LC/MS analysis of oligonucleotides using new polymer-based HILIC column having diol group

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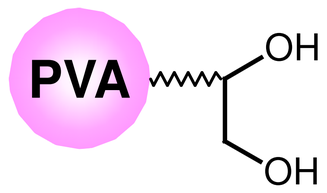
Introduction

Nucleic acid drug development and quality control are expected to drastically increase within the pharmaceutical sector. LC/MS measurements are a highly sensitive and highly selective analytical tool required for quality control. Conventional ion pair reverse phase mode has been widely used for the analysis of oligonucleotides. However, the ion pair agent tends to remain in the instrument.

In this study, a novel column Shodex™ HILICpak™ VN-50 2D column was applied for LC/MS measurement of oligonucleotides. A method was developed to separate oligonucleotides under a gradient condition of volatile salt solution and acetonitrile without using ion pairing agent and to conduct highly sensitive analysis.

Shodex® HILICpak® VN-50 series

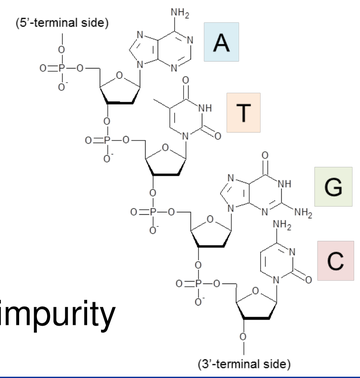
- Packing material: Poly vinyl alcohol particle with diol group
- Housing: PEEK
- Usable pH: 2～13 — *Wide pH range*
- Usable temp.: 4～60℃
- Usable solvent: H_2O, CH_3CN, CH_3OH (mixable at any ratio)
- Hydrophilic substance can be retained without derivatization or ion pair agent
- Suitable for HILIC analysis of oligosaccharides and oligonucleotides



Product name	Plate number (TP/Column)	Functional group	Particle size (μm)	Pore size (Å)	Column size (mm) I.D. × Length
HILICpak VN-50 4D	≥ 10,000	Diol	5	100	4.6 × 150
HILICpak VN-50G 4A	(guard column)	Diol	5	100	4.6 × 10
HILICpak VN-50 2D	≥ 3,500	Diol	5	100	2.0 × 150
HILICpak VN-50G 2A	(guard column)	Diol	5	100	2.0 × 10

Experimental

<Sample>
Synthetic oligodeoxyribonucleotides(salt-free grade)
(1) 20mer 5'-ATACCGATTAAGCGAAGTTT-3'
(2) 20mer 5'-ATACCAATTAAACAAAATTT-3'
(3) 62mer 5'-CATGAGAAGTATGACAACAGCCCCACACCGG
CTGTTGTCATACTTCTCATGGTTCTTCGGAA-3'
* All samples contain trace amounts of imperfect oligo-DNAs as impurity



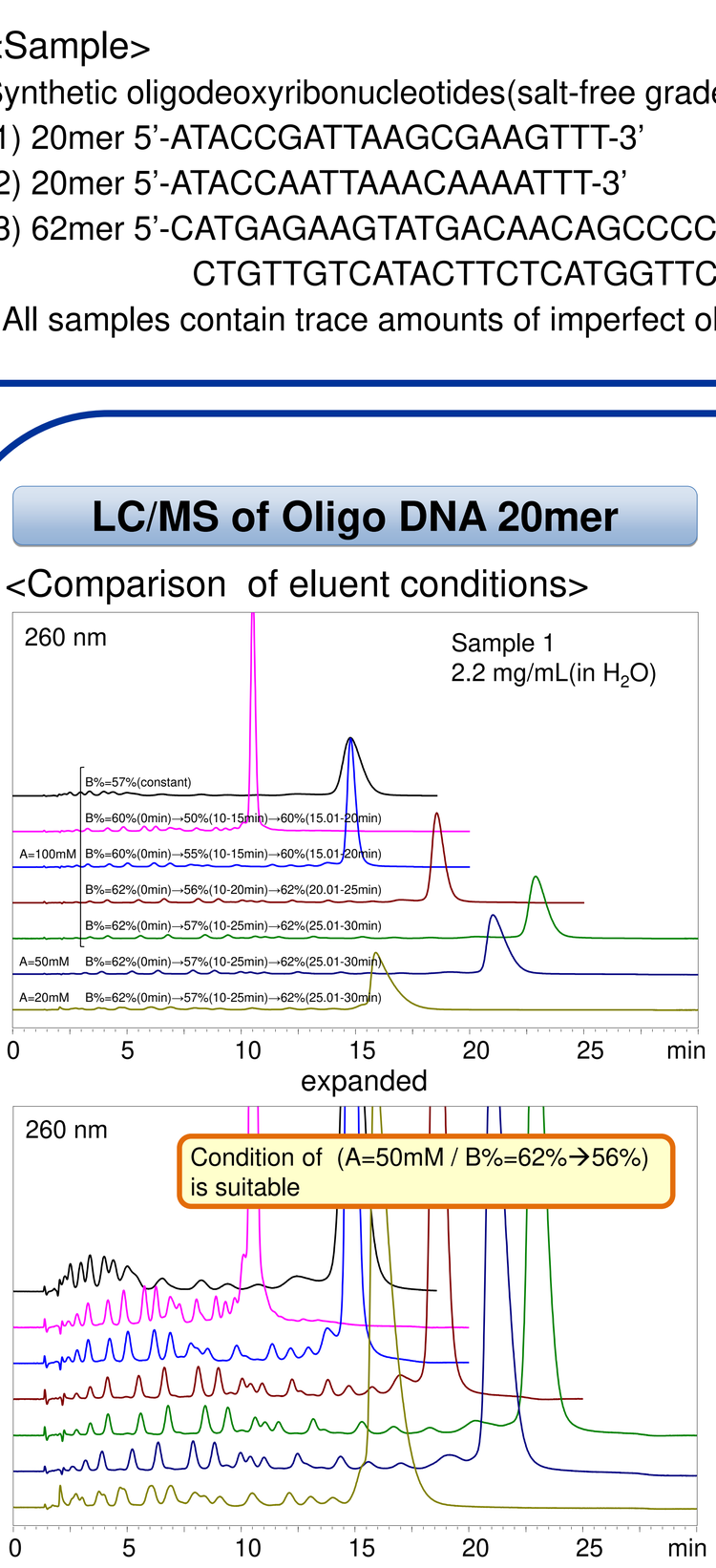
<Analytical conditions>
Instrument : Shimadzu Nexera / LCMS-8030 Plus
Column : Shodex HILICpak VN-50 2D (2.0 mm I.D. x 150 mm)
Eluent : (A) 50 mM $HCOONH_4$ aq. / (B) CH_3CN
Linear gradient (High pressure)
Flow rate : 0.2 mL/min
Detector : PDA (190-350 nm) & ESI-MS SIM(-)
Column temp. : 40℃ Injection vol. : 1 μL

