Separation of oligonucleotides modified with disulfides using YMC-Triart Bio C4

Antisense DNA and siRNA are widely used for gene silencing in research and medical applications. An effective delivery of the oligonucleotides into cells is important for clinical applications. As oligonucleotides are negatively charged the efficiency of the cell membrane permeability is low. Previous methods took several hours to deliver oligonucleotides to cytoplasm. Oligonucleotides modified with low molecular weight disulfide groups at their terminal residues reached the cytoplasm in 10 minutes as a result of disulfide exchange reactions with the thiol groups on cell surface.[1)]

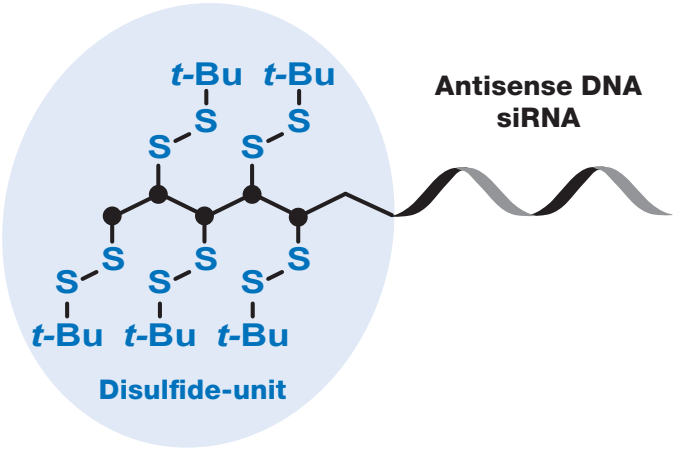

Figure 1: Structure of disulfide modified oligonucleotides.

Due to the hydrophobic character of the disulfide units, a less hydrophobic stationary phase is necessary for the analysis of the modified oligonucleotides. Even C18 columns with lower hydrophobicity such as Hydrosphere C18 achieve poor peak shapes. Also, the target disulfide modified oligonucleotides are not completely eluted.

In this application good peak shapes are achieved using the less hydrophobic, widepore YMC-Triart Bio C4 column. The disulfide modified oligonucleotides were analysed using 50 mM TEAA buffer/acetonitrile and acetonitrile as eluents at an elevated temperature of 50 °C.

Table 1: Chromatographic conditions.

Column:	5 µm, 250 x 4.6 mm ID
Product code:	TB30S05-2546PTH
	HS12S05-2546WT
Eluent:	A) 50 mM TEAA* (pH 7.0)/acetonitrile (95/5)
	B) acetonitrile
Gradient:	5–95%B (0-30 min), 95%B (30–35 min),
	95–5%B (35-35.1 min), 5%B (35.1–45 min)
Flow rate:	1 mL/min
Temperature:	50 °C
Detection:	UV at 260 nm
Sample:	Crude reaction mixture

*Triethylammonium acetate

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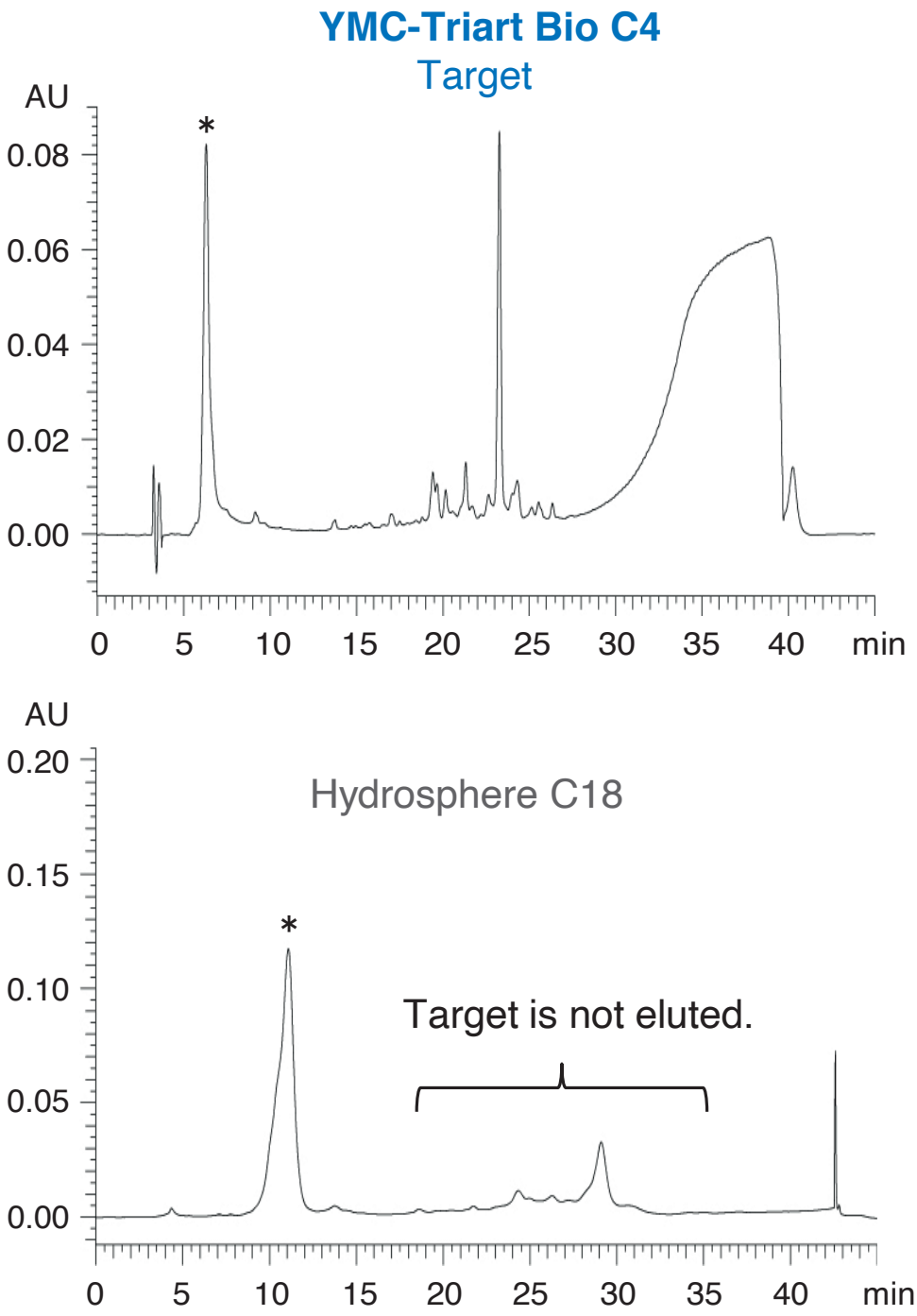

Figure 2: Analysis of disulfide modified oligonucleotides using YMC-Triart Bio C4 and Hydrosphere C18.

By courtesy of Saki Kawaguchi, Chemistry Department, Nagoya University, Japan

Literature:
1) Zhaome Shu et al. (2019) Disulfide-Unit Conjugation Enables Ultrafast Cytosolic Internalization of Antisense DNA and siRNA. Angew. Chem, 131, 6683-6687