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Accelerating Oligonucleotide Research Using Novel Bioinert LC, QTOF and TQ LC-MS

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Overview

- Nucleic acid drugs offer a new avenue for treating a wide range of illnesses by targeting disease causes at the genetic level.
- Chemical synthesis of nucleic acid drugs can result in impurities, requiring proper separation and purification techniques.
- To achieve optimal analytical performance, an inert UHPLC system, Q-TOF and triple quad mass spectrometers with an ion-pairing buffer system were used.
- These studies successfully confirmed the molecular weight and achieved sensitive quantification of oligonucleotides, showing promise for safe and effective nucleic acid drug development.

1. Introduction

Oligonucleotide therapeutics consist of a few to several dozen bases (including modified bases) linked together. The molecules are produced via chemical synthesis and act directly on organisms without being translated into proteins. The ability of oligonucleotide therapeutics to target specific diseases makes them unique. In practice, these therapeutics are rapidly degraded and excreted by blood and cell exonucleases and endonucleases after administration. To address this issue, modified oligonucleotides are being developed to improve chemical stability in vivo, as well as lesion-targeted DDS (drug delivery systems). Demonstrated here is the use of bioinert UHPLC and LC-MS to analyze oligonucleotides to accurately speed up the oligonucleotide development and quality control.

2. Methods

- **Sample Preparation**. The unmodified single-stranded DNA synthesized was purified using HPLC and diluted with water to a concentration of 5 µmol/L to prepare an oligonucleotide mixture. To obtain a 5 µg/mL solution for LC-MS analysis, a DNA oligonucleotide ladder with 10, 15, 20, 25, 30, 40, 50, and 60 bases was dissolved in Milli-Q water. All other oligonucleotides were prepared in mobile phase A or water for LC and LC-MS analysis.
- **LC Separation**.
  - Shim-pack Velox column (2.1 x 100 mm; 2.7um)
  - Flow rate 0.3 mL/min
  - Binary gradient; water and methanol with 2 mM ammonium formate and 0.002% formic acid
  - Cycle time 17 minutes
- **Mass Spectrometry Detection**. The experiments utilized the QTOF LCMS-9030 with external mass calibration for positive ESI, as well as the LCMS-8060.
- **Data processing**. LabSolutions was utilized for acquiring and analyzing the data. The mass spectra of the multi-charged ions were deconvoluted using the "ReSpect" algorithm, which is an option available in the LabSolutions Insight Explore™ software.

3. Results for LC

A successful analysis of oligonucleotides has been developed using ion exchange chromatography with Nexera XS inert and Shim-pack Bio IEX. This enabled separation of the target oligonucleotide from impurities and emphasizes the importance of pH control in achieving stable analysis. This study highlights the significance of metal-free flow paths in nucleotide analysis, eliminating the need for metal suppression treatments. The combination of Nexera XS inert and Shim-pack Scepter C18 metal-free columns provides highly sensitive, accurate, and robust analytical results.

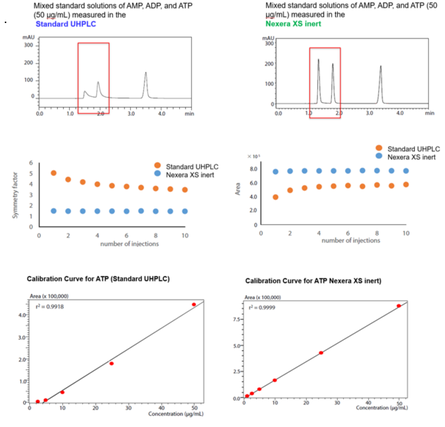

Figure 1: The figure illustrates the strategies for achieving improved sensitivity and ensuring reliable analytical performances in nucleotide analysis.

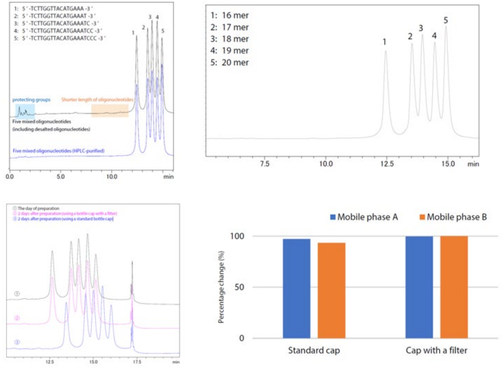

Figure 2: The figure illustrates the analysis of oligonucleotides using Ion Exchange Chromatography and examines the impact of pH variations in the mobile phase on the separation process.

