Improved LC Analysis of Human Serum Albumin Tryptic Digest Using 1.8-µm ZORBAX Rapid Resolution HT SB-C18 Column

Application

Proteomics

William E. Barber

Introduction

The liquid chromatography (LC) analysis of complex biological fluids is significantly improved using fine particle columns. This is demonstrated by a comparison of two columns, differing in length and particle size, on the analysis of a tryptic digest of human serum albumin (HSA). The chromatographic comparison is shown in Figure 1.

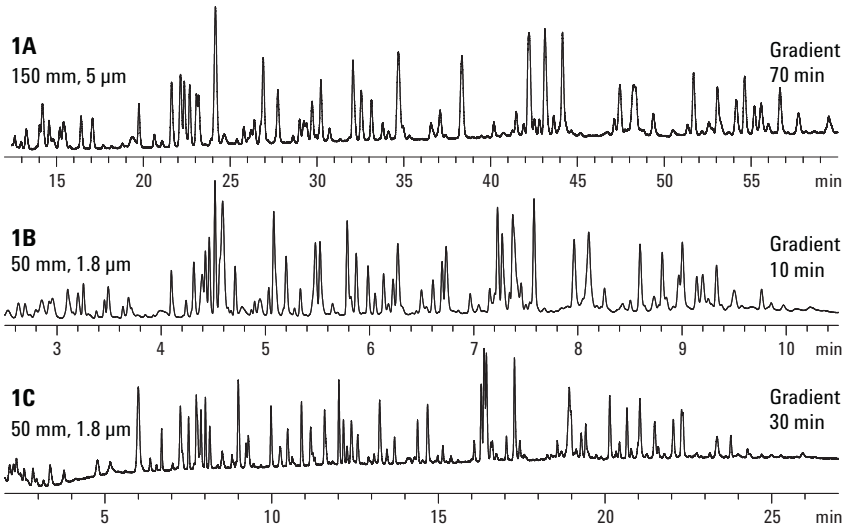

Figure 1. Comparison of LC chromatograms achieved using ZORBAX SB-C18 columns differing in column length, particle size, and gradient profile.

Conditions

Instrument	Agilent 1100LC
Column	**1A: ZORBAX SB-C18** (2.1 × 150 mm, 5 µm) p/n 883700-922 **1B and 1C: ZORBAX Rapid Resolution HT SB-C18** (2.1 × 50 mm, 1.8 µm) p/n 822700-902 SB-C18 was 80 Å for all columns
Temperature	50 °C
Sample concentration	15 pmol/µL
Injection	8 µL (120 pmol total on-column)

The Agilent 1100 LC was optimized to minimize peak broadening by using 0.12-mm id tubing from the autosampler to the diode array detector (DAD) and a 1.7-µL flowcell. Peaks were measured using the 214-nm absorption band.

Highlights

- High resolution and rapid analysis using Agilent Rapid Resolution High Throughput (RRHT) columns is demonstrated for a sample of HSA tryptic digest
- ZORBAX SB-C18, 2.1 × 50 mm, 1.8-µm columns provide better resolution than, and comparable sensitivity to 150-mm columns, but in one-sixth the time.

Mobile phase

A: 0.1% TFA in H_2O

B: 0.1% TFA in ACN

Gradient timetable

Run	Flow mL/min	Gradient 2% to 50% B
1A	0.2	70 min
1B	0.5	10 min
1C	0.5	30 min

Observations

Run	X/Y Peaks*	Window, min	Peak widths
1A (5 µm)	90/120	60	12–18 s
1B (1.8 µm)	102/125	10	3–6 s
1C (1.8 µm)	134/156	25	6–12 s

* There were X total peaks for the window shown out of Y peaks for the entire chromatogram

Results

Figure 1A shows the results obtained for a column (2.1 × 150 mm, 5 µm) and gradient conditions in common use for the gradient HPLC analysis of HSA tryptic digests. Using a shorter 50-mm column of the same phase, but with smaller (1.8 µm) particles, higher resolution is obtained in one-sixth the time, or 500% faster, (Figure 1B) due to the combined effects of using a much smaller particle, reducing the column length by a third, increasing the flow rate by a factor of 2.5 and reducing the gradient time from 70 min to 10 min. The resolution can be increased further (Figure 1C) by increasing the gradient time (shallower gradient) from 10 min to 30 min while the analysis time is still less than half that of the original conditions.

Conclusion

LC separation of HSA tryptic digest components is efficiently performed using short RRHT columns with 1.8-µm particles.

Column Types and Part Numbers for ZORBAX Columns Used in this Study

Run	Column type	Dimensions	Part number
1A	ZORBAX SB-C18	2.1 × 150 mm, 5 µm	883700-922
1B	ZORBAX SB-C18	2.1 × 50 mm, 1.8 µm	822700-902
1C	ZORBAX SB-C18	2.1 × 50 mm, 1.8 µm	822700-902

For More Information

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The author, William E. Barber, is a LC Applications Specialist based at Agilent Technologies, Wilmington, Delaware.

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