SHIMADZU
Excellence in Science

Application News

Measurement of Oligonucleotide Impurities Using Shimadzu Nexera™ XS Inert Coupled To LCMS-9030 QTOF

Shannie Tay[1], Derrick Tan[1], Celine Sim[2]
1 Shimadzu Asia Pacific, Singapore 2 Nanyang Technological University, Singapore

User Benefits

- End-to-end workflow of oligonucleotide characterization using Shimadzu Nexera XS Inert, LCMS-9030 QTOF and LabSolutions Insight™ Biologics software.
- LabSolutions Insight Biologics software provides complete characterization of oligonucleotide main products and impurities.
- Keywords: Oligonucleotide, High-Resolution Mass Spectrometry (HRMS), LabSolutions Insight Biologics, Nexera XS Inert, LCMS-9030 QTOF, Shim-pack™ Scepter Claris, LCMS

■ Introduction

The biopharmaceutical industry is experiencing significant growth in the market for oligonucleotide therapeutics, such as antisense oligonucleotides and small interfering RNA (siRNA). To effectively characterize these oligonucleotides, High-Resolution Mass Spectrometry (HRMS) plays a crucial role in ensuring their quality, safety, and efficacy. Typically, impurities in oligonucleotides can arise from several factors, which include incomplete synthesis, degradation, or chemical modifications during the manufacturing process. Therefore, regulatory guidelines mandate the analysis of oligonucleotide impurities to evaluate product quality and ensure its compliance with regulatory standards.

This application note presents an end-to-end workflow for quantifying oligonucleotide impurities by spiking the oligonucleotides into a Full-Length Product (FLP). To ensure complete inertness of the sample flow path for optimal chromatographic separation, Nexera XS inert UHPLC system (Fig. 1) is used along with bioinert-coating metal-free Shim-pack Scepter™ Claris C18-300 column.

Fig. 1 Nexera™ XS Inert Coupled To LCMS-9030 QTOF

For HRMS analysis, LCMS-9030 QTOF (Fig. 1) and LabSolutions Insight Biologics software are used. The repeatability, intermediate precision, and accuracy data are collected following the guideline outlined in ICH Q2 (R2).

■ Experimental

Reagents and Chemicals

20-mer antisense oligonucleotides Tofersen and its 19-mer n-1 (5′) impurities were obtained from Integrated DNA Technologies (IDT). Torast-H Bio Vial (P/N: 370-04350-00) was obtained from Shimadzu. 1,1,1,3,3,3-Hexafluoropropan-2-ol (HFIP), Triethylamine (TEA) and Methanol (HPLC grade) were obtained from commercial suppliers.

Sample preparation

5% of n-1 (5′) impurities were spiked into FLP and diluted to a final concentration of 20 µM FLP using nuclease-free water. Samples were then transferred to a Shimadzu TORAST-H Bio Vial for injection and analysis by Shimadzu LCMS-9030 QTOF. The analytical conditions are shown in Table 1.

Table 1 Analytical conditions on LCMS-9030 QTOF

LC Conditions	
Column	Shim-pack Scepter™ Claris C18-300 2.1 × 100 mm, 1.9 µm (P/N: 227-31209-02)
Flow Rate	0.3 mL/min
Mobile Phase	A: 100 mM HFIP, 10mM TEA in Water B: 100 mM HFIP, 10mM TEA in Methanol
Elution mode	Gradient elution in 20 mins
Gradient Program	%B conc.: 0-1.0 min., 5%; 1.0-11.0 min.; 5% to 60%; 11-20 min., 5%
Oven Temp.	60 ˚C
Injection Vol.	2.5 µL

Interface Conditions (LCMS-9030)	
Interface	Heated ESI at 4.50 kV
Interface Temp.	350 °C
DL Temp.	25 °C
Heat Block Temp.	350 °C
Nebulizing Gas	2 L/min
Heating Gas Flow	10 L/min
Drying Gas Flow	10 L/min
Data acquisition (QTOF)	
MS Mode	DDA negative
TOF m/z range	700-2200 m/z

Data files were uploaded onto LabSolutions Insight Biologics software with the following target modification as shown in Table 3. Software will automatically calculate the theoretical mass after inputting the sequence as per Table 2.

