Optimization of nSEC-MS for Sustainable Oligonucleotide Analysis Using Design of Experiments

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Introduction

Oligonucleotides (ONs) possess the ability to interfere with gene expression through complementary Watson-Crick base pairing. Consequently, they have shown great potential to treat or manage currently undruggable illnesses.

Understanding the intact ON and degradants is crucial, as the impurities present in the ON may have the potential to impact drug safety and efficacy.

At the time of the study, native size exclusion chromatography-mass spectrometry (nSEC-MS) had not yet been trialed for routine use for the analysis of ONs. Currently ion pair-reversed phase liquid chromatography (IP-RPLC) is the dominant separation mode used. However, it has the potential to contaminate the system and decrease sensitivity for other applications.

This study evaluates a stressed ON using ion-pair free analytical techniques on a TOF-MS coupled with a UPLC-UV system.

Methodology

ONs are typically highly polar molecules with multiple charge states, making conventional reversed-phase separations challenging.

IP-RP-LC improves chromatographic performance through an increase in retention. HFIP is also typically used to suppress isomer separation and improve the quality of the mass spectra. However, these reagents have the potential to contaminate the mass spectrometer and decrease sensitivity for other applications.

nSEC has shown promise for the analysis of ONs due to the inherent compatibility with large molecules and different . Other columns such as HILIC or BEH can easily get contaminated with large molecules as they get retained on the column and cannot be washed off.

A chromatographic method which maximised theoretical plates and MS sensitivity while minimising reagent consumption was developed using MODDE Design of Experiments (DoE) software. The DoE approach enabled the determination of optimal parameters by the simultaneous variation of multiple factors per experiment. This enabled the collection of the most information from the least number of runs. The mobile phase flow rate, column temperature, mobile phase buffer salt concentration and injection volume were investigated.

A desolvation rating was utilised to assess the quality of the MS data. Desolvation rating is the ratio between desired product (intact ON) and undesired product (adducts). A statistical model was produced to determine the optimal conditions demonstrated below.

Final Method Parameters
Column - Acquity Premier Protein SEC 250Å, 1.7 µm, 4.6 mm x 30 mm
Column Temp – 40°C
Salt concentration of mobile phase – 50 mM
Flow Rate - 0.08 mL/min
Injection Volume - 10 uL

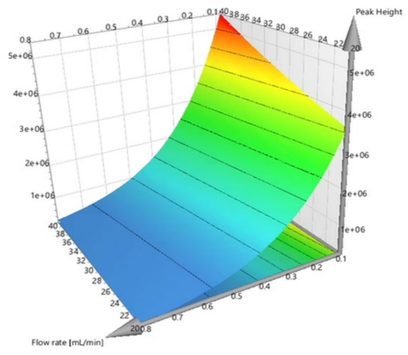

Figure 1 - Visual representation of the relationship between peak height and flow rate

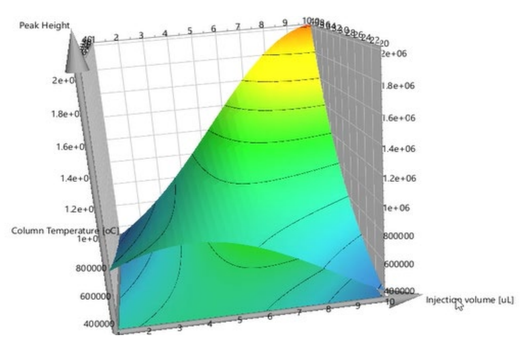

Figure 2 - Visual representation of the relationship between peak height, column temperature and injection volume

Results

The ON was stressed under acidic conditions to assess the methods suitability for related substances. Figure 3 shows the m/z spectra of the sample compared with control.

