YMC SEPARATION TECHNOLOGY

HPLC DATA SHEET

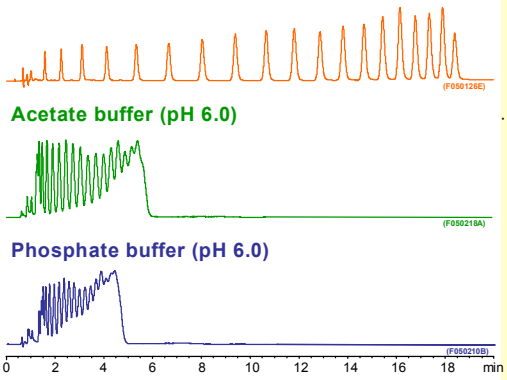
High resolution analysis of Oligonucleotides on reversed phase chromatography

F121018AE

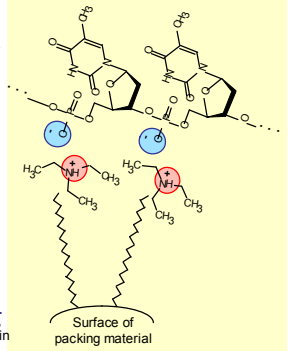
Oligonucleotides analysis on reversed-phase ion-pair chromatography (RP-IPC)

Comparison of retention and separation under various mobile phase conditions

Oligodeoxythymidylic acid, [d(T)$_{2\text{-}20}$]



Retention mechanism of oligonucleotide on RP-IPC (Image)



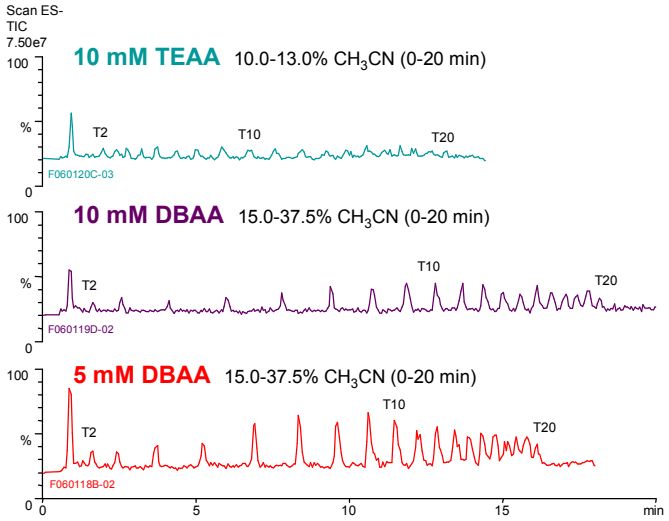
Column	: Hydrosphere C18 (3 µm, 12 nm) 50 X 4.6 mmI.D.	Flow rate	: 1.0 mL/min
Eluent	: A) 100 mM buffer B) 100 mM buffer/acetonitrile (80/20) 50-65%B (0-20 min)	Temperature	: 35ºC
		Detection	: UV at 269 nm
		Injection	: 5 µL (5 nmol/mL)

- Separations of oligodeoxythymidylic acid [d(pT)$_{2\text{-}20}$] under three different buffer systems are compared.
- d(pT)$_{2\text{-}20}$ is well retained under TEAA buffer system, and good separation even by one-nucleotide difference is achieved. On the other hand, retention and separation of d(pT)2-20 under acetate buffer and phosphate buffer are insufficient.
- Ion-pairing reagents that have both positive charge and hydrophobic moiety in molecule, such as triethylamine (TEA) or dibutylamine (DBA), form ion pair with negatively charged oligonucleotides. This interaction contributes to long retention and improvement on resolution.

Applicability to LC/MS analysis

Impact of concentration and types of ion-paring reagent on resolution and signal intensity

Oligodeoxythymidylic acid, [d(T)$_{2\text{-}20}$]

