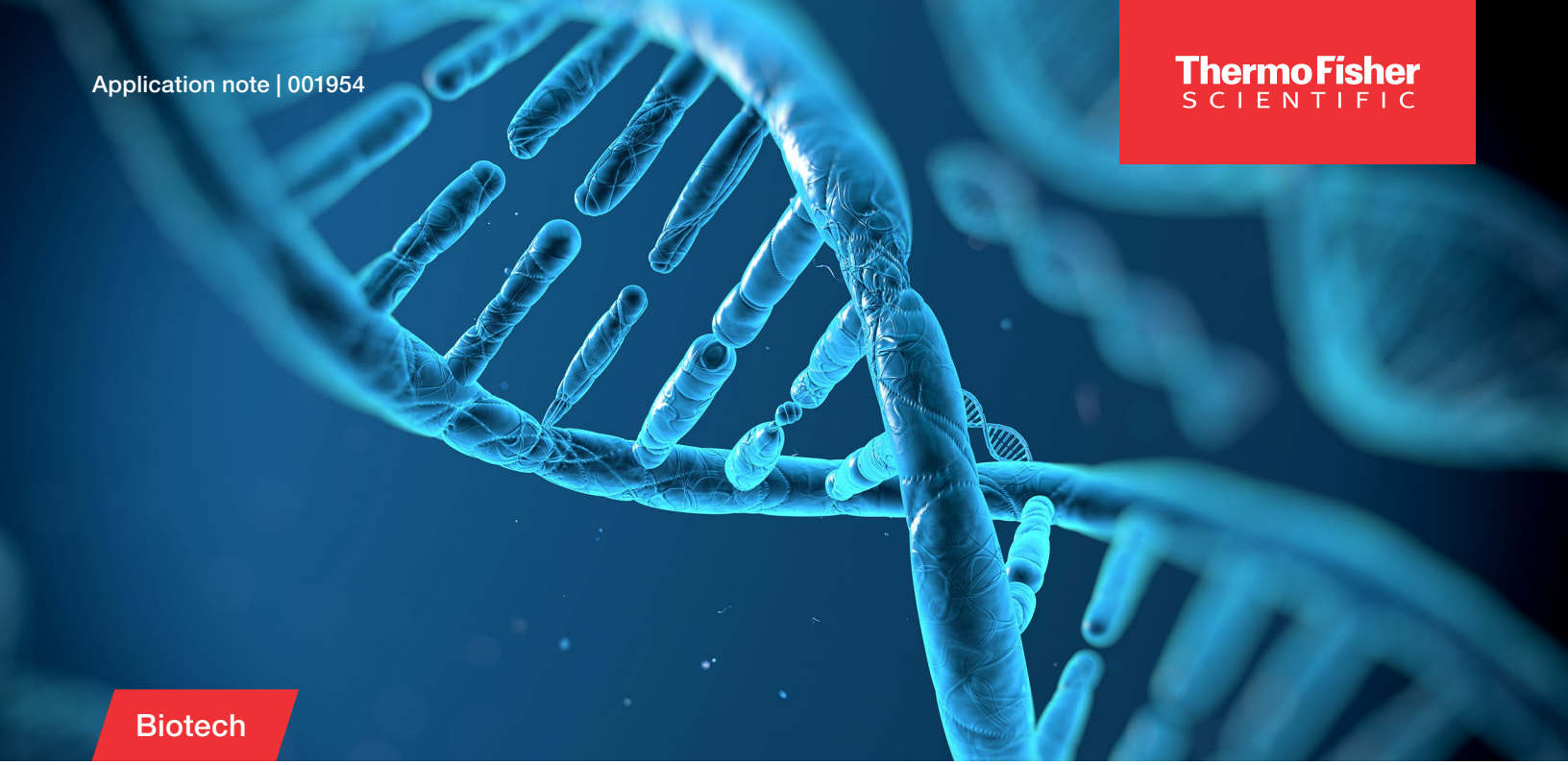

Application note | 001954

Biotech

An integrated LC-MS system performance evaluation test for oligonucleotide analysis

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Keywords

Oligonucleotide analysis, oligonucleotide standard, salt adduct, system performance evaluation test, intact mass determination, liquid chromatography high-resolution accurate-mass mass spectrometry (LC-HRAM-MS), ion-pairing reverse phase liquid chromatography (IPRP-LC), Vanquish Horizon UHPLC, Vanquish Flex UHPLC, Orbitrap Exploris 240 mass spectrometer, Orbitrap Exploris MX mass detector, DNAPac reverse phase column, Chromeleon CDS, eWorkflow procedure

Application benefits

- Effortless deployment of an integrated LC-MS system performance evaluation test, based on customized oligonucleotide standards, a set of instrument and processing methods, and a fit-for-purpose report template with pass/fail criteria
- Quick confirmation of oligonucleotide mass and evaluation of relative abundance of salt adducts using full MS data collected on both the Thermo Scientific™ Orbitrap Exploris™ 240 system and Thermo Scientific™ Orbitrap Exploris™ MX system by using a Thermo Scientific™ Chromeleon™ CDS eWorkflow™ procedure
- Automated data acquisition, processing, and reporting under compliance-ready and enterprise-deployable software to satisfy the needs of regulated environments

