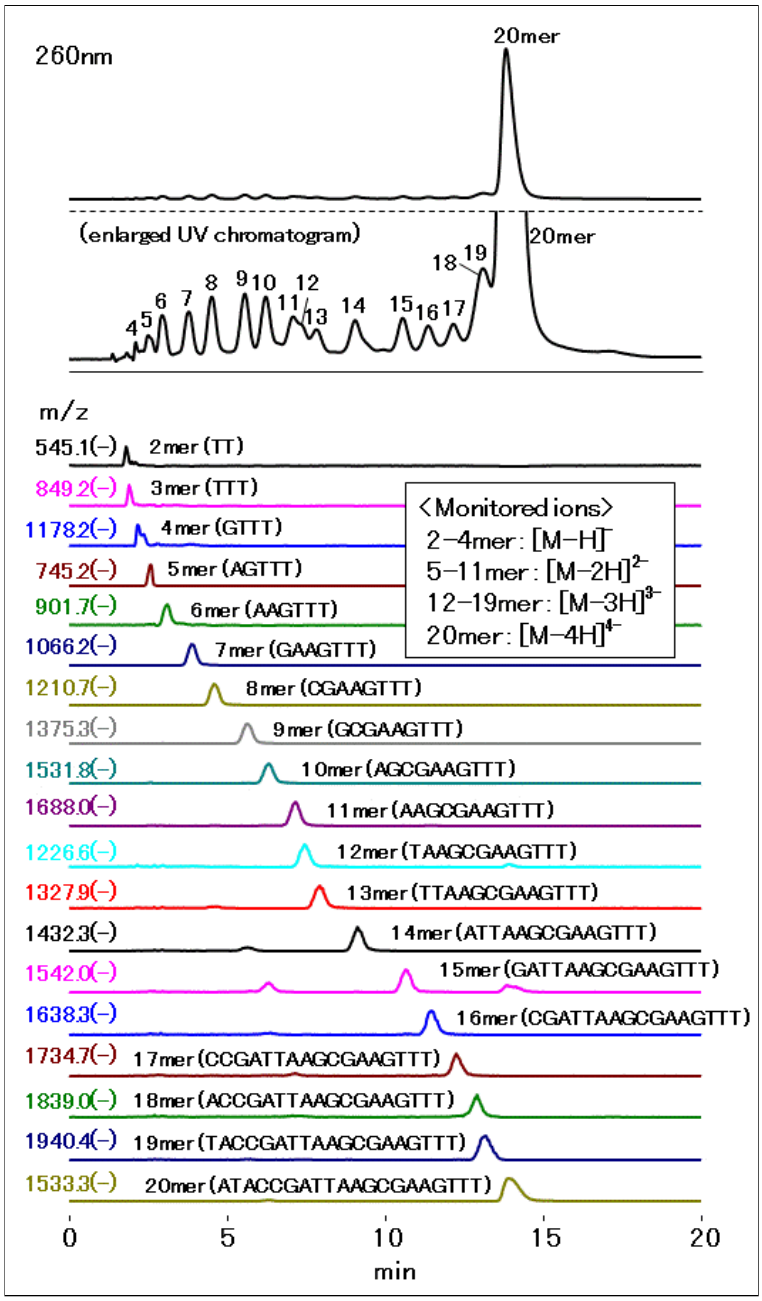
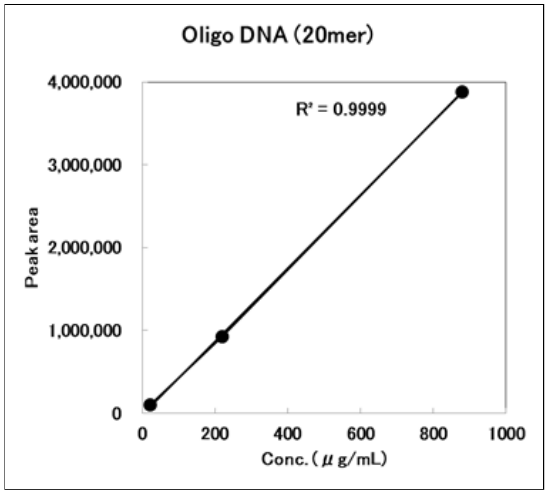
Highly sensitive and selective analytical methods are required for the development and the quality control of nucleic acid drugs, which are expected to be the next-generation pharmaceuticals. Conventionally, the ion pair reverse phase mode and the ion exchange mode are frequently used for analysis of oligonucleotides. The ion pair agent tends to remain in the device and the ion exchange mode, which requires high concentration salt as the eluent, is not suitable for MS detection. In this application, an unpurified oligodeoxyribonucleotide (oligo-DNA) synthesis product (ATACCGATTAAGCGAAGTTT) was analyzed using HILICpak VN-50 2D, a polymer-based HILIC mode column, with LC/UV/MS detection. The ion pair reagent is not required in this condition using HILIC mode, and good oligomer separation was obtained from dimer to 20 mer, a main component, by gradient elution of 50 mM ammonium formate aqueous solution and acetonitrile. Furthermore, it was confirmed that the calibration curve with MS detection has high linearity, quantification with high sensitivity and high selectivity was also possible.





Sample : 1 μL
Synthesized oligo-DNA 20 mer (ATACCGATTAAGCGAAGTTT; crude)
2.2 mg/mL (in H_2O)

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Column       : Shodex HILICpak VN-50 2D (2.0 mm I.D. x 150 mm)
Eluent       : (A)50 mM HCOONH4 aq./(B) CH3CN
               Linear gradient ;
               (B%) 60 % (0 to 10 min), 60 % to 55 % (10 to 15 min), 60 % (15 to 20 min)
Flow rate    : 0.2mL/min
Detector     : UV (260 nm) (small cell volume), ESI-MS (SIM Negative)
Column temp. : 40 °C
```