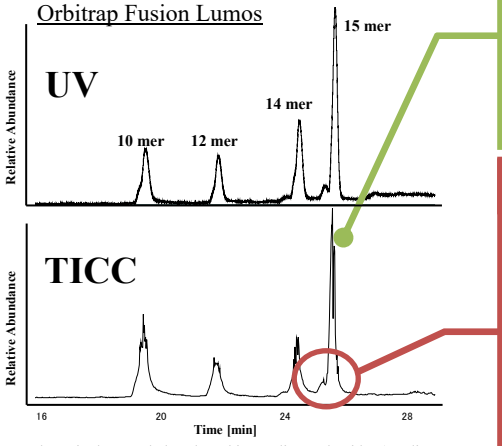
Analytical Technologies Accelerating the Research and Development of Oligonucleotide Therapeutics (Ultra-high Performance LC-MS, NMR, and CD Analyses)

In the R&D of oligonucleotide therapeutics, the analysis of impurities is essential for evaluating medicinal actions properly. The higher-order structure and complex formation of oligonucleotide therapeutics are important evaluation items because they probably have impacts on quality. The following are the examples of the nucleic acid analyses using ultra-high performance LC-MS, NMR, and CD.

Analysis of single-strand phosphorothioate oligonucleotides using ultra-high performance LC-MS (high-resolution, accurate-mass Orbitrap mass spectrometry)

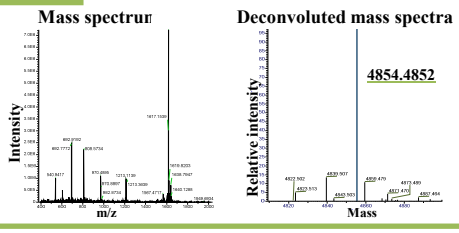
Analysis of nucleic acids using high-resolution, accurate-mass spectrometry

Orbitrap Fusion Lumos

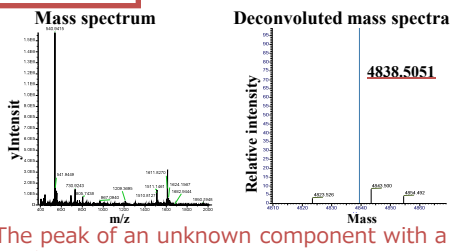


Sample: a single-strand phosphorothioate oligonucleotides (S-Oligo, 5´CACGTTGAGGGGCAT3´) and the 3 kinds of base deleted S-Oligo equally mixed solution
Conditions: 0.6 nM DNA, 5 mM HFIP in MeOH, 5 mM HFIP in Water

Intact Phosphorothioate oligonucleotides (15 mer)



Unknown e.g. Degradants, Impurities



The peak of an unknown component with a MW* different from insufficiently separated intact were successfully detected with the LC.

*: molecular weight

S-Oligo	Monoisotopic mass		Deviation (ppm)
	Calculated exact mass	Measured accurate mass	
15 mer	4854.4896	4854.4852	-0.9
14 mer	4534.4664	4534.4632	-0.7
12 mer	3900.4081	3900.4054	-0.7
10 mer	3210.3487	3210.3491	0.1

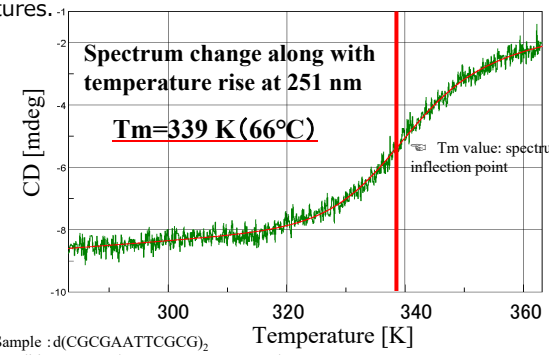
Deviations from the calculated exact mass were less than 1 ppm!

Estimation of unknown component structure is also possible by calculating accurate-mass using deconvolution tool.
From the comparison of exact mass and accurate mass, it was presumed that a part of 15 mer phosphorothioate oligonucleotides were not phosphorothiated and remained as oxygen.

Analysis of impurities, estimation of metabolites, and quantitative determination using accurate-mass.

Analysis of double strand nucleic acids using Circular Dichroism (CD)

CD spectrum sensitively reflects the higher-order structure of nucleic acids. By measuring the CD spectrum while changing the temperature, the melting process of double-strand structure by heat is trucked to calculate the melting temperature (Tm) at which 50% of double-stranded DNAs dissociate and the double-strand structures turn to single strand structures.



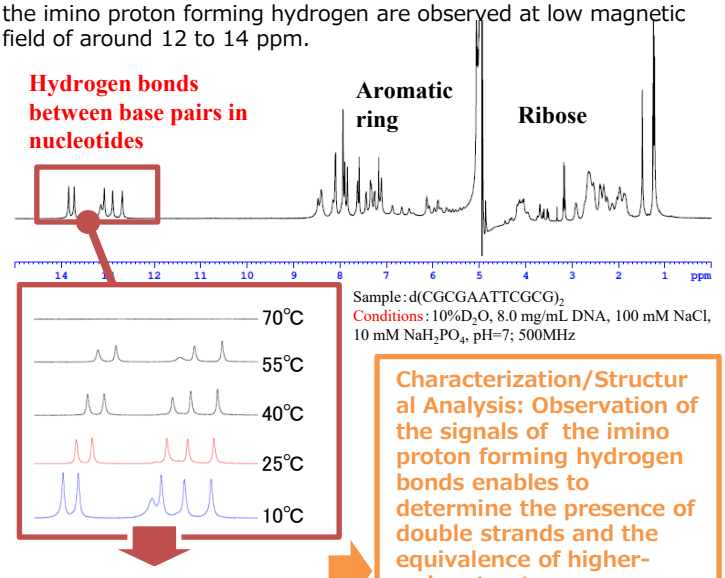
Sample : d(CGCGAATTCGCG)$_2$
Conditions: 0.2 mg/mL DNA, 100 mM NaCl, 10 mM NaH_2PO_4, pH=7

Using the melting temperature (Tm), the heat stability of nucleic acid higher order structure is evaluated.

Characterization analysis: The Tm of nucleic acids changes dependent on the sequence and concentration of nucleic acids, and solvent, and it is an important evaluation item to characterize oligonucleotide therapeutic products.

Analysis of double-strand nucleic acids using Nuclear Magnetic Resonance (NMR)

When ^{1}H NMR spectrum of nucleic acids is analyzed, the signals of the imino proton forming hydrogen are observed at low magnetic field of around 12 to 14 ppm.



Sample: d(CGCGAATTCGCG)$_2$
Conditions: 10%D_2O, 8.0 mg/mL DNA, 100 mM NaCl, 10 mM NaH_2PO_4, pH=7; 500MHz

Characterization/Structural Analysis: Observation of the signals of the imino proton forming hydrogen bonds enables to determine the presence of double strands and the equivalence of higher-order structure.

Formation of double strands can be judged by signal observation.
As temperature rises, more hydrogen bonds cleave, and the double strand dissociation ratio of nucleic acids rises. The imino proton signals disappear at a temperature higher than the Tm value of respective nucleic acid.

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