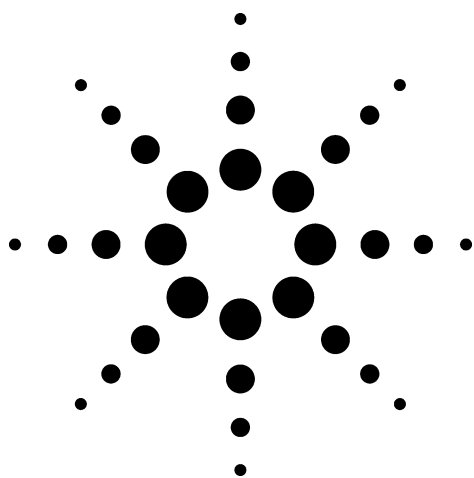
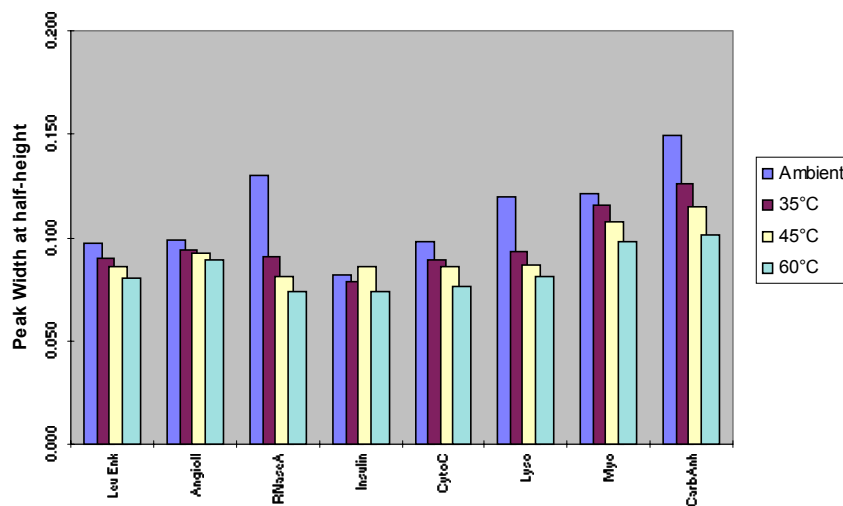




Effect of Temperature Elevation on Resolution and Retention of Polypeptides

Application
Technical
Robert Ricker

Separation of peptides and large proteins using conventional HPLC methods at room temperature can result in poor resolution and peak shape. Using elevated temperatures for the reverse-phase separation of peptides and proteins can increase column efficiency and improve peak shape.



Highlights

- Wide-pore, sterically protected ZORBAX Stablebond columns show excellent improvement in peak width of proteins and peptides when temperature is elevated from ambient to 60°C.
- Sterically protected ZORBAX bonded phases are extremely stable at high temperature and low pH (TFA).

	PW1/2*					Retention Time (min)			
	Ambient	35°C	45°C	60°C		Ambient	35°C	45°C	60°C
Leu Enk	0.097	0.090	0.086	0.080	Leu Enk	5.21	4.72	4.42	4.00
Angiot	0.099	0.094	0.092	0.089	Angiot	5.66	5.20	4.89	4.43
RNaseA	0.130	0.091	0.081	0.074	RNaseA	8.62	8.48	8.32	7.99
Insulin	0.082	0.079	0.086	0.074	Insulin	10.21	10.58	10.84	11.06
CytoC	0.098	0.089	0.086	0.076	CytoC	11.09	11.00	10.84	10.58
Lyso	0.120	0.093	0.087	0.081	Lyso	13.13	13.03	12.82	12.36
Myo	0.121	0.116	0.108	0.098	Myo	16.50	16.64	16.56	16.16
CarbAnh	0.149	0.126	0.115	0.101	CarbAnh	17.53	17.90	18.03	18.00

*PW1/2 = peak width at half-height

Conditions:

ZORBAX 300 SB-C3 (4.6 x 150 mm, Agilent P/N: 883995-909)

Mobile Phase: 15-53% in 20 min., posttime 12 min.,

A: 5:95 ACN:Water with 0.10 % TFA (v/v%) B: 95:5 ACN:Water with 0.085% TFA (v/v%)

Injection 10µL, 2- 6µg protein (in 6M Guanidine HCl pH 7.0),

1.0mL/min., ambient- 60° C, Detect. UV(215 nm)



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