Goal

- Develop a robust IPRP-LC-MS method for highly reproducible separation and detection of oligonucleotides with lengths ranging from 10 to 55mer using the Thermo™ Scientific™ DNAPac™ RP column on a Thermo Scientific™ Vanquish™ UHPLC coupled with an Orbitrap Exploris 240 mass spectrometer and an Orbitrap Exploris MX mass detector
- Demonstrate the use of an easily deployable LC-MS system performance evaluation test for assessing the expected performance based on a customized oligonucleotide sample and defined LC and MS performance-related attributes that are relevant for oligonucleotide intact mass analysis

Introduction

Advances in synthetic biology have propelled the rapid growth of synthetic oligonucleotides with diverse lengths and modifications for novel therapeutic and diagnostic applications.[1] A comprehensive characterization of these oligonucleotides involving mass confirmation, purity assessment, and identification and quantification of impurities can be challenging. Currently, ion-pairing reverse phase liquid chromatography (IPRP-LC) coupled to mass spectrometery is by far the most widely used method for oligonucleotide analysis. Numerous published reports have shown the impact of the mobile phase composition on chromatographic and mass spectrometric behavior for the analysis of oligonucleotides.[2-4] Mobile phase containing a triethylamine (TEA) / 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) combination is the most applied for LC-MS analysis due to the fact that this combination improves both the chromatographic resolution and the detectability of the oligonucleotide.[4] Despite these advantages, inconsistent chromatography and reduced MS sensitivity over time are still present when using this combination due to the fact that both TEA and HFIP have relatively low boiling points.[5] Another well-known phenomenon in IPRP-LC-MS analysis of oligonucleotides is adduct formation as the phosphodiester backbone of the oligonucleotide has strong affinity for salts. The presence of salts in the flow path, mobile phase, or samples could influence the amount of salt adduct formation and thus affect the quality of the MS spectra. Hence, it is advised to flush the fluidic path with MS grade solvents and prepare fresh mobile phase with the highest purity of HFIP available to reduce salt adduct formations for oligonucleotide applications.

Given the complexity of this type of analysis, a system performance evaluation test is essential to ensure the overall instrument setup, including solvents, columns, and LC and MS instruments, is suitable for the intended purpose. To address this need, we developed an oligonucleotide system performance evaluation test (oligo SET) using a customized six-oligonucelotide mixture, which can be easily implemented across different LC-MS systems with streamlined data acquisition, processing, and reporting under compliance-ready software. This oligo SET was used to monitor LC-MS performance attributes that are relevant for oligonucleotideanalysis based on a comprehensive set of acceptance criteria. These attributes include retention time/peak area/peak width reproducibility, peak height range, mass accuracy of the full-length product (FLP), and % salt adducts. To demonstrate the applicability of this test for Orbitrap-based instruments, we executed this test on a Orbitrap Exploris 240 mass spectrometer and two Orbitrap Exploris MX mass detectors using a Chromeleon eWorkflow procedure, and the obtained results were consistent across three instruments.

This method can be easily adopted and modified for simultaneous oligonucleotide mass confirmation and purity assessment with similar oligonucleotide standard mixtures and implemented for quality control of synthetic oligonucleotides.

Experimental

Reagents and consumables

- Oligonucleotide standards, see Table 1 for sequence information (Life Technologies)
- 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), 99.9% (Acros Chemicals, AC293410500)
- Triethylamine (TEA), 99% (Acros Chemicals, 157911000)
- Water, UHPLC-MS grade, Thermo Scientific™ (P/N W8-1)
- Acetonitrile, UHPLC-MS grade, Thermo Scientific™ (P/N A956-1)
- Thermo Scientific™ DNAPac™ RP HPLC column, 2.1 × 50 mm, 4 µm (P/N 088924)
- Thermo Scientific™ 9 mm screw thread vials, polypropylene, 12 × 32 mm, 400 µL (P/N C4000-11)
- Thermo Scientific™ 9 mm autosampler vial screw thread caps, polypropylene (P/N C5000-50)

Oligonucleotide sample preparation

A 25 pmol/µL oligonucleotide standard solution containing six oligonucleotides ranging from 10mer to 55mer was prepared in UHPLC grade water. An injection sequence containing 10 replicate injections of the standard was created for oligo SET to evaluate the system performance related attributes that are relevant for oligonucleotide analysis.