Table 2 Oligonucleotide sequence and theoretical mass

20-mer FLP sequence	mCe-sAe-pGe-sGe-pAe-sTd-sAd-smCd-sAd-sTd-sTd-sTd-smCd-sTd-sAd-smCe-pAe-sGe-pmCe-sTe
Mono-isotopic Mass	7123.15891
Most Abundant Mass	7127.16499
Average Mass	7127.84848

Table 3 LabSolutions Insight Biologics Target Modification

Target Modifications	
Max Modifications	1
Short mers (n-x)	1
A Loss	☑
C Loss	☑
G Loss	☑
T Loss	☑

■ Results and Discussion

LabSolutions Insight Biologics software will automatically deconvolute the mass spectrum for the characterization of oligonucleotides and their impurities. The software displays the measured impurities with respect to the FLP area and component chromatography (Fig. 2 and 3). The software also displays sequence coverage based on the MS2 fragmentation spectra in either the fill mode or branch mode (Fig. 4 and 5).

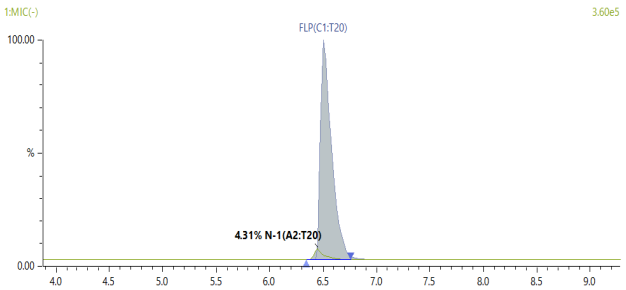

Fig. 2 Component Chromatogram of FLP and spiked n-1 (5') impurities

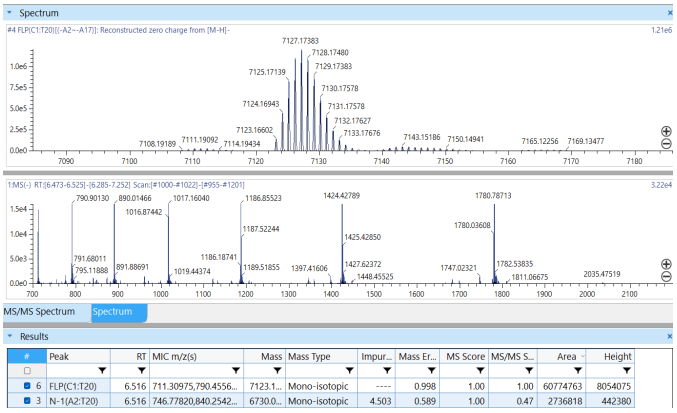

Fig. 3 FLP Mass Spectrum (Top) and Deconvoluted Mass Spectrum (Bottom). Impurities ratio is automatically calculated by LabSolutions Insight Biologics Software

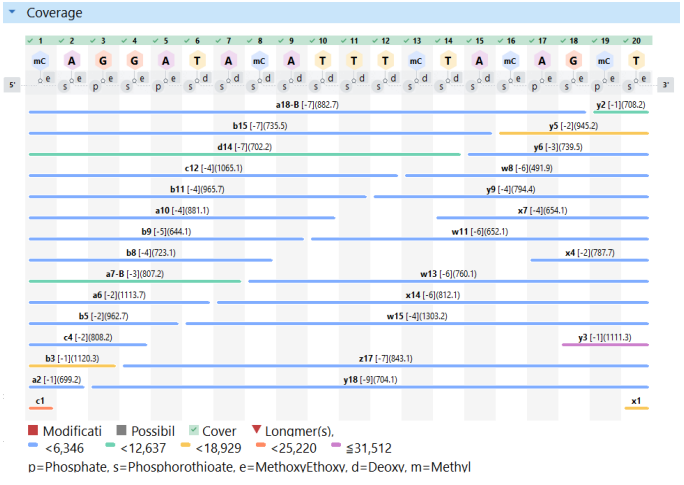

Fig. 4 Tofersen Sequence Coverage (Fill Mode)

