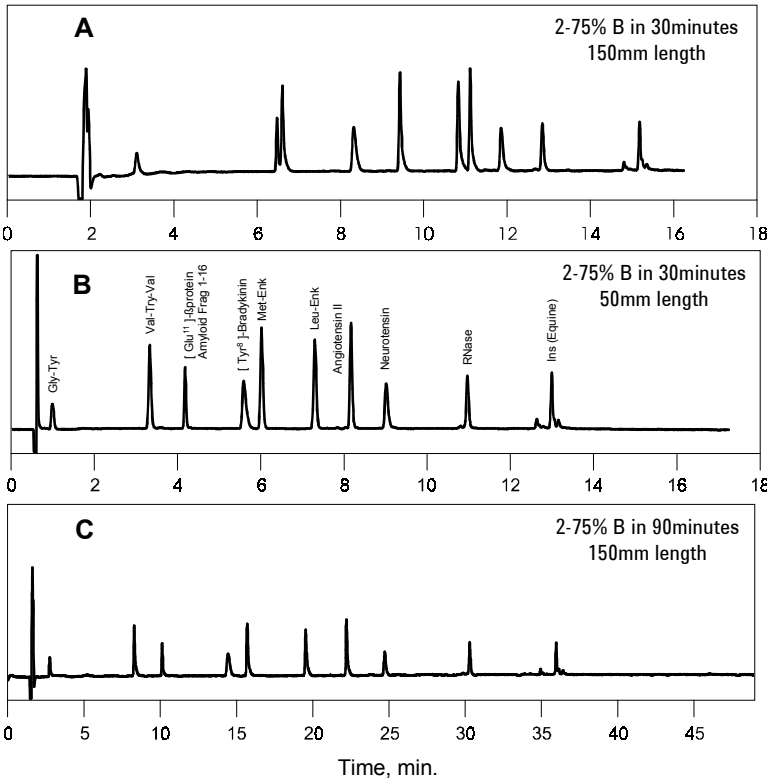
Improving Gradient Resolution by Reducing Column Length: ZORBAX 300SB-C8

Application

Technical

Robert Ricker

Improvement in gradient resolution may be accomplished by decreasing column length (column volume, Vm) without increasing analysis time. A sample containing peptides and proteins was chromatographed on ZORBAX 300SB-C8's of different lengths, keeping all other parameters constant. The results in panel B (50-mm length) show an increase in resolution for most components when compared to Panel A (150-mm column length). Panel C illustrates the increase in run time necessary to achieve the improved separation using a 150-mm column.



Conditions:
ZORBAX 300SB-C8, (3.5µm; 4.6 x 50 mm, 150mm) (Agilent P/N: 865973-906, 863973-906)
Mobile Phase: A: 95:5 Water:ACN, 0.10% TFA (v/v%) B: 5:95 Water:ACN, 0.085% TFA (v/v%)
Gradient: as indicated in panels
Injection: 10µL, 1-6 µg protein, 1 mL/min, 35°C, Detect. UV(215 nm) micro flow cell

Highlights

- *Resolution, selectivity, and signal response can be improved by reducing column length instead of changing gradient conditions.*
- *ZORBAX 300SB-C8 columns are highly stable and reproducible batch-to-batch and for various column configurations.*

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Printed in the USA
April 25, 2002
5988-6436EN

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