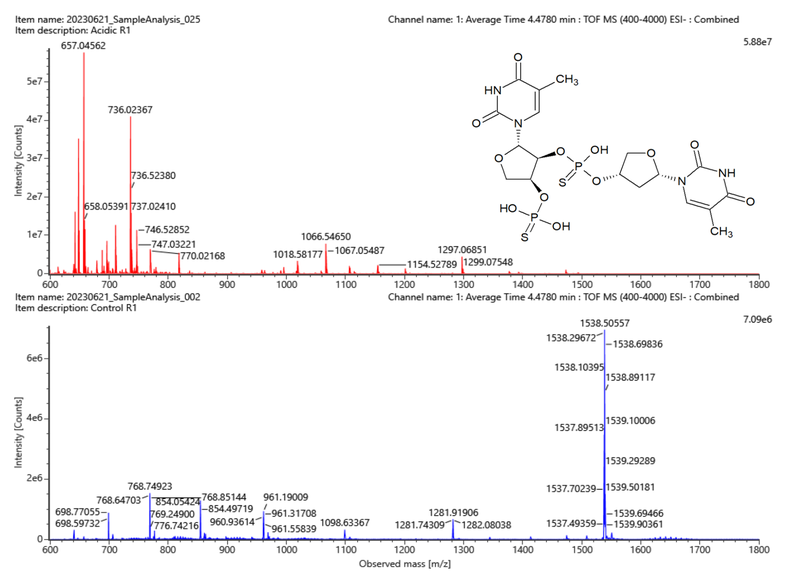

Figure 3 - m/z spectra of acidic stressed sample (top) compared with control sample spectra (bottom)

The deconvoluted accurate mass spectra for the detected components were extracted using waters_connect UNIFI. These component spectra were subsequently used for the identification of the related substances in the sample.

To evaluate whether the developed method had a reduced environmental impact, the reagent consumption to the method was compared with a high-throughput IP-RP-MS method in the literature. (Figures 4 and 5)

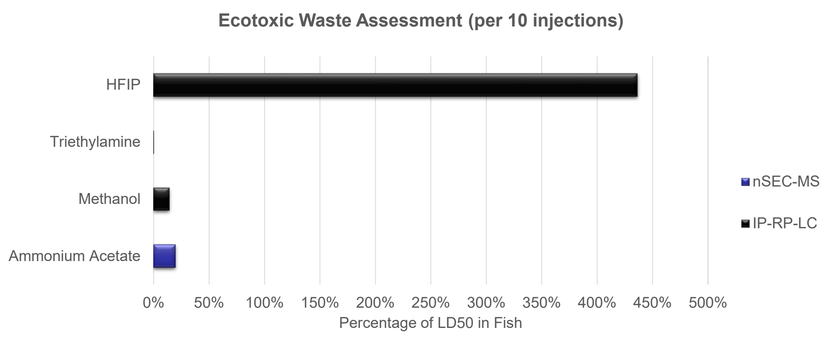

Figure 4 - Comparison of the of waste ecotoxicity for the nSEC-MS and IP-RP-MS approaches.

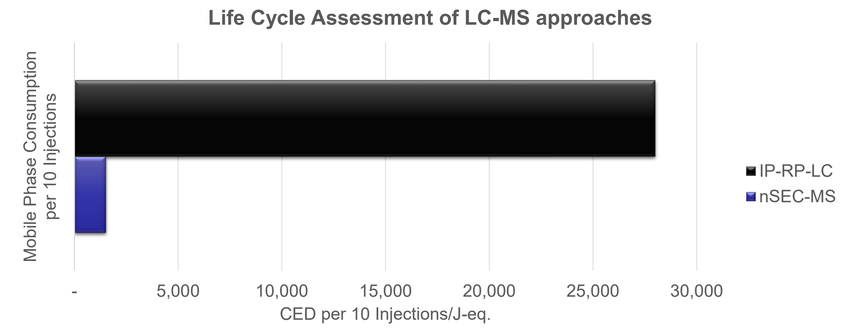

Figure 5 - Comparison of the LCA of the nSEC-MS and IP-RP-MS approaches.

Comparison of the developed nSEC-MS method with the IP-RP-MS method illustrates the potential for nSEC-MS as a sustainable alternative for oligonucleotide analysis.

Conclusions

An optimized nSEC-MS method was successfully developed for the characterization of oligonucleotides and related substances in 7.5 minutes without the need for ion-pairing reagents.

Relative to existing IP-RP-MS methodologies, the developed method provides a more environmentally friendly alternative for the analysis of oligonucleotides.

References

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