To obtain a calibration curve, ATP standard solutions ranging from 1-50 µg/mL were measured six times. However, the linearity of the "metal-based" calibration curve was found to be reduced due to metal adsorption. Quantification of QC controls at 2, 20, and 45 µg/mL using this calibration curve led to deviations in quantitation values, which resulted in low linearity. In contrast, the "metal-free" calibration curve exhibited excellent linearity (r^2 = 0.9999, as shown in Fig. 1) and reproducibility at low-level calibration points and QC controls. These findings suggest that using a Nexera XS inert and metal-free column can effectively suppress metal adsorption and enable the analysis of compounds with phosphate groups, such as nucleotides.

When analyzing oligonucleotides using ion exchange chromatography, a salt concentration gradient is typically used for separation and elution of samples. However, a high pH mobile phase can lead to carbon dioxide absorption and pH changes, which can significantly impact analytical results since the separation mechanism is based on charge differences of analytes. Therefore, it is crucial to avoid changes in mobile phase pH during the analysis. To prevent such pH changes, we compared standard mobile phase bottle caps with caps containing filters that suppress solvent evaporation. We performed analyses using each cap on the day of preparation and after two days, and the chromatograms of mixed oligonucleotide samples are shown in Fig. 2, depicting the rates of pH change for both mobile phases. We found that using a standard cap resulted in an average 8% increase in retention time as pH changed compared to the day of preparation, whereas a cap with a filter did not change substantially, resulting in stable analysis results.

3.1 Results for LC-MS

Oligonucleotide therapeutics are synthetic molecules that can effectively bind to target genes or proteins, making them promising treatments for a variety of diseases. Currently, there are several types of approved oligonucleotide therapeutics, many of which are approximately 20 bases in length. In this article, the Q-TOF mass spectrometer LCMS-9030 was used to analyze a 2'-MOE modified oligonucleotide therapeutic with 20 bases. The accurate mass spectrometry technique employed by the LCMS-9030 allowed for the determination of the molecular mass of the therapeutic with an error of only 3 mDa, equivalent to 0.05 ppm. The device was calibrated using the multiple reaction monitoring (MRM) mode, which allowed for linearity to be observed within a range of 1 to 1000 ng/mL. Like triple quadrupole devices, the LCMS-9030 can perform highly sensitive quantification using the MRM mode. In this study, the octavalent ion at m/z 803.4626 was selected as a precursor ion, and the product ion at m/z 94.9358 (PSO2-) was used as a monitor ion.

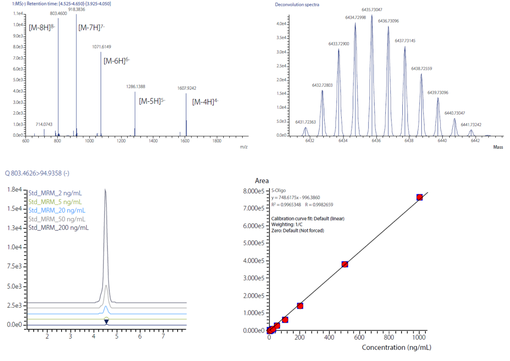

Figure 3: Upper panel shows the mass spectra of oligonucleotide therapeutics and deconvoluted spectra. As shown in the lower panel, linearity was observed within a concentration range of 1-1000 ng/mL. This finding suggests that the analytical method used is reliable and accurate for detecting the analyte within this concentration range.

Fig. 3 demonstrates the results obtained by a high-resolution ion-pair reversed-phase (IP-RP) LC-MS system, which enables efficient separation and accurate mass analysis of oligonucleotides. The system was designed to achieve a mass accuracy of less than 2.5 ppm for all targets, which is a significant improvement over previous system. The methods were utilized both high-resolution and triple quad (TQ) mass spectrometers to quantify oligonucleotides using the multiple reaction monitoring (MRM) acquisition mode. This involves selectively detecting product ions using a triple quadruple mass spectrometer. By focusing on a specific m/z range for production scans, higher sensitivity can be achieved, making it easier to detect and quantify oligonucleotides. Overall, this approach provides a robust and reliable method for oligonucleotide analysis. It has the potential to enhance our understanding of oligonucleotide structure and function, which can have important implications in various fields such as genetics, biotechnology, and pharmaceuticals.

4. Conclusions

- Oligonucleotide therapeutics hold great promise for targeted disease treatment, but their chemical instability in vivo presents a challenge.
- The novel bioinert UHPLC and LC-MS methods are used to analyze oligonucleotides accurately and reproducibly, allowing for faster development and quality control.
- The metal-free flow path in the Nexera XS liquid chromatograph and column allows for accurate separation of oligonucleotides, with the shorter sequences eluting first.
- The calibration curve obtained with the metal-free method shows excellent linearity, making it a superior choice to the metal-based method.
- The use of a quadrupole time-of-flight mass spectrometer and a triple quadrupole mass spectrometer allows for high-resolution separation and mass analysis, as well as quantification using MRM and SRM acquisition modes.