Table 1. Oligonucleotide standard sequences and their theoretical monoisotopic mass

Oligonucleotide length	Sequence	Theoretical mass (Da)
10mer	GAG CGG CTG T	3082.5493
20mer	GAG CGG CTG TGA GCG GCT GT	6227.0543
30mer	GAG CGG CTG TGA GCG GCT GTG AGC GGC TGT	9371.5593
40mer	GAG CGG CTG TGA GCG GCT GTG AGC GGC TGT GAG CGG CTG T	12516.0643
50mer	GAG CGG CTG TGA GCG GCT GTG AGC GGC TGT GAG CGG CTG TGA GCG GCT GT	15660.5693
55mer	GAG CGG CTG TGA GCG GCT GTG AGC GGC TGT GAG CGG CTG TGA GCG GCT GTG AGC G	17249.8309

Chromatography

The Thermo Scientific™ Vanquish™ Horizon and Thermo Scientific™ Vanquish™ Flex UHPLC systems were used for the applied gradient detailed in Table 2. The modules included in the system are listed in Table 3. 1 µL or a total of 25 pmol of standards was injected onto the DNAPac column for oligo SET. The systems were set up to collect both UV and MS data in a single run. UV data were acquired at 260 nm with a sampling rate of 20 Hz.

Table 2. Chromatographic conditions

Parameter	Value
UHPLC column	DNAPac RP 2.1 x 50 mm, 4 µm
Flow rate	0.2 mL/min
Solvent A	20 mM TEA and 80 mM HFIP in water
Solvent B	20 mM TEA and 80 mM HFIP in 20% acetonitrile
Gradient	Time (min) %B 0 5 1 5 11 25 11.5 90 14 90 14.5 5 20 5
Injection volume	1 µL
Thermostatting mode	Still Air
Column oven temperature	50 °C

Table 3. Vanquish Horizon and Vanquish Flex UHPLC system modules and part numbers

Modules	Vanquish Flex (P/N)	Vanquish Horizon (P/N)
Vanquish System Base F/H	VF-S01-A-02	VF-S01-A-02
Vanquish Binary Pump F/H	VF-P10-A-01	VH-P10-A-01
Vanquish Split Sampler FT/HT	VF-A10-A-02	VH-A10-A-02
Vanquish Column Compartment H	VH-C10-A-03	VH-C10-A-03
Vanquish Variable Wavelength Detector F	VF-D40-A	VF-D40-A
Semi-micro Bio, 2.5 µL, 7 mm, 50 bar UV cell	6077.0300	6077.0300

Mass spectrometry

For all analyses, a full MS method was developed on the Orbitrap Exploris 240 mass spectrometer and executed on both the Orbitrap Exploris 240 mass spectrometer and the Orbitrap Exploris MX mass detector. Detailed instrument methods and source parameters for both MS systems are summarized in Table 4.

Table 4. Instrument method and ion source parameters for the Orbitrap Exploris 240 mass spectrometer and Orbitrap Exploris MX mass detector (Note: Unless otherwise indicated, default parameters were used.)

Instrument	Orbitrap Exploris 240 mass spectrometer	Orbitrap Exploris MX mass detector
MS source parameters		
Negative ion (V)	2,500	Same as Orbitrap Exploris 240
Sheath gas (Arb)	35	
Aux gas (Arb)	10	
Sweep gas (Arb)	0	
Ion transfer tube temperature (°C)	320	
Full scan parameters		
Expected LC peak width (s)	6	Same as Orbitrap Exploris 240
Resolution	120,000	
Scan range (*m/z*)	420–1,600	
Time range (min)	1–12 minutes	
AGC target	1E6	
Maximum injection time (ms)	50	
Sweep gas	1 a.u.	

Targeted MS processing using Chromeleon CDS 7.3.1

A targeted MS processing method was created and used to evaluate relevant LC-MS system performance attributes in the oligo SET. Similar to the previously described approach,[6] a list of detected charge states with 10 isotopes per charge state were included in the Chromeleon MS component table of the targeted MS processing method. RT of individual charge state was adjusted, and peak integration parameters were optimized to ensure accurate component detection and consistent peak integration across datasets. The associated charge states for the individual oligonucleotide sequence were grouped into a single component, and the peak area for that component was used for quantitation. The following settings were applied to the MS processing method:

- MS detection algorithm: ICIS
- Manually defined mass tolerance: 10 ppm
- Inhibit integration for TIC channel
- Gaussian smoothing: 5 points

Intact mass deconvolution using Chromeleon CDS 7.3.1

An intact mass deconvolution processing method with six different retention time windows was developed for intact mass analysis of oligonucleotide standards. For each oligonucleotide, a specific retention time range was selected to generate a source spectrum by averaging the spectra across that window. The averaged full MS spectrum was deconvoluted using the Xtract algorithm, or isotopically resolved, algorithm with the following settings:

- Output mass range from 1,000 to 20,000
- Signal to noise threshold set to 10
- Charge state range from -2 to -30
- Minimum number of detected charge states set to 3
- Isotope table set to nucleotide
- Select *true* for negative ion under advanced settings

Intensities of the deconvoluted mass were used to evaluate the fractional abundance of the salt adducts and FLP.

