Monitoring the Purification of Synthetic Amyloid Peptide Ab (1-42) by High-Temperature Reversed-Phase HPLC

Application

Biochemical

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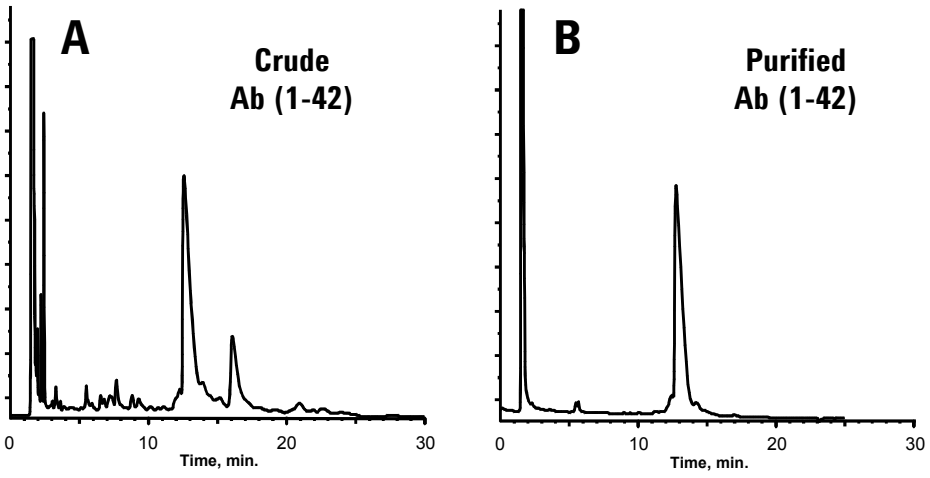
This example illustrates the use of elevated column temperature to examine the purity of isolated Ab (1-42) peptide. The high column temperature, 80°C, is required to obtain good recovery (>75%) and peak shape for this highly hydrophobic and aggregation-prone peptide. Chromatography at lower temperature results in increasing loss of the peptide (5% recovery at 25°C), and marked band-tailing. This example compares the purity of the crude synthetic peptide in Panel A, to the purified peptide in Panel B. The analysis takes advantage of isocratic separation (no re-equilibration time) using highly efficient 3.5 µm particle diameter, 300Å pore size, Agilent ZORBAX SB-C18 column packing material. The high stability of the StableBond packing permits operation at elevated temperature and low pH, without damage to the column.

Highlights

- *High temperature separation enhances recovery and improves peak shape for this badly behaved peptide.*
- *Highly stable, sterically-protected silane chemistry permits the use of extreme separation conditions, without the consequence of rapid column break down.*
- *Small particle-diameter packing material results in improved resolution.*



Conditions:
LC: HP1090
Columns: ZORBAX 300SB-C18, 3.5µm, 4.6 x 150mm, Agilent P/N: 863973-902
Mobile Phase: 29% ACN / 0.1% TFA in Water; 80°C
Sample: 20µL, Ab(1-42); Flow: 1.0 mL / min.; UV 210 nm

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