Full Characterization and Confirmation of Diverse Oligonucleotides by Ion-pairing Chromatography Coupled with the Q Exactive HF-X HRMS

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ABSTRACT

Oligonucleotides were characterized by ion-pairing chromatography using the Thermo Scientific™ Q Exactive™ HF-X mass spectrometer. Oligonucleotide sequences varying in length from 17 to 120 nucleotides were detected with excellent mass accuracy and reproducibility. Oligo Primers.

INTRODUCTION

Oligonucleotides are used in many molecular biology applications including gene therapy, antisense and small interfering RNA (siRNA) therapy for sequencing and amplification in polymerase chain reactions (PCR), to select complementary DNA and RNA strands via hybridization reactions, and in oligonucleotides designed to land specific molecular targets. Although these are seemingly simple entities comprised of combinations of only four different bases, the potential for numerous structural modifications and substitutions can make characterization of these diverse biopolymers complex. However, continuing advancements in LC-MS/MS technology provide increasingly sensitive and reliable methods that have facilitated improved qualitative and quantitative analysis.

MATERIALS AND METHODS

Sample Preparation

Oligonucleotide standards (17 to 120 nucleotides in length) were purchased from Integrated DNA Technologies and resuspended in 100 µL of a 0.1 M sodium cacodylate buffer supplemented with 1% methanol to a final concentration of either 1 µM (10 pmol mix) or 5 µM (90 and 120mer).

Liquid Chromatography Method

System: UHPLC 3000 HPLC Column: DNA-Pac RP analytical column Mobile Phases: A: 40% methanol, 16.3 mM TEA Buffer B: Water Gradient: See Figure 1

RESULTS

Experiment # 1: Full MS of Oligonucleotide Primer Mix Separated

Figure 4. Full MS deconvoluted with Biopharma Finder 3.0. Full MS spectra were deconvoluted using sliding windows and the XIC deconvolution feature. All oligos were detected with excellent mass accuracy and reproducibility.

Table 4. Deconvolution of 10 oligonucleotide primer mix using Biopharma Finder 3.0 software.

Table 5. Deconvolution of 10 oligonucleotide primer mix using Biopharma Finder 3.0 software.

Figure 7. 90mer DNA Oligonucleotide analyzed at 240,600 resolution with protein and standard mode: Charge state distribution of 20 pmol of the 90mer oligonucleotide with three times improvement in signal intensity.

Figure 8. 90mer DNA oligonucleotide analyzed at 120,000 resolution with protein mode and standard mode: Charge state inspection of +5. 10% demonstrates improved sensitivity when using reduced trapping gas pressure available with protein mode.

Table 6. Mass accuracy observed in duplicate analysis of 90mer oligonucleotide for different methods.

Table 7. Characterization of 90mer DNA oligonucleotide at high resolution.

Figure 9. 120mer DNA oligo at 240,600 resolution with protein mode: Total ion chromatogram of duplicate injections of 20 pmol of 90mer oligonucleotide. Duplicated injections showed strong reproducibility signal.

Figure 10. 120mer DNA oligo at 240,600 resolution with protein mode: Charge state distribution of 20 pmol of the 120mer oligonucleotide and charge inspection of charge state +5. 10%.

CONCLUSIONS

Oligonucleotides are a class of biomolecules that are traditionally not been well characterized by mass spectrometry due to the difficulty in acquiring high-resolution accurate mass data. In this study, we demonstrate the ease of acquiring high resolution accurate mass spectrometry data using the Q Exactive HF-X mass spectrometer in decreased trapping gas pressure mode, available with the Protein Mode option. We were able to fully resolve oligonucleotides ranging in size from 17 to 90 nucleotides with a mass accuracy of < 3 ppm. Additionally, mixtures of oligonucleotides can be easily fractionated using ion-pairing chromatography in conjunction with the DNA-Pac RP analytical column with an ion-pairing gradient that is effective in resolving oligonucleotides ranging from 17 to 190 nucleotides in length.

TRADEMARKS/LICENSING

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