Oligo SET injection sequence

The oligo SET injection sequence contained 12 injections with one instrument method and seven processing methods (one for targeted MS processing only, and six intact mass deconvolution processing methods) as shown in Figure 1. Ten oligonucleotide standard injections were sandwiched by two blank injections that were used to pre-condition the columns and prepare them for storage, respectively. Two custom variables were created to evaluate the mass accuracy of the deconvoluted FLP. Custom variable "ExpectedMass" lists the theoretical masses that were calculated from the oligonucleotide sequences. And custom variable "TargetAccuracy" defines the expected mass tolerance for the measured monoisotopic mass and is set to 10 ppm. As shown in Figure 1, six oligonucleotide sequences were evaluated individually using specific intact mass deconvolution processing methods (e.g., processing method "Oligo SST – 10mer" for 10mer).

Oligo SET report

A report template was built for optimal presentation of the obtained results. For oligo SET, the critical system performance attributes and acceptance criteria were defined and applied as summarized in Table 5. The first six critical system performance attributes were evaluated using the targeted MS processing method, whereas the last two critical system performance attributes were evaluated using the intact mass deconvolution processing method.

Table 5. Oligonucleotide SET critical system performance attributes and acceptance criteria

System performance attributes	Acceptance criteria
Retention time reproducibility	Retention time %RSD ≤ 2%
Peak area reproducibility	Peak area %RSD ≤ 10%
Peak height range	Peak height range between 2E7 to 2E9 counts
Peak width at 10% height reproducibility	Peak width at 10% height %RSD ≤ 10%
Peak width at 10% height	Peak width at 10% height ≤ 0.5 min
Mass accuracy of deconvoluted FLP mass	Mass accuracy of deconvoluted FLP mass ≤ 10 ppm
% Na, K, and Fe adducts	% relative abundance of Na and K adducts ≤ 10%

The oligo SET is defined and reflected as "pass" when all measured critical system performance attributes for all monitored oligonucleotides meet the acceptance criteria. An example of obtained results representing the monitored LC-MS system performance attributes using the targeted MS processing method are shown in Figure 2, which includes a summary of the sequence details and acceptance criteria in the upper section, followed by a peak summary table listing the results for monitored attributes with a pass/fail status for every oligonucleotide.

Sequence Preview

#	Chromatogram	Name	Type	*ExpectedMass [Da]	*TargetAccuracy [ppm]	Level	Position	Volume [µl]	Instrument Method	Processing Method	Status
1	*None*	Blank	Blank	0.0000	0.0		R:A1	1.00	OligoSST_10-25B...		*Idle*
2	*None*	Oligo_SST_MSOnly_01	Unknown	0.0000	0.0		R:A1	1.00	OligoSST_10-25B...	Oligo SST	*Idle*
3	*None*	Oligo_SST_MSOnly_02	Unknown	0.0000	0.0		R:A1	1.00	OligoSST_10-25B...	Oligo SST	*Idle*
4	*None*	Oligo_SST_MSOnly_03	Unknown	0.0000	0.0		R:A1	1.00	OligoSST_10-25B...	Oligo SST	*Idle*
5	*None*	Oligo_SST_MSOnly_04	Unknown	0.0000	0.0		R:A1	1.00	OligoSST_10-25B...	Oligo SST	*Idle*
6	*None*	Oligo_SST_MSOnly_IPD_05	Unknown	3082.5493	10.0		R:A1	1.00	OligoSST_10-25B...	Oligo SST - 10mer	*Idle*
7	*None*	Oligo_SST_MSOnly_IPD_06	Unknown	6227.0543	10.0		R:A1	1.00	OligoSST_10-25B...	Oligo SST - 20mer	*Idle*
8	*None*	Oligo_SST_MSOnly_IPD_07	Unknown	9371.5593	10.0		R:A1	1.00	OligoSST_10-25B...	Oligo SST - 30mer	*Idle*
9	*None*	Oligo_SST_MSOnly_IPD_08	Unknown	12516.0643	10.0		R:A1	1.00	OligoSST_10-25B...	Oligo SST - 40mer	*Idle*
10	*None*	Oligo_SST_MSOnly_IPD_09	Unknown	15660.5693	10.0		R:A1	1.00	OligoSST_10-25B...	Oligo SST - 50mer	*Idle*
11	*None*	Oligo_SST_MSOnly_IPD_10	Unknown	17249.8309	10.0		R:A1	1.00	OligoSST_10-25B...	Oligo SST - 55mer	*Idle*
12	*None*	Blank	Blank	0.0000	0.0		R:A1	1.00	OligoSST_10-25B...		*Idle*

Figure 1. Overview of oligo SET injection sequence

LC-MS System Performance Metrics for Oligonucleotide Analysis

Sequence Details

Name:	Oligo_SET_SOM_OE240_withUV_13_Sep_202	*RT %CV*	< or =	*2%*	13/Sep/22 14:59:36	
Directory:	Oligos	*Peak Area %CV*	< or =	*10%*	Administrator	
Data Vault:	ChromeleonLocal	*Min Peak Height*	> or =	*2.00E+07*	19/May/22 22:24:47	
No. of Injections:	13	*Max Peak Height*	< or =	*2.00E+09*	Administrator	
		Max. Peak Width 10%	< or =	*0.5*		
		Peak Width 10% Heigh	< or =	*10%*		

