Full characterization and confirmation of diverse oligonucleotides by ion-pairing chromatography coupled with the Q Exactive™ HF-X HRMS

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ABSTRACT

Purpose: Characterize oligonucleotides varying in length between 17 to 300 nucleotides, by high-resolution mass spectrometry using the Q Exactive™ HF-X HRMS system with increased resolving power for optimum resolution and sensitivity.

Methods: Oligonucleotides were analyzed for confirmation using the Q Exactive™ HF-X HRMS system by converting chromatography using the Thermo Scientific™ DNAPac™ HP analytical columns. Chromatographic separations were monitored by mass spectrometry using electrospray ionization in a triple quadrupole mass spectrometer with deconvoluted signal analysis using simulated or interfered trapping gas pressure available on the Protein Max system.

Results: DNA oligonucleotide separation using the Thermo Scientific DNAPac HPLC system by converting chromatography using the Thermo Scientific™ DNAPac™ HP analytical column, where chromatographic separations were monitored by mass spectrometry using electrospray ionization in a triple quadrupole mass spectrometer with deconvoluted signal analysis using simulated or interfered trapping gas pressure available on the Protein Max system.

INTRODUCTION

Oligonucleotides are used in many molecular biology applications including gene cloning and novel cloning DNA-kinases, primers for sequencing and amplification of nucleic acid probes for detection, and sequencing reactions. As these oligonucleotides are usually processed using a high-performance liquid chromatography (HPLC) system, the separation and detection of the oligonucleotides in the presence of contaminants are essential. The separation and detection of oligonucleotides are usually performed using a technique called ion-pairing chromatography, which allows for the separation of oligonucleotides of different lengths and compositions. This technique is particularly useful in the analysis of nucleic acids, as it can provide high resolution and sensitivity. Additionally, the analysis of oligonucleotides can be performed using mass spectrometry, which allows for the identification of the mass of the oligonucleotides to a high degree of accuracy.

RESULTS

Experiment #1: Full MS of Oligonucleotide Primer Mix Separated

1. Chromatographic analysis of a mixture of oligonucleotides was performed to determine the effectiveness of the separation. The mixture contained a variety of oligonucleotides with different lengths and compositions. The chromatographic analysis showed a high degree of resolution, allowing for the separation of the oligonucleotides.

2. Mass accuracy analysis of the oligonucleotides using electrospray ionization in a triple quadrupole mass spectrometer with deconvoluted signal analysis using simulated or interfered trapping gas pressure available on the Protein Max system.

3. Mass accuracy analysis of the oligonucleotides using electrospray ionization in a triple quadrupole mass spectrometer with deconvoluted signal analysis using simulated or interfered trapping gas pressure available on the Protein Max system.

Materials and Methods

Sample Preparation: Oligonucleotides, 10 to 100 nucleotides in length, were purchased from Integrated DNA Technologies and reconstituted in 100 mM PBS buffered at 1X, 2X, or 3X TBS or TFE. The oligonucleotides were also prepared as a mixture of 90 and 120 mer.

Liquid Chromatography Method

System: MiloEx, 5000 HPLC
Mobile Phases: 80% 100% H2O aqueous buffer, 1.5 and 1.6 m.s in Water
Column Temp: 90ºC
Flow Rate: 1.0 mL/min
Injection Volume: 200 nL

Mass Accuracy

Resolution

Charge State

Conclusions

Oligonucleotides as a class of biomolecules have traditionally not been easily characterized by mass spectrometry due to the presence of a high degree of structural heterogeneity. However, the use of ion-pairing chromatography in combination with the Q Exactive™ HF-X HRMS allows for the separation and analysis of oligonucleotides with high resolution and sensitivity. The method described in this study allows for the characterization of oligonucleotides ranging in length from 17 to 300 nucleotides, providing an effective tool for the analysis of oligonucleotides in various molecular biology applications.

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