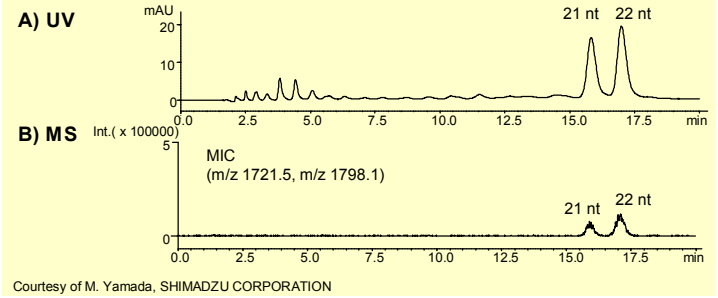


Column	: Hydrosphere C18 (3 µm, 12 nm), 50 X 2.0 mmI.D.
Eluent	: A) 10 mM triethylamine-acetic acid (pH 6.0)
	B) 10 mM triethylamine-acetic acid (pH 6.0)/acetonitrile (80/20) 50-65%B (0-20 min)
	A) 10 mM di-n-butylamine-acetic acid (pH 6.0)
	B) 10 mM di-n-butylamine-acetic acid (pH 6.0)/acetonitrile (50/50) 30-75%B (0-20 min)
	A) 5 mM di-n-butylamine-acetic acid (pH 6.0)
	B) 5 mM di-n-butylamine-acetic acid (pH 6.0)/acetonitrile (50/50) 30-75%B (0-20 min)
Flow rate	: 0.2 mL/min
Temperature	: 35ºC
Detection	: ESI-negative mode
Injection	: 5 µL (5 nmol/mL)

- TEA and DBA are both volatile ion-pairing reagents, and applicable to LC/MS analysis. When comparing the separation characteristics of d(pT)$_{2\text{-}20}$ with those reagents under the same buffer concentration, signal intensity and retention with DBA is superior to that with TEA.
- At 5 mM dibutylamine-acetic acid buffer (DBAA) condition, higher signal intensity of oligonucleotides is achieved even though retention and resolution is slightly decreased. On LC/MS analysis of oligonucleotides, 5-10 mM DBAA/acetonitrile (or methanol) is recommended to be used as a standard mobile phase. And then, it is effective to optimize the types of organic solvent and gradient condition.

LC/MS analysis of miRNA

5'-pUGG AGU GUG ACA AUG GUG UUG-3' (21 nt, MW 6890.1)
5'-pUGG AGU GUG ACA AUG GUG UUG U-3' (22 nt, MW 7196.3)



Courtesy of M. Yamada, SHIMADZU CORPORATION

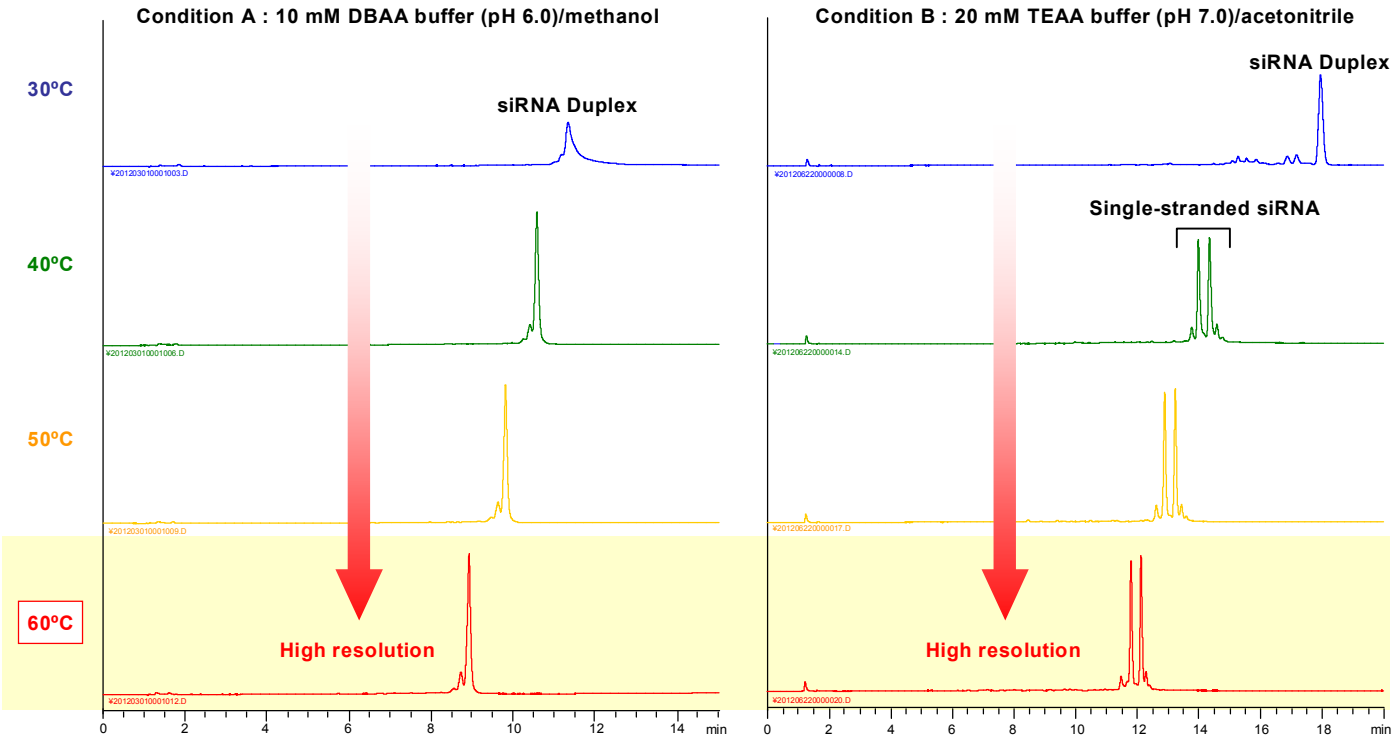
Column	: YMC-Triart C18 (3 µm, 12 nm), 150 X 2.0 mmI.D.
Eluent	: A) 10 mM di-n-butylamine-acetic acid (pH 7.5)
	B) 10 mM di-n-butylamine-acetic acid (pH 7.5)/acetonitrile (50/50) 62-72%B (0-20 min)
Flow rate	: 0.2 mL/min
Temperature	: 30ºC
Detection	: A) UV at 260 nm
	B) ESI-negative mode
Injection	: 4 µL (5 nmol/mL)
System	: LC) Shimadzu Prominence
	MS) Shimadzu LCMS2020

