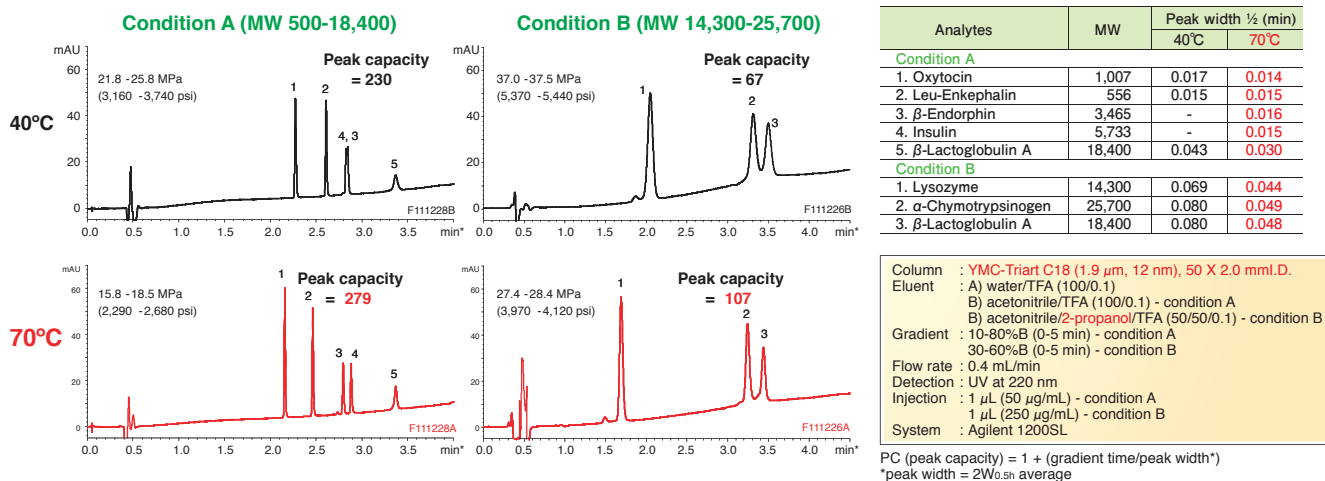


Effect of column temperature on separation of peptides and proteins



The effect of temperature on separation of peptides and proteins with a variety of molecular weights (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, and improved resolution especially for larger molecules is obtained. Generally, larger molecules diffuse very slowly compared to small molecules.

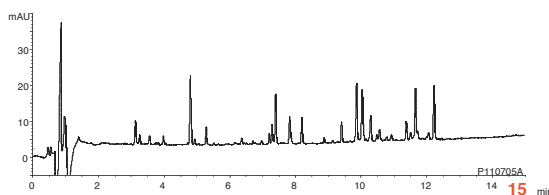
An elevated temperature can improve efficiency and peak shape by lowering mobile phase viscosity and improving mass transfer. Temperature is a simple and effective tool to increase resolution in separation of proteins and peptides.

Improvement of resolution by increasing column temperature and coupling of 1.9 μ m columns

40°C

1.9 μ m, 100 X 2.0 mm I.D.
 15 min gradient
 46.5-48.5 MPa (6,740-7,030 psi)

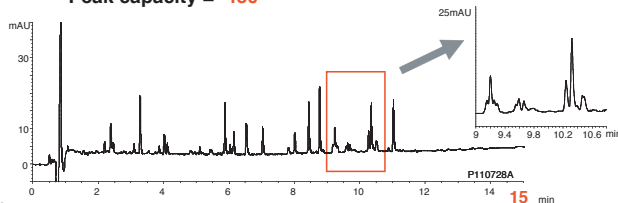
Peak capacity = 365



70°C

1.9 μ m, 100 X 2.0 mm I.D.
 15 min gradient
 27.6-28.6 MPa (4,000-4,150 psi)

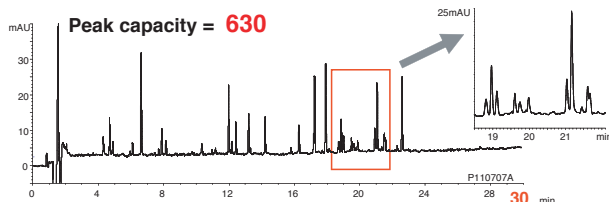
Peak capacity = 450



Coupling of two columns

Two coupled
 1.9 μ m, 100 X 2.0 mm I.D.
 30 min gradient
 58.1-61.6 MPa (8,430-8,930 psi)

Peak capacity = 630



Column : YMC-Triart C18 (1.9 μ m, 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 μ L for a single column
 20 μ L for two coupled columns
 Sample : Tryptic digest of Bovine Hemoglobin
 System : Agilent 1290

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 μ m columns reduces co-elution of peaks and allows the precise separation in an analysis of complicated samples, such as peptide mapping.