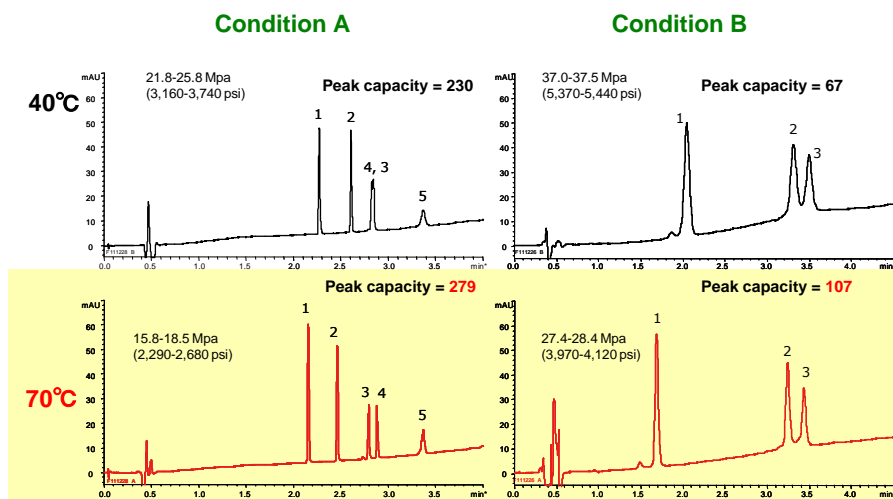


## Tips for optimization of peptides and proteins separation

R150610AE

### Effect of column temperature on separation of peptides and proteins

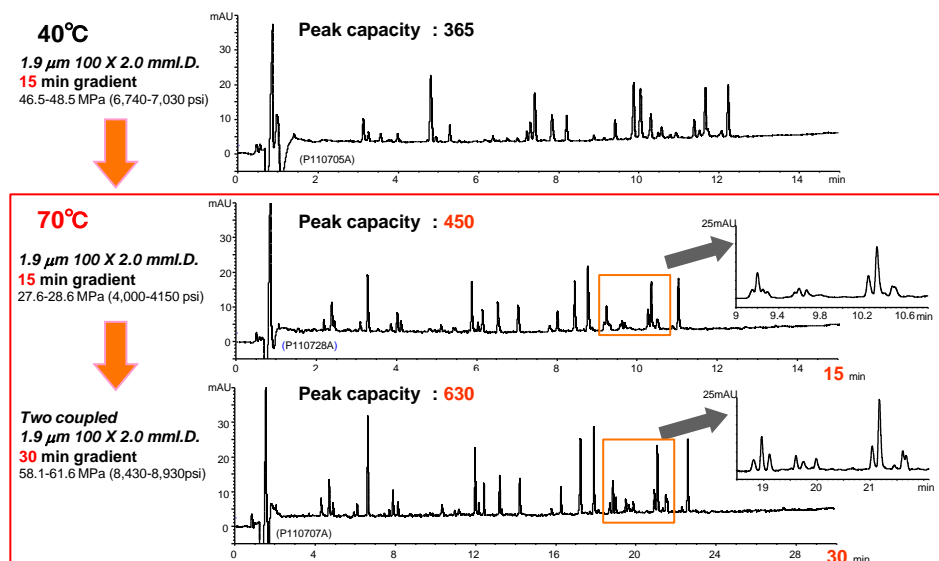


Analytes	MW	Peak width 1/2(min)	
		40°C	70°C
<b>Condition A</b>			
1. Oxytocin	1,007	0.017	0.014
2. Leu-Enkephalin	556	0.015	0.015
3. $\beta$ -Endorphin	3,465	-	0.016
4. Insulin	5,733	-	0.015
5. $\beta$ -Lactoglobulin A	18,400	0.043	0.030
<b>Condition B</b>			
1. Lysozyme	14,300	0.069	0.044
2. $\alpha$ -Chymotrypsinogen	25,700	0.080	0.049
3. $\beta$ -Lactoglobulin A	18,400	0.080	0.048

Column : YMC-Triart C18 (1.9  $\mu$ m, 120  $\text{\AA}$ ), 50 X 2.0 mmI.D.  
 Eluent : A) water/TFA (100/0.1)  
 B) acetonitrile/TFA (100/0.1) – condition A  
 C) acetonitrile/2-propanol/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

- The effect of temperature on separation of peptides and proteins with a variety of molecular weight (MW) is estimated. The separations at 40°C and 70°C are compared.
- By increasing column temperature to 70°C, selectivity change is observed, and peaks become sharper. Thus, improved resolution especially for larger molecules is obtained. 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.
- Temperature is a simple and effective tool to increase resolution in separation of proteins and peptides.

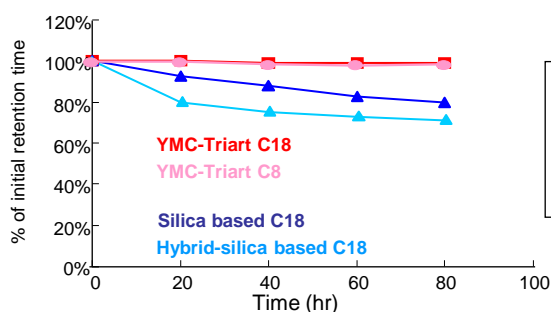
### Improvement of resolution by increasing column temperature and coupling of 1.9 $\mu$ m columns



- 23% more peaks can be resolved by increasing the column temperature to 70°C in the separation of tryptic digest of Hemoglobin.
- The outstanding efficiency obtained by a coupling of two 100 mm length of Triart 1.9  $\mu$ m 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  $\mu$ m, 120  $\text{\AA}$ )  
 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  
 Sample : Tryptic digest of Bovine Hemoglobin

### Durability in pH 1 (1% TFA), 70°C



**Test conditions**  
 Column : 5  $\mu$ m, 50 X 2.0 mmI.D.  
 Eluent : acetonitrile/water (60/40)  
 Flow rate : 0.2 mL/min  
 Temperature : 37°C  
 Sample : butyl benzoate

The columns are kept in acetonitrile/water/TFA (10/90/1, pH 1) at 70°C, and tested for performance every 20 hours

- YMC-Triart C18, which offers excellent durability even under elevated temperature and low-pH conditions, is effective for the separation of peptides and proteins.