Peak Summary

Oligonucleotide	Avg. RT (min)	%CV (RT)	%CV (Area)	Min. Peak Height (counts/min)	Max. Peak Height (counts/min)	%CV (Peak Width 10% Height)	Max. Peak Width 10% Height (min)	Pass or Fail
10mer	2.11	0.28%	0.70%	4.41E+07	4.73E+07	0.91%	0.21	Pass
20mer	5.06	0.19%	0.67%	6.85E+08	7.34E+08	1.15%	0.16	Pass
30mer	7.41	0.17%	0.80%	7.09E+08	7.80E+08	1.38%	0.20	Pass
40mer	9.44	0.08%	1.01%	9.41E+08	1.09E+09	1.71%	0.22	Pass
50mer	10.95	0.23%	0.93%	9.89E+08	1.09E+09	2.69%	0.23	Pass
55mer	11.42	0.18%	0.98%	7.53E+08	8.81E+08	2.92%	0.23	Pass

Figure 2. Example oligo SET report showing the sequence details and acceptance criteria and the peak summary table with obtained results for the defined critical system performance attributes

Chromeleon eWorkflow procedure

A Chromeleon eWorkflow procedure was created for oligo SET and executed on an Orbitrap Exploris 240 mass spectrometer and two Orbitrap Exploris MX mass detectors. This eWorkflow procedure bundles the instrument method, both targeted processing and intact mass deconvolution methods, the above injection sequence, a data processing view setting, and oligo SET report.

Software

Chromeleon CDS version 7.3.1 was used for all data acquisition, targeted MS processing, intact mass analysis, and reporting.

Results and discussion

The oligo SET was developed to evaluate relevant LC-MS performance attributes that are critical for oligonucleotide analysis. This test evaluates chromatographic reproducibility, mass accuracy, and relative abundance of salt adducts. The results are assessed against pre-defined acceptance criteria that are based on using the customized oligonucleotide sample. It can be easily adopted to assess the system performance metrics using other oligonucleotide standards. As shown in Table 5, this test is intended to determine whether the LC-MS system setups are deemed fit for intended oligonucleotide applications. A Chromeleon eWorkflow procedure was developed to facilitate seamless execution of the oligo SET on an Orbitrap Exploris 240 mass spectrometer and two Orbitrap Exploris MX mass detectors, and the obtained results are discussed in the following sections.

Chromatographic reproducibility

Mobile phases containing a TEA-HFIP combination have been widely employed for the IPRP-LC separation of oligonucleotides. This mobile phase not only provides predictable elution, it also drastically increases the ionization efficiency as HFIP is volatile and thus increases the evaporation rate of the droplet during the electrospray process. During the method optimization, we found the use of a combination of 20 mM TEA and 80 mM HFIP in water (Solvent A), and in 20% acetonitrile (Solvent B) resulted in good separation of the six oligonucleotides as well as high MS intensities for the analysis. As shown in Figure 3, the oligonucleotides elute in the order of increasing length and the injected amount. This method delivered robust chromatography across the entire oligo SET duration (~4.5 hours), as we consistently observed less than 0.4% retention time variability, less than 2% peak area variability, and less than 5% peak width at 10% height for the monitored oligonucleotides across 10 replicate injections as illustrated in Figure 3.

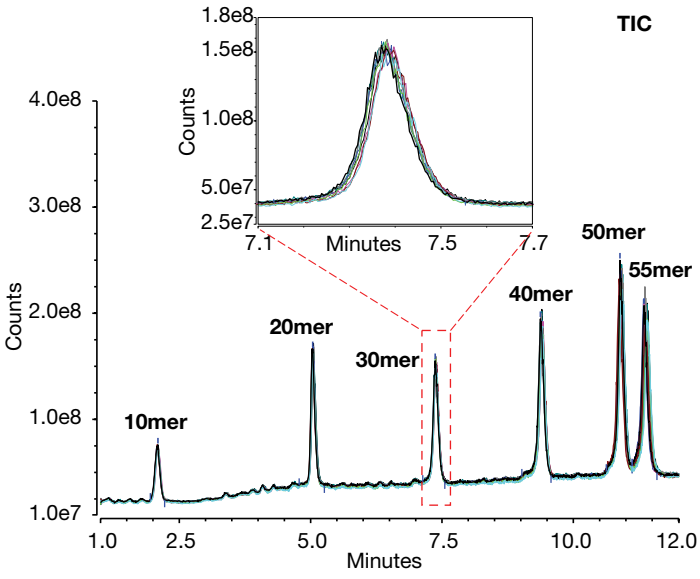


Figure 3. Separation of six oligonucleotides using the mobile phase containing 20 mM TEA and 80 mM HFIP. Shown here is the data collected from the Vanquish Horizon UHPLC coupled to an Orbitrap Exploris 240 mass spectrometer. Ten TIC traces are overlaid, and the inset shows the peak consistency for the analysis of 30mer.

