YMC SEPARATION TECHNOLOGY

HPLC DATA SHEET

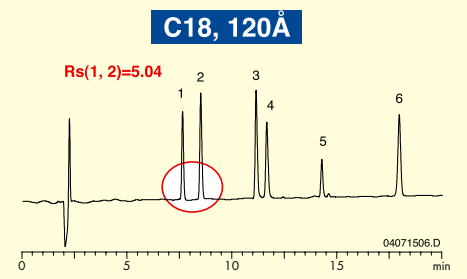
For setting separation conditions

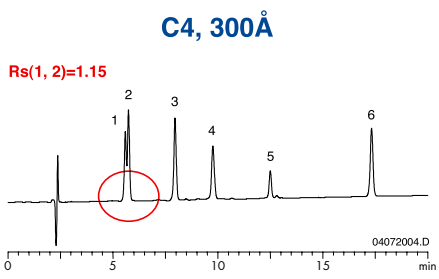
Resolution improvement using columns with different pore sizes and functional groups.

S050715A

During the optimization of separation conditions, changing the composition of the mobile phase is an effective way to influence retention and resolution of compounds. Proper column selection can also change retention and resolution between peaks.
Wide pore columns are normally used for separation of proteins and peptides with high molecular weight. To achieve better separation, it is important to select appropriate functional groups and pore sizes depending on the molecular weight and the characteristics of the targeted compounds.

Separation of peptides (MW 574-3465)





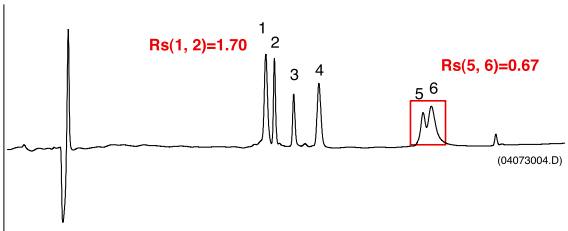
1. Oxytocin (MW 1007)
2. Met-Enkephalin (MW 574)
3. Leu-Enkephalin (MW 556)
4. Neurotensin (MW 1673)
5. γ-Endorphin (MW 1859)
6. β-Endorphin (MW 3465)

Column	: 150 X 4.6 mmI.D.
Eluent	: A) water / TFA (100/0.1), B) acetonitrile / TFA (100/0.1) 20-40%B (0-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm

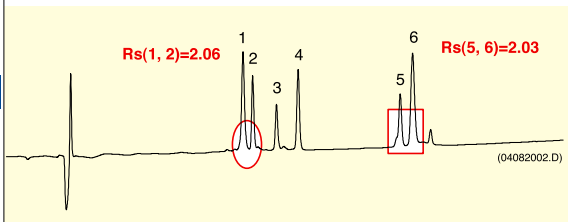
C18 column in 120Å is suitable for separation of peptides with molecular weights below 5000.

Separation of peptides and proteins (MW 4300-17000)

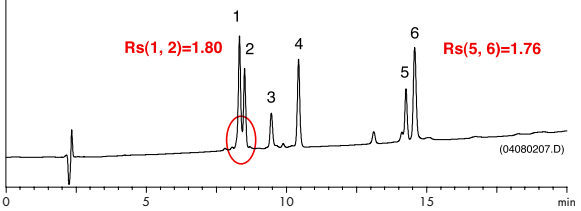
C18, 120Å



C8, 200Å



C4, 300Å

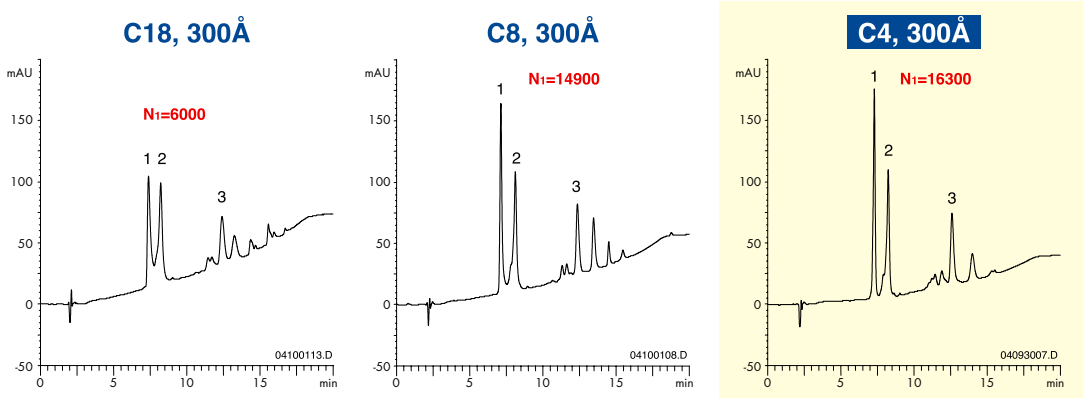


1. Cytochrome *c* (MW 12400)
2. Insulin (MW 5700)
3. Amyloid β-protein (MW 4300)
4. Lysozyme (MW 14400)
5. α-Lactalbumin (MW 14200)
6. Myoglobin (MW 17000)

Column	: 150 X 4.6 mmI.D.
Eluent	: A) water / TFA (100/0.1) B) acetonitrile / TFA (100/0.1) 25-60%B (0-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm

In analysis of proteins and peptides with molecular weights from approximately 5000 to 20000, C8 column in 200Å can obtain better separation than that on C18 column in 120Å and C4 column in 300Å.

Separation of proteins (MW 66000-96000)



1. BSA (MW 66000)
2. Conalbumin (MW 77000)
3. Lipoxidase (MW 96000)

Column	: 150 X 4.6 mmI.D.
Eluent	: A) water / TFA (100/0.1), B) acetonitrile / 2-propanol / TFA (50/50/0.1) 30-75%B (0-15 min), 75%B (15-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 210 nm

For separation of proteins with molecular weight more than 20000, C4 that has low hydrophobicity is a suitable column in 300Å pore size to effect adequate separations of these higher molecular weight species.