Agilent ZORBAX 300SB-C18 1.8 µm Rapid Resolution High Definition Columns for Proteins

Technical Overview

Introduction

Agilent ZORBAX RRHD 300SB-C18 1.8 µm is a new reverse phase media for UHPLC of proteins and peptides. The use of 1.8 µm particles in a column designed for UHPLC systems significantly reduces analysis time in HPLC, critical for increasing the efficiency of QC for protein primary structure analysis.

The eluents routinely employed for reverse phase analysis are acidic, containing trifluoroacetic acid or formic acid, which can limit the lifetime of many HPLC columns. However, by using StableBond technology it is possible to produce a 300Å pore-size media that is stable under acidic conditions, to provide the robust reproducible separations required for protein analysis.

Intact protein analysis

Short 50 mm columns are used to separate and resolve intact proteins. In these examples, different flow rates, from 0.5 mL/min to 1.0 mL/min, and temperatures, from 60 °C to 50 °C, are used to demonstrate the effect of flow rate on efficiency. As expected, higher flow rates improve efficiency.

The effect of three different flow rates is shown in Figures 1, 3, and 5. Figure 7 shows the separation at 50 °C, with a slight improvement in separation at this temperature which is below the boiling point of the solvent. Base line separations are given in Figures 2, 4, 6 and 8.

Conditions

Column	Agilent ZORBAX RRHD 300SB-C18, 2.1 x 50 mm, 1.8 µm (p/n 857750-902)
Sample	Sigma Protein Standards (ribonuclease A, cytochrome C, transferrin, myoglobin)
Sample conc	1 mg/mL
Inj vol	5 µL
Eluent	A, 0.1% TFA in water; B, 0.085% TFA in ACN
Gradient	20% B 0.5 min, 20-60% B 2 min, 60-90% B 0.5 min, 90% B 1 min, 90-20% B 0.1 min, 20% B 0.9 min
Temp	as indicated
Flow rate	as indicated
Pressure	as indicated
System	Agilent 1290 Infinity LC

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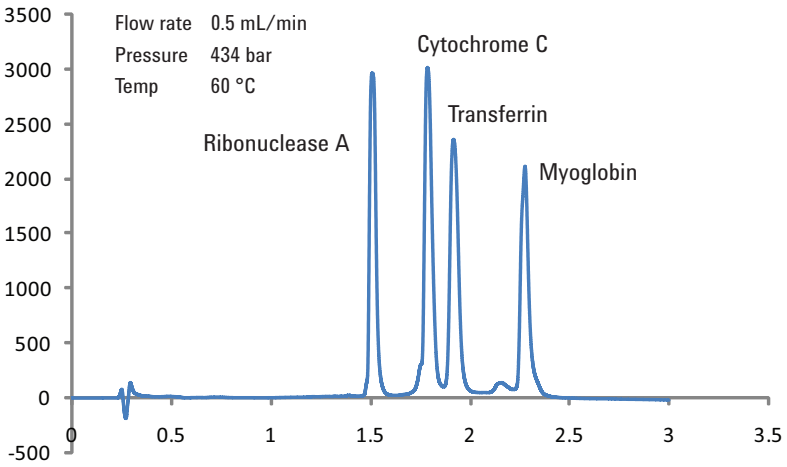

Figure 1. Protein standards on an Agilent ZORBAX RRHD 300SB-C18, 2.1 x 50 mm, 1.8 µm column at 0.5 mL/min.

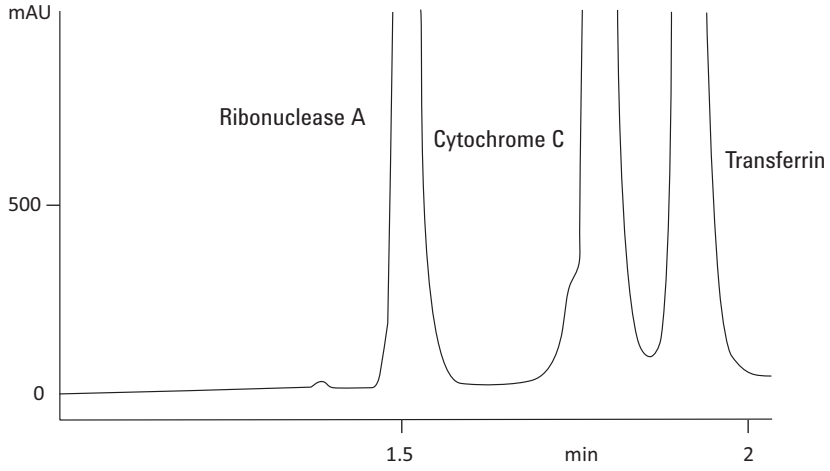

Figure 2. Base line expansion of Figure 1.