Mass accuracy evaluation

The masses of the oligonucleotide standards were confirmed via intact mass deconvolution, and mass accuracy of the FLP was calculated as the mass difference (e.g., Δppm) between the measured and theoretical monoisotopic mass. With a resolution setting of 120,000 (@ *m/z* 200), the detected MS peaks were completely isotopically resolved, which enabled the determination of monoisotopic masses with typical mass accuracy of less than 2 ppm as shown in Figure 4. As illustrated in Figure 5a, a charge state profile ranging from -11 to -21, with baseline resolved isotopic pattern for each charge state, was observed for the analysis of the 55mer. We could even detect the monoisotopic peak, *m/z* 1013.6874, for charge state of -17 as shown in Figure 5b, which allowed for the determination of monoisotopic mass for the FLP with 0.9 ppm mass accuracy.

Deconvoluted Mass Identification

Sequence Details

Name:	Oligo_SET_SOM_OE240_withUV_13_Sep_2022 14_03	13/Sep/22 14:59:36
Directory:	Oligos	Thermo
Data Vault:	ChromeleonLocal	24/Oct/22 15:29:35
No. of Injections:	13	hao.yang

Deconvoluted mass overview

Component 1

Inj. No.	Oligonucleotide Name	Position	TargetAccuracy ppm	ExpectedMass Da	Component Identification	Measured Mass Da	Delta Mass ppm	Pass/Fail
6	)ligo_SET_MSOnly_IPD_0	R:A1	10.0	3082.5493	Full length product	3082.5454	1.3	Pass
7	)ligo_SET_MSOnly_IPD_0	R:A1	10.0	6227.0543	Full length product	6227.0467	1.2	Pass
8	)ligo_SET_MSOnly_IPD_0	R:A1	10.0	9371.5593	Full length product	9371.5500	1.0	Pass
9	)ligo_SET_MSOnly_IPD_0	R:A1	10.0	12516.0643	Full length product	12516.0519	1.0	Pass
10	)ligo_SET_MSOnly_IPD_0	R:A1	10.0	15660.5693	Full length product	15660.5596	0.6	Pass
11	)ligo_SET_MSOnly_IPD_1	R:A1	10.0	17249.8309	Full length product	17249.8152	0.9	Pass

Figure 4. Example report showing the summary of deonvoluted monoisotopic masses and the resulting mass accuracies for all six oligonucleotides. If the measured delta mass ppm is equal or less than the target mass accuracy set to 10 ppm, the identity of the FLP has been confirmed resulting in a pass status.

