A Microchip CE-MS Based End-to-End Solution for the Analysis of Synthetic Oligonucleotides

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Highlights

- Microfluidic CE/MS analysis of oligos by ZipChip-MS with no ion pairing reagents
- Simple & fast workflow: minimum sample prep, short analysis time & easy data deconvolution
- Method can be easily adopted for oligo intact mass and impurity analysis etc.
- Entire workflow is vendor agnostic (compatible with MS from ThermoFisher, Sciex & Bruker)

Introduction

Due to their unique chemical and physiological properties, and proven efficacy towards a range of disease conditions, synthetic oligonucleotides continue to gain traction with strong growth outlook from the pharmaceutical industry. However, obtaining synthetic oligos with high purity continues to remain a challenge.

In this work, we present a simple, and easy workflow that is vendor agnostic end to end. The workflow is microchip CE-MS based coupled with a vendor neutral informatics platform as a complete solution for the analysis of synthetic oligos. This technology known as the ZipChip relies on separation of analytes based on their electrophoretic mobilities first, then having these separated analytes electrosprayed directly into a mass spectrometer. The workflow provides simple and easy methodology for the analysis of a range of oligos using high resolution accurate mass in positive mode. Mixtures of oligos including the full-length product (FLP) along with its truncated shortmer impurities can be analyzed with efficiency within 5-6 minutes. The positive ESI mode data significantly simplifies the raw mass spectra for oligos enabling easier post-processing of the CE-MS data.

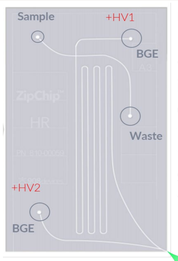
Materials & Methods

Samples: All oligonucleotides including synthetic ssRNA, DNA and oligonucleotides with same sequence and modifications as Nusinersen and its n-1, n-2 and n-3 shortmer impurities on 5' and 3' ends were purchased from Integrated DNA Technologies. All standards were reconstituted with the Oligos BGE (908 Devices Inc.) and diluted to appropriate concentrations in the Oligos diluent prior to analysis.

Instrumentation: The ZipChip™ Device was used for all analyses (908 Devices Inc.).[1] The High Resolution Bare (HRB) Glass Chip and the Oligonucleotides BGE, both part of the Oligonucleotides kit (908 Devices Inc.) were used. An on-chip injection volume was 1 nL with separation run at a field strength of 500 V/cm. MS analysis was performed on a Thermo Fisher QExactive HF or Sciex 6600+ TripleTOF mass spectrometer.

Data Processing: The data was visualized using the Qual Browser data analysis software (Thermo Fisher Scientifc) or Sciex OS (Sciex) depending on the mass spectrometer used. The data was deconvoluted using Byos software (v 4.2) (Protein Metrics Inc.)

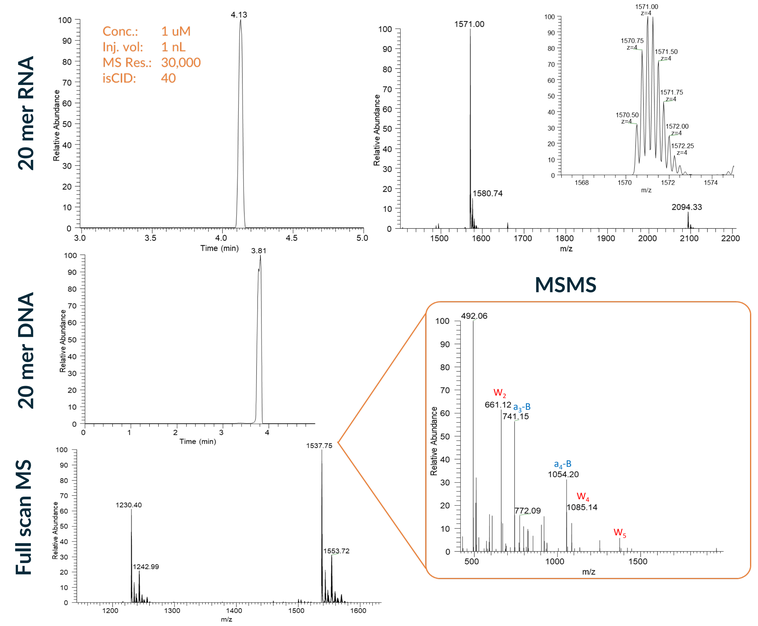
Bare Glass Chip Separation Mechanism



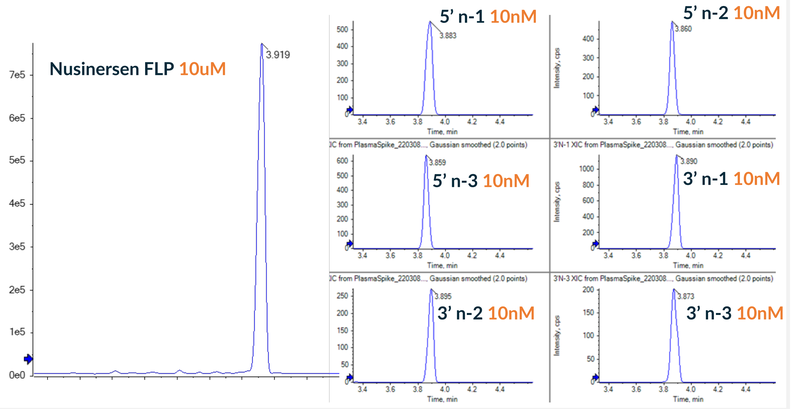
Unlike the standard neutral polymer coated chips,[2] the channels in the HRB chips contain significantly charged bare silica surface. When a voltage potential difference is applied across the separation channel, it induces a steady unidirectional motion of the BGE resulting in electroosmotic flow (EOF) which results in the migration of all analytes towards the ESI sprayer. Positively charged species migrate first, followed by neutrals which in turn are followed by negatively charged species, thus allowing for electrophoretic separation of analytes with varying charges.

