Oligonucleotide Analysis via Microfluidic CE-MS

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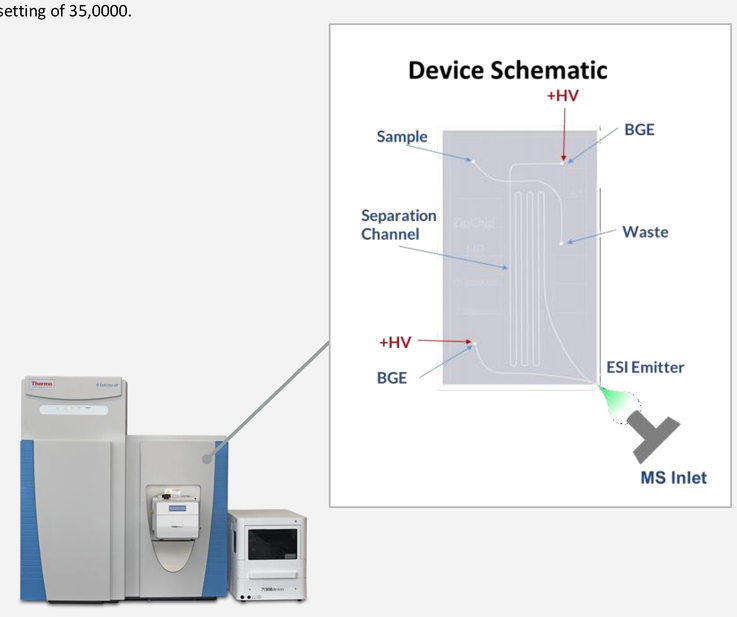
Overview

- A new microfluidic CE-MS approach that does not require ion pairing agents and utilizes positive ESI for enhanced system robustness
- A mixture of 5 different ssDNA primers was analyzed with the developed method, and all 5 primers were separated and detected as protonated ions via positive electrospray
- 100 automated replicates were run to assess migration time and peak area reproducibility of this method
- A dilution series ranging from 5 nM to 16 µM was run to assess dynamic range

Methods and Materials

Samples: 5 different single-strand DNA primers were purchased from Integrated DNA Technologies, with 10 µg of dried primer in each vial. 600 µL of LC-MS grade water was sequentially added to each vial to create the mix of 5 primers. This mix was transferred to an LC-MS vial and loaded in the autosampler for the reproducibility experiment. A single primer was resuspended in 100 µL of deionized water, and then serially diluted 5x to create the dilution series from 5 nM to 16 µM. Each of the diluted samples was loaded into the PATsmart™ ZipChip® System autosampler for automated analysis.

Instrumentation: The ZipChip System (Repligen) was used for all analyses. The ZipChip System utilizes microfluidic technology to harness the inherent speed and efficiency of zone electrophoresis separations. The microfluidic device design, as seen below, incorporates an injection cross, serpentine separation channel, and an integrated ESI emitter where electrospray is generated directly off the corner of the device. HRB type chips (Repligen) were used for analysis. A prototype background electrolyte containing ammonium acetate adjusted to pH 8.5 was used. An on-chip injection volume of 1 nL was performed and the separation was run at a field strength of 500 V/cm. MS analysis was performed using a Thermo Fisher Exactive Plus EMR Orbitrap mass spectrometer at a resolution setting of 35,0000.



Mixture of 5 Oligonucleotides

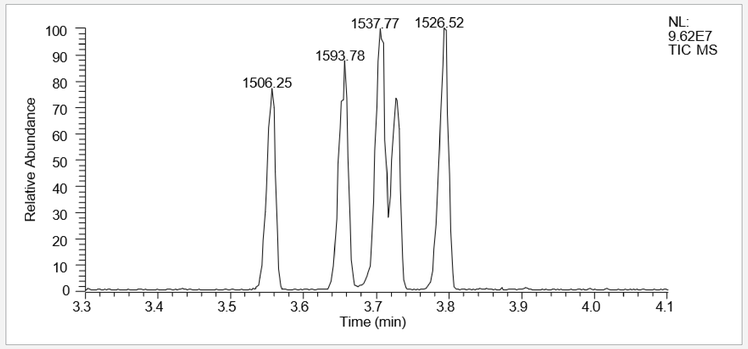


Figure 1. Separation of a 5-component mixture of ssDNA primers. Almost all species are baseline resolved in less than 4 minutes.

Figure 2. Example raw mass spectrum for peak 2 in Figure 1. The primers are detected as protonated ions. In the spectrum above the most abundant charge state detected is +4. As shown in the inset, isotopic resolution for primers of this size is easily achieved at an orbitrap resolution setting of 35,000.

Table 1. Deconvoluted Masses for ssDNA Primers

Peak #	Monoisotopic Mass (Da)	Theoretical Mass (Da)	Mass Error (ppm)
1	6018.979	6018.96503	2.3
2	6369.100	6369.08531	2.3
3	6145.056	6145.05147	0.74
4	6098.949	6098.93136	2.9
5	6100.062	6100.05255	1.5

Dynamic Range and Reproducibility

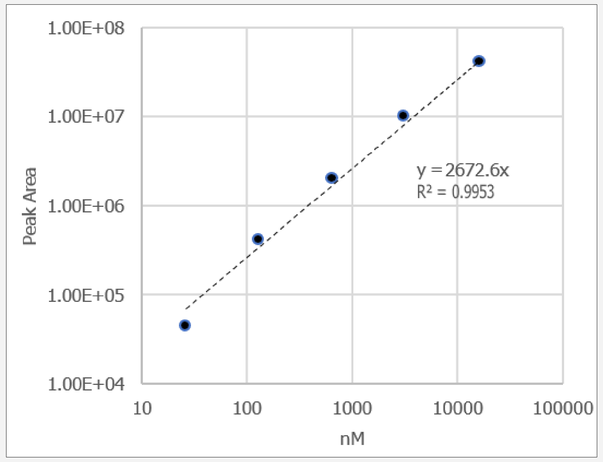


Figure 3. Dilution series of a ssDNA primer. Response is linear from 26 nM to 16 µM. The 5 nM sample was only detected in one replicate and was therefore omitted from this plot. The limit of detection was estimated to be approximately 10 nM.

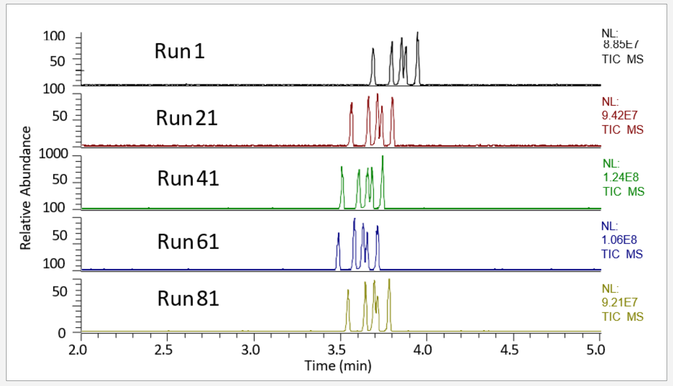


Figure 4. Select runs from replicates of the 5-component mixture. Migration time RSDs were calculated at 1.6% and peak are RSDs calculated at 13%.

Summary

This new method for oligonucleotide analysis has demonstrated the ability to achieve separation of ssDNA primer molecules, while also producing high quality mass spectra in positive ESI mode. Importantly, this method functions with a simple ammonium acetate buffer as the background electrolyte, removing the need for ion-pairing agents.

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