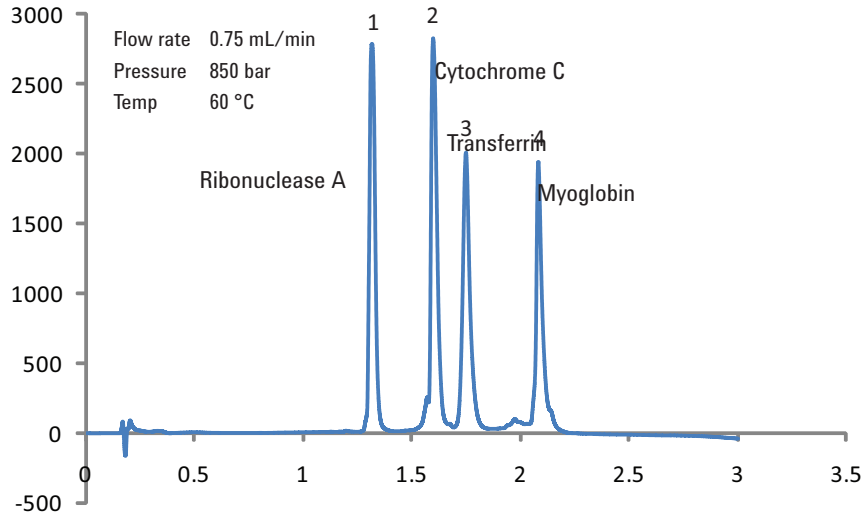

Figure 3. Protein standards on Agilent ZORBAX RRHD 300SB-C18, 2.1 x 50 mm, 1.8 µm at 0.75 mL/min.

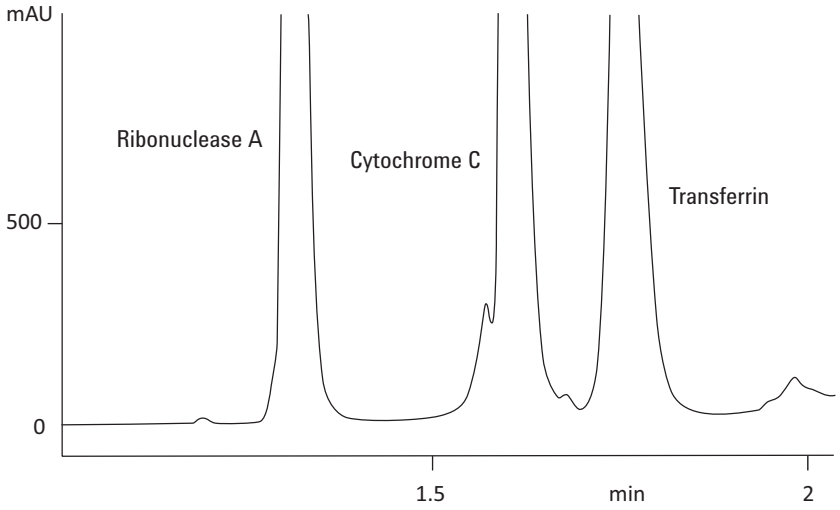

Figure 4. Base line expansion of Figure 3.