Characterization of Oligonucleotides

Figure below displays electropherograms for 1 uM of a 20 mer ssRNA (top left) and 1 uM solution of 20 mer DNA (middle left) and their MS spectra. An insert in the mass spectrum shows a zoomed-in view of the $[M+4H]^{4+}$ species that was detected as the dominant charge states for the ssRNA standard. These analyses were accomplished using a BGE without ion-pairing agents. MSMS characterization for oligos was also be performed on ZipChip-MS in positive mode ionization using standard MSMS workflows such as DDA or MRM. An example of DDA analysis for a 20 mer DNA is shown below. Information rich MSMS data can be generated for such oligos in positive mode. The data can either be processed manually or using commercial software packages such as Byos (Protein Metrics Inc.).

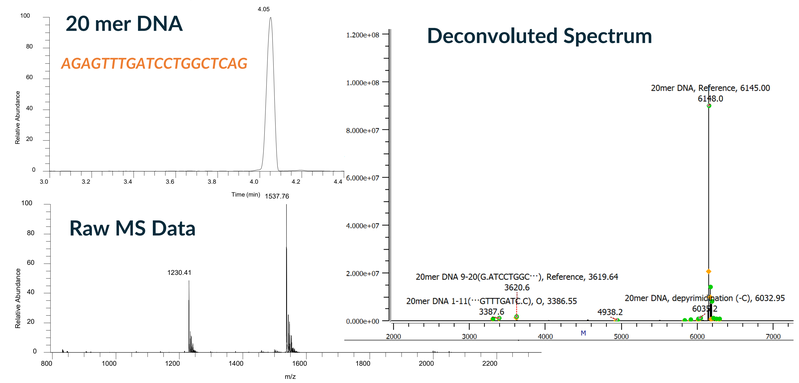


A 10uM standard of an oligonucleotide with the same sequence and modifications as an FDA approved drug Nusinersen was spiked with six different shortmer impurities *viz.* 5' n-1, 5' n-2, 5' n-3, 3' n-1, 3' n-2 and 3' n-3 each at a concentration of 10nM (~0.1% relative to the Full Length Product (FLP)). Extracted ion electropherograms for the FLP and impurities are shown below. Similar to the 20 mer DNA and RNA examples shown above, all the species in this sample showed only one or two different charge states. Even though the FLP and the impurities had migration times very close to one another, the data analysis was simplified because only one or two different charge state for each species were observed making the raw mass spectrum much simpler to analyze. This data shows that low level sequence length impurities (~0.1%) can be easily analyzed using the ZipChip

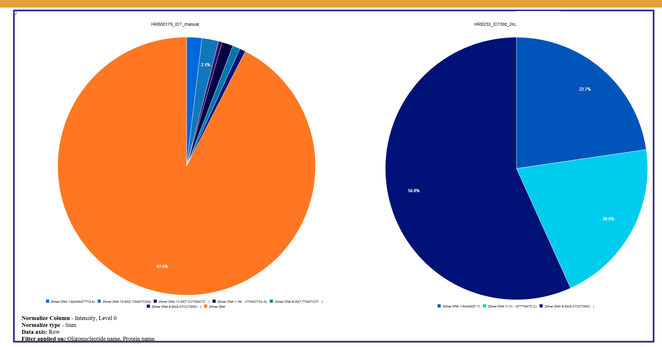


Data Processing for Oligonucleotides

Positive ESI mode oligonucleotides data acquired on the ZipChip was seamlessly processed using vendor neutral software (Protein Metrics' Byos). Figure below displays electropherogram and raw mass spectrum for 1 uM of 20-mer DNA (left) and the corresponding deconvoluted spectrum (right) as obtained from Byos. The software detected and identified the FLP using both the average and monoisotopic masses. Impurities in the sample such as sequence truncations, as well as the modifications along the phosphate backbone and the bases were identified automatically.



Automated Data Processing



Automated, templated oligonucleotide workflows analyze data and can be edited for specialist cases, molecules, or processes. The deconvolution algorithm makes better use of modern instrument capabilities, and manages data from isotope spacing, charge states, and known mass differences simultaneously ("three channel deconvolution"). Automated reporting lets users create visualizations for non-experts to make the most efficient use of human and instrument capital. The figure above shows a visual representation for the impurities of a 20 mer DNA relative to the FLP (left pie chart). The pie chart on the right shows a visual representation of relative amounts of just the impurities present in a different batch of the same 20 mer DNA.

Conclusions

- The ZipChip seamlessly integrates CE with mass spectrometers from multiple vendors
- Hassle-free method for characterization of oligos without the use of ion-pairing agents
- Generate data in positive ESI mode and allows for both MS and MSMS analysis
- Highly sensitive method for length/intact mass confirmation and impurity analysis of oligos
- Data processing made easy with processed using a commercially available software Byos from Protein Metrics Inc.

References

1. Tech Note 1.0; 908 Devices; *ZipChip: What are they and how they work.*
2. Redman, E. A.; Batz, N. G.; Mellors, J. S.; Ramsey, J. M. *Anal. Chem.* **2015**, *87*, *4*, *2264-2272.*

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The technologies discussed in this poster are the subject of one or more granted/pending patents. www.908devices.com/patents/