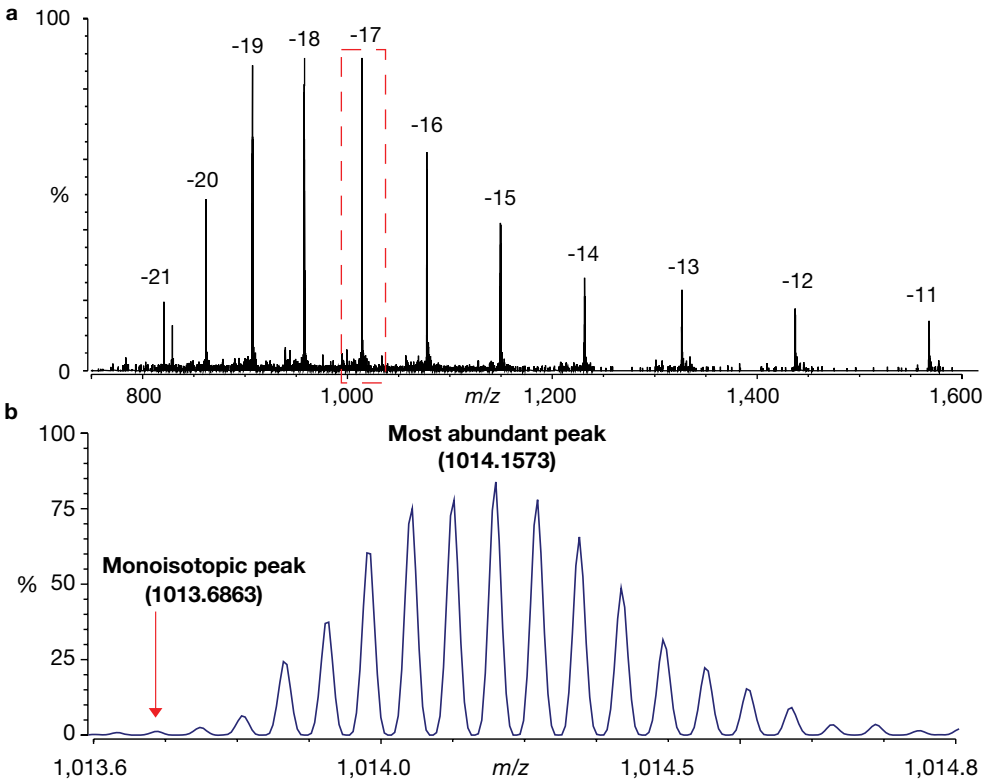
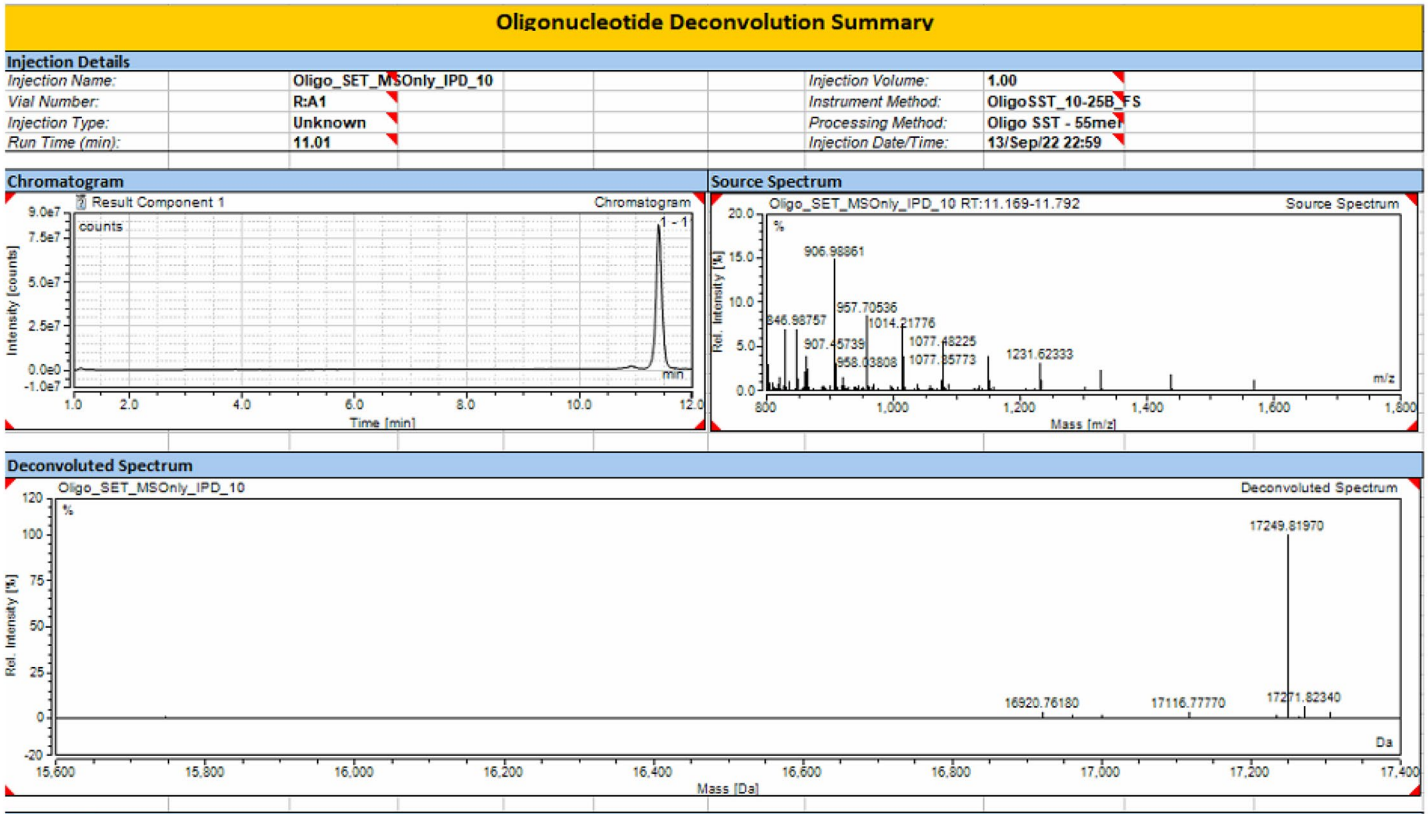


Figure 5. Example analysis of the 55mer using an Orbitrap Exploris 240 mass spectrometer at a resolution setting of 120,000. A charge state profile ranging from -11 to -21 was detected in the collected full MS spectrum taken at peak apex (a). For charge state -17, baseline resolution of the isotope pattern was observed (b).

Product purity and salt adducts evaluation

In addition to mass confirmation of the FLP, mass deconvolution allows for a quick evaluation of product purity, which is calculated as the sum of fractional abundance of FLP and salt adducts. Typically, the amount of salt adducts tends to increase with increasing length of oligonucleotides, and the presence of salts can affect MS sensitivity and impede accurate mass determination. It is imperative to monitor the salt content in the system and develop effective cleaning strategies to alleviate this inevitable reduction in performance. This oligo SET allows for quick assessment of salt adducts by checking the fractional abundance of these adducts and determines whether the system is sufficiently clean for oligonucleotide analysis. As shown in Figure 6, deconvolution results of the 55mer revealed about 5% Na adducts. In addition to salt adducts, other impurities were found based on the mass differences against the FLP and their abundance levels could be used for estimating the level of purity. The ability to simultaneously confirm the identity of FLP and estimate its purity is very useful for high throughput applications such as quality control of synthetic oligo primers.