- Mixture of miRNA of 21 nt and 22 nt is separated by using 10mM DBAA/acetonitrile as a mobile phase and detected with MS.

High temperature analysis of oligonucleotides with YMC-Triart C18

Effect of mobile phase and column temperature on separation of siRNA duplex

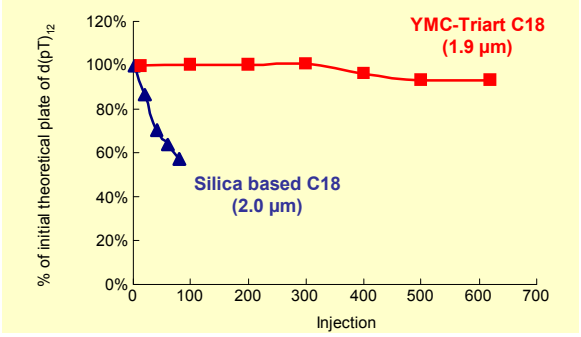
Crude synthetic siRNA duplex (19 bp) : 5'-CGU ACG CGG AAU ACU UCG AdTdT-3'
3'-dTdTGCA UGC GCC UUA UGA AGC U-5'



Column	: YMC-Triart C18 (1.9 µm, 12 nm) 100 X 2.0 mmI.D.	**Condition A** Eluent	: A) 10 mM di-n-butylamine-acetic acid (pH 6.0) B) methanol 35-60%B (0-15 min)
Flow rate	: 0.2 mL/min		
Detection	: UV at 269 nm	**Condition B** Eluent	: A) 20 mM triethylamine-acetic acid (pH 7.0) B) acetonitrile 5-12%B (0-20 min)
Injection	: 1 µL (5 nmol/mL)		
System	: Agilent 1290		

- Separation of siRNA duplex under different mobile phase conditions at various temperatures with YMC-Triart C18 is shown.
- Under both condition A and condition B, peak shape and resolution between immediate peaks is improved by increasing the column temperature.
- Due to the improvement of dispersion and distribution velocity when increasing column temperature, bio-macro molecules such as RNA and DNA generally exhibit sharper peak shape and improved resolution.
- Under condition B at 40 ℃ or higher temperature, two peaks of single-stranded RNA that is generated by denaturation of siRNA duplex are observed. This HPLC technique that is utilizing high temperature to generate single-stranded RNA is called "Denaturing HPLC", and widely used in the field of gene mutation analysis.
- As shown above, denaturation of duplex DNA or RNA is also influenced by ionic strength (type and concentration), pH and polarity as well as temperature. Those analysis conditions (temperature and mobile phase) are recommended to be optimized depending on characteristics of target analyte and purpose of analysis.

Durability at pH 6.0 (DBAA buffer) and 65℃



Test condition		
	Column	: 1.9 µm or 2.0 µm, 12 nm, 50 X 2.0 mmI.D.
	Eluent	: A) 10 mM di-n-butylamine-acetic acid (pH 6.0) B) methanol 30-50%B (0-20 min)
	Flow rate	: 0.4 mL/min
	Detection	: UV at 269 nm
	Temperature	: 65°C
	Sample	: Oligodeoxythymidylic acid, $[d(T)_{2\text{-}20}]$
	Injection	: 1 µL (5 nmol/mL)
	System	: Agilent 1290

- Combination of neutral buffer containing amino ion-paring reagent and high temperature is useful for high-throughput analysis of oligonucleotides or denaturing HPLC. However, conventional silica-based reverse-phase column can hardly used with such condition due to the poor durability.
- YMC-Triart C18 using inorganic/organic hybrid silica with thorough surface modification offers excellent durability at elevated temperature and pH. YMC-Triart C18 is ideal for oligonucleotides analysis.