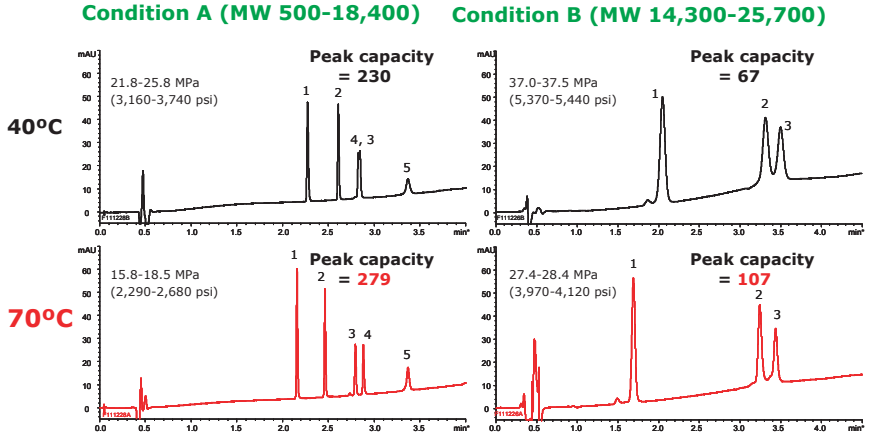
YMC SEPARATION TECHNOLOGY

HPLC DATA SHEET

High efficiency RP-HPLC separation of peptides and proteins using high-temperature

S120515AE

Comparison of separation of peptides and proteins between at 40℃ and 70℃

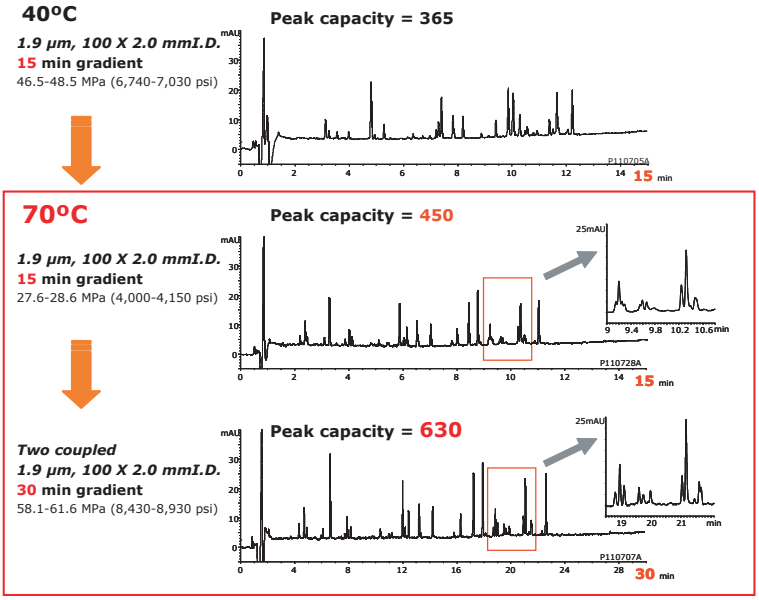


Analytes	MW	Peak width ½ (min)	
		40℃	70℃
Condition A			
1. Oxytocin	1,007	0.017	0.014
2. Leu-Enkephalin	556	0.015	0.015
3. β-Endorphin	3,465	-	0.016
4. Insulin	5,733	-	0.015
5. β-Lactoglobulin A	18,400	0.043	0.030
Condition B			
1. Lysozyme	14,300	0.069	0.044
2. α-Chymotrypsinogen	25,700	0.080	0.049
3. β-Lactoglobulin A	18,400	0.080	0.048

Column : YMC-Triart C18 (1.9 µm, 12 nm), 50 X 2.0 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1) - condition A
B) acetonitrile/IPA/TFA (50/50/0.1) - condition B
Gradient : 10-80%B (0-5 min) - condition A
30-60%B (0-5 min) - condition B
Flow rate : 0.4 mL/min
Detection : UV at 220 nm
Injection : 1 µL (50 µg/mL) - condition A
1 µL (250 µg/mL) - condition B
System : Agilent 1200SL

- The separation of peptides and proteins with a variety of molecular weight (MW) is compared increasing column temperature from 40℃ to 70℃.
- Although adding stronger solvent like IPA to acetonitrile of the mobile phase (condition B) is effective to reduce larger protein retention and improve peak shape, the molecules with MW >10,000 still result in peak broadening at 40℃, as shown in the upper chromatograms.
- Increasing column temperature to 70℃ provides selectivity change, sharper peaks, and therefore, improved resolution especially for larger molecules. Generally, larger molecules diffuse very slowly compared to small molecules. An elevated temperature can improve efficiency and peak shape by lowering mobile phase viscosity and improving mass transfer, and the appropriate MW range for pore size of packing materials can be more expanded than that at a lower temperature.
- Temperature is a simple and effective tool to increase resolution in separation of proteins and peptides.

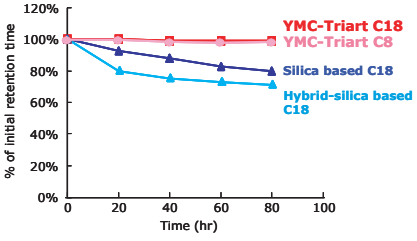
Improvement of resolution by increasing column temperature and coupling of 1.9 µm columns



- 23% more peaks can be resolved by increasing the column temperature to 70℃ in the separation of tryptic digest of Hemoglobin.
- The outstanding efficiency obtained by a coupling of two 100 mm length of Triart 1.9 µm columns reduces co-elution peaks and allows the precise separation in an analysis of complicated samples, such as peptide mapping.

Column : YMC-Triart C18 (1.9 µm, 12 nm)
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.08)
5-40%B (0-15 min) for a single column
5-40%B (0-30 min) for two coupled columns
Flow rate : 0.4 mL/min
Detection : UV at 220 nm
Injection : 10 µL for a single column
20 µL for two coupled columns
Sample : Tryptic digest of Bovine Hemoglobin
System : Agilent 1290

Comparison of retention stability of RP columns at pH 1 (1% TFA) and 70℃



Test condition

The columns are kept in acetonitrile/water/TFA (10/90/1, pH 1) at 70°C, and tested for performance every 20 hours under the condition of column inspection below.

[Column inspection]
Column : 5 µm, 50 X 2.0 mmI.D.
Eluent : acetonitrile/water (60/40)
Flow rate : 0.2 mL/min
Temperature : 37°C
Sample : butyl benzoate

- Newly developed hybrid particles and surface modification of Triart C18 and C8 provide excellent durability in the difficult conditions such as strongly acidic at elevated temperature (1% TFA, 70℃).This advantage enables a rapid and efficient method development of a complex mixture of peptides and proteins.