

Effect of Elevated Temperature on Resolution and Retention of Polypeptides

Application **Biochemical**

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, change selectivity, and improve peak shape.



Highlights

- Wide-pore (300Å), sterically protected, ZORBAX StableBond columns show an improvement in peak width when the temperature of the column is elevated from ambient to 60°C.
- Sterically protected ZORBAX 300SB bonded-phases are extremely stable at high temperature and low pH (TFA).
- Selectivity of peaks 4 and 5 is ٠ dramatically changed (peak position is reversed) with increase in column temperature.
- High temperature brings out hidden minor components preceding peaks 3 and 6.

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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