not using ion pair agent and HFIP

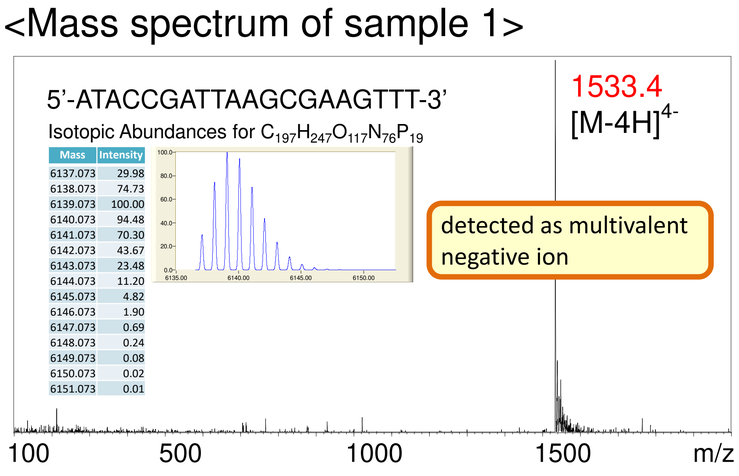
Results and Discussion

LC/MS of Oligo DNA 20mer

<Comparison of eluent conditions>



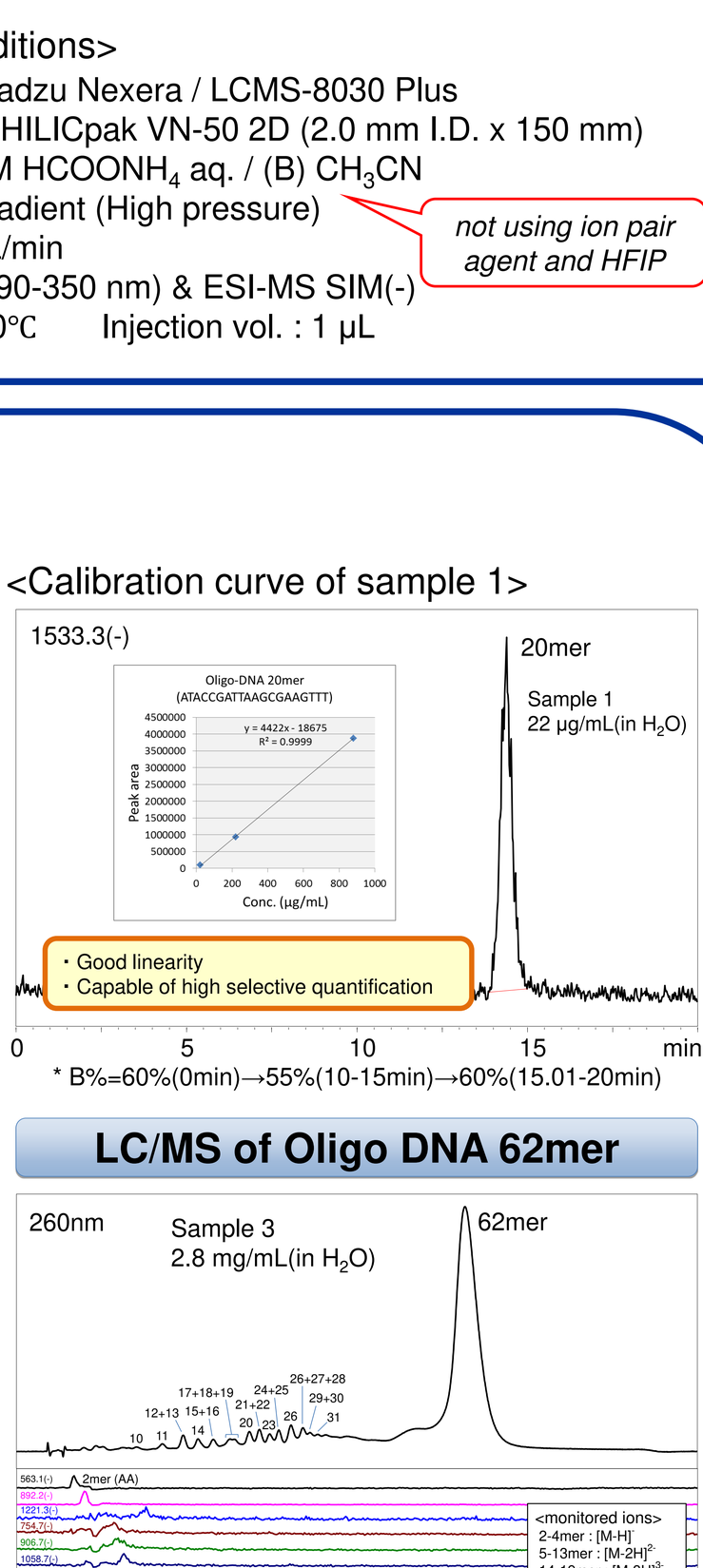
<Mass spectrum of sample 1>



<Comparison of chromatograms>

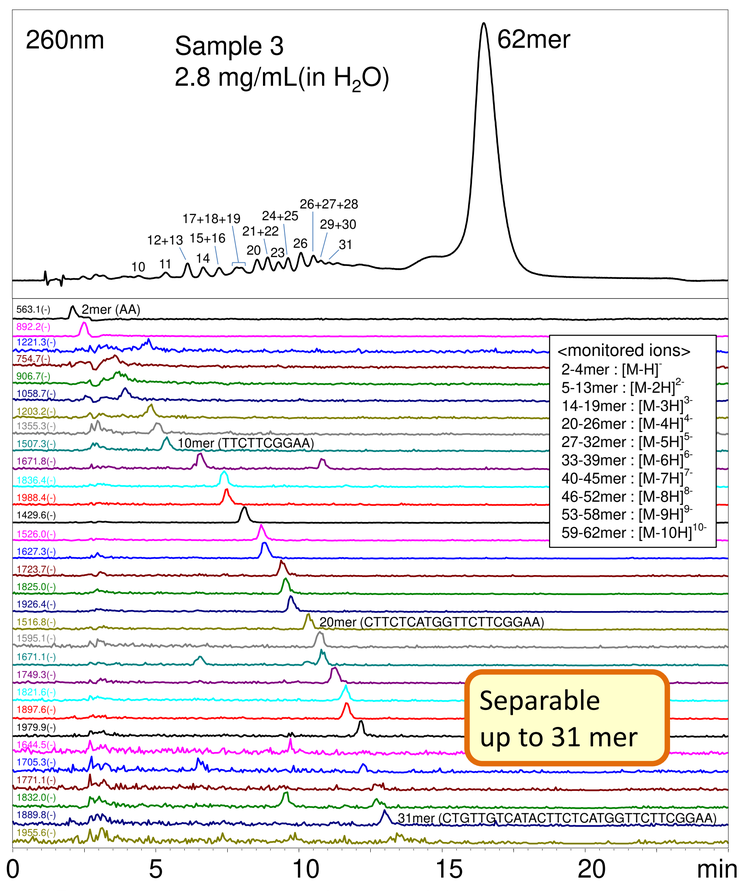
* B%=62%(0 min)→56%(10-20 min)→62%(20.01-25 min)

<Calibration curve of sample 1>



* B%=60%(0min)→55%(10-15min)→60%(15.01-20min)

LC/MS of Oligo DNA 62mer



* B%=60%(0 min)→50%(10-20 min)→60%(20.01-25 min)

Conclusions

Shodex™ HILICpak™ VN-50 2D, a newly developed polymer-based diol-type HILIC column, was used with LC/UV/ESI-MS to elute oligonucleotides in order of polymerization degree. It was confirmed that highly selective analysis was possible. The eluent was a gradient of 50 mM ammonium formate aqueous solution and acetonitrile, a condition that does not require the addition of an ion pair reagent in reverse phase mode or addition of a high concentration salt in ion exchange mode. This condition seems to have superiority in terms of simplification of desalting process even for preparative applications. Alkaline washing is also possible. The VN-50 series is a useful column for the development and quality control of nucleic acid medicine.