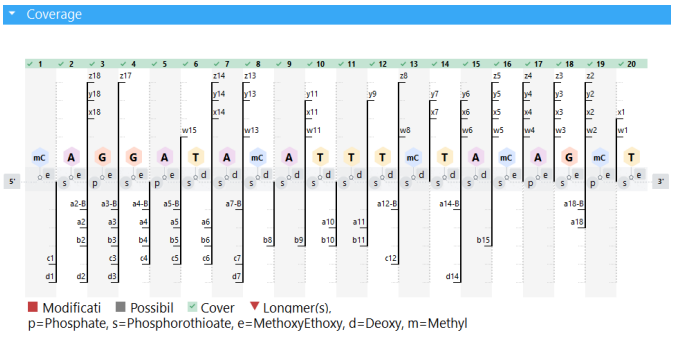

Fig. 5 Tofersen Sequence Coverage (Branch Mode)

Table 3 Repeatability, Intermediate Precision, and Accuracy data

Day 1	MS Score	Impurity ratio %	Recovery	Mean	Std deviation	Repeatability (%)
1	0.93	4.523	90.46%	4.573	0.040	0.865
2	0.98	4.618	92.36%			
3	0.92	4.555	91.10%			
4	0.91	4.539	90.78%			
5	0.95	4.570	91.40%			
6	1.00	4.631	92.62%			

Day 2	MS Score	Impurity ratio %	Recovery	Mean	Std deviation	Repeatability (%)
1	0.96	4.248	84.96%	4.291	0.051	1.183
2	0.97	4.220	84.40%			
3	1.00	4.362	87.24%			
4	0.99	4.262	85.24%			
5	0.99	4.336	86.72%			
6	0.94	4.317	86.34%			

Day 3	MS Score	Impurity ratio %	Recovery	Mean	Std deviation	Repeatability (%)
1	0.99	4.346	86.92%	4.336	0.040	0.922
2	0.99	4.379	87.58%			
3	0.93	4.394	87.88%			
4	0.99	4.287	85.74%			
5	0.91	4.312	86.24%			
6	1.00	4.300	86.00%			
					Intermediate Precision	0.990

6 sets of data were collected over 3 days to assess the repeatability, intermediate precision, and accuracy of the method. The recovery rate was within the acceptable range of 80% to 120%. Both repeatability and intermediate precision were found to be below 5%, with MS scores ranging from 0.91 to 1. These results adhere to the guideline outlined in ICH Q2 (R2).

■ Conclusion

The combination of Shimadzu Nexera XS Inert, Shim-pack Scepter Claris C18-300 column and LCMS-9030 QTOF mass spectrometer offers a reliable platform for analyzing oligonucleotide impurities. LabSolutions Insight Biologics software, a specialized software, is designed specifically for oligonucleotide characterization and impurity quantification, and it can ensure compliance with regulatory standards.

Furthermore, the data is collected according to ICH Q2 (R2) guidelines, which demonstrates that this is a thorough workflow that effectively meets regulatory requirements.

■ References

1. 01-00595-EN An Oligonucleotide Impurity Analysis Workflow Using LabSolutions Insight Biologics Software (shimadzu.com)
2. Current State of Oligonucleotide Characterization Using Liquid Chromatography–Mass Spectrometry: Insight into Critical Issues J. Michael Sutton, Guilherme J. Guimaraes, Vidya Annavarapu, William D. van Dongen, and Michael G. Bartlet. J. Am. Soc. Mass Spectrom. 2020, 31, 1775−1782

04-AD-0305-EN First Edition: Oct 2024

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU (Asia Pacific) Pte. Ltd,
www.shimadzu.com.sg

Please fill out the survey

Related Products Some products may be updated to newer models.

Nexera XS inert
Ultra High Performance Liquid Chromatograph

LCMS-9030
Quadrupole Time-of-Flight Liquid Chromatograph Mas···

Shim-pack Scepter LC Columns
HPLC Column

Related Solutions

Oligonucleotide and mRNA Therapeutics

Pharmaceutical and Biopharmaceutical

Price Inquiry

Product Inquiry

Technical Service / Support Inquiry

Other Inquiry