Deconvolution Results

Result Component	RT min	Mass Da	Std. Dev. Da	Std. Dev. ppm	Delta Mass Da	Intensity counts	Fractional Abundance counts	Rel. Abundance %	Number of Charge States	Identification
Result Component 1	11.41	17249.820	0.00	0.00	0.000	920221	79.9969	100.00	11	full length product
Result Component 2	11.40	17271.823	0.00	0.00	22.004	60145	5.2285	6.54	8	Na adduct
Result Component 3	11.43	16920.762	0.00	0.00	-329.058	35342	3.0724	3.84	7	M-G
Result Component 4	11.42	17116.778	0.00	0.00	-133.042	33199	2.8861	3.61	6	G Depurination
Result Component 5	11.42	17306.751	0.00	0.00	56.932	32840	2.8549	3.57	6	na
Result Component 6	11.42	17233.816	0.00	0.00	-16.004	15574	1.3539	1.69	5	Sulphur loss
Result Component 7	11.44	17000.730	0.00	0.00	-249.090	14860	1.2918	1.61	5	na
Result Component 8	11.30	16960.765	0.00	0.00	-289.055	14642	1.2729	1.59	5	M-C
Result Component 9	11.46	15746.539	0.00	0.00	-1503.281	12363	1.0747	1.34	4	na
Result Component 10	11.42	17264.802	0.00	0.00	14.982	11135	0.9680	1.21	3	na

Figure 6. Example oligonucleotide deconvolution summary for the analysis of 55mer. The report shows the XIC of FLP, averaged source spectrum across the set retention time window, deconvoluted spectrum, and deconvolution result table showing the top 10 detected components and their identifications based on the measured delta mass against the FLP.

Since the oligo SET is based on a full MS method, it is applicable to all Orbitrap-based MS instruments. As a proof of principle, a Chromeleon eWorkflow procedure was developed to facilitate direct method transfer between the Orbitrap Exploris 240 mass spectrometer and the Orbitrap Exploris MX mass detector. The fractional abundance of Na adduct was evaluated using three instruments, and the results were compared. While the deconvolution results did not detect any K adducts, the amount of Na adduct increased with increasing length of the oligonucleotides. As shown in Figure 7, obtained results were consistent across all three LC-MS systems used in this study. The fractional abundance of Na adduct was well below 10%. These results serve as a baseline for the evaluated instruments; passing the test would reflect the required performance level of the instrument, and deem fit for oligonucleotide analysis.

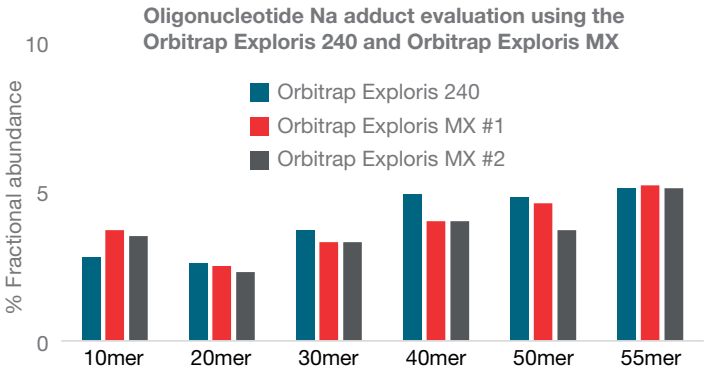


Figure 7. Cross comparison of fractional abundance of Na adduct using Orbitrap Exploris 240 and two Orbitrap Exploris MX instruments

Conclusions

We developed an integrated LC-MS system performance evaluation test using a mixture of six oligonucleotide standards, a DNAPac column, a Vanquish Flex or Vanquish Horizon UHPLC system coupled to an Orbitrap Exploris 240 or Orbitrap Exploris MX system, and Chromeleon CDS software for quick evaluation of critical LC-MS performance attributes that are relevant for oligonucleotide analysis.

This oligo SET contains the following:

- A comprehensive set of acceptance criteria, modifiable to meet the requirements of each project, to assess critical LC-MS performance attributes with a pass/fail status for the monitored oligonucleotides, and customizable using other standards
- A full MS method for simultaneous evaluation of oligonucleotide mass and amount of salt adducts via an intact mass deconvolution method
- A Chromeleon eWorkflow procedure that encompasses all instrument methods, processing methods, view setting, report template, and a pre-defined injection sequence for simplistic execution of oligo SET on Orbitrap Exploris 240 mass spectrometer and Orbitrap Exploris MX mass detector, which supports the generation of consistent results

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