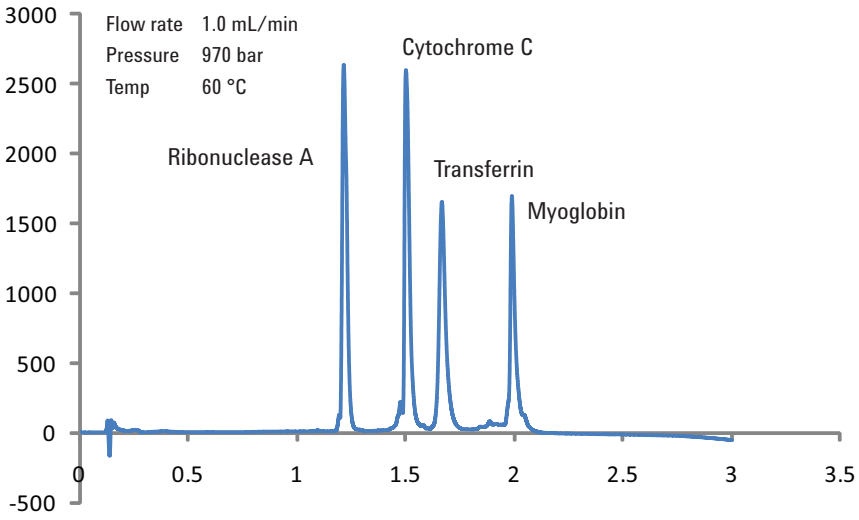

Figure 5. Protein standards on an Agilent ZORBAX RRHD 300SB-C18, 2.1 x 50 mm, 1.8 µm column at 1.0 mL/min.

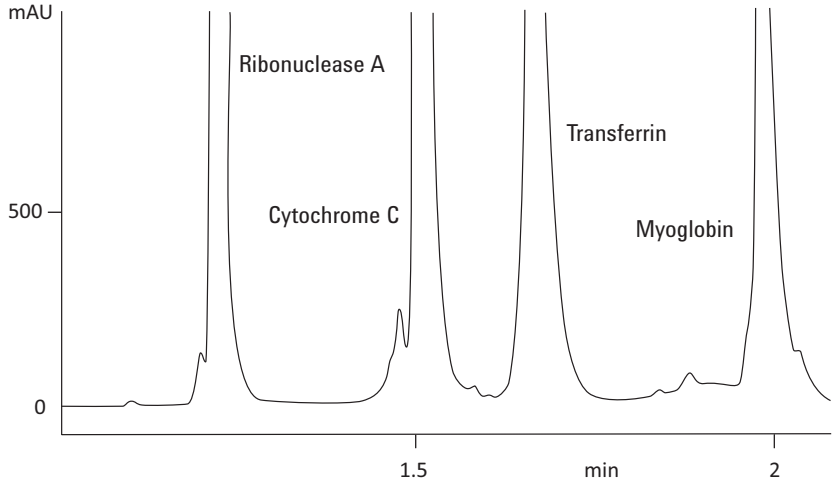

Figure 6. Base line expansion of Figure 5.

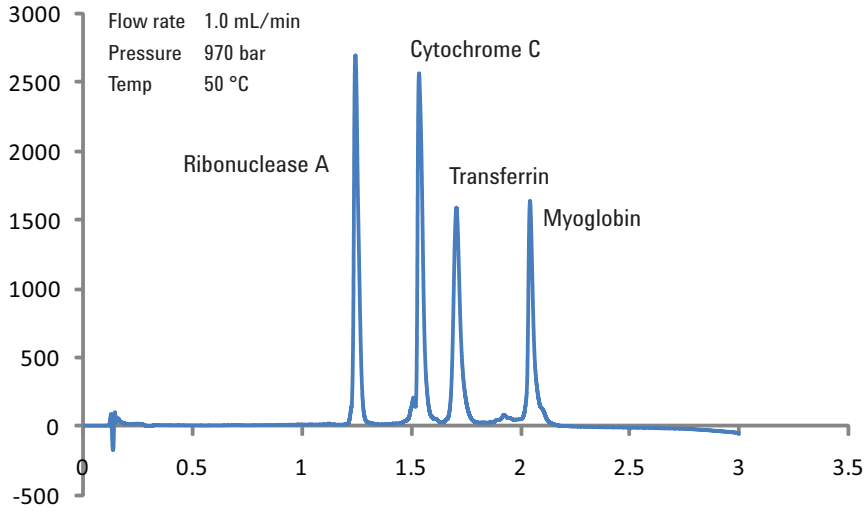

Figure 7. Protein standards at reduced temperature on an Agilent ZORBAX RRHD 300SB-C18, 2.1 x 50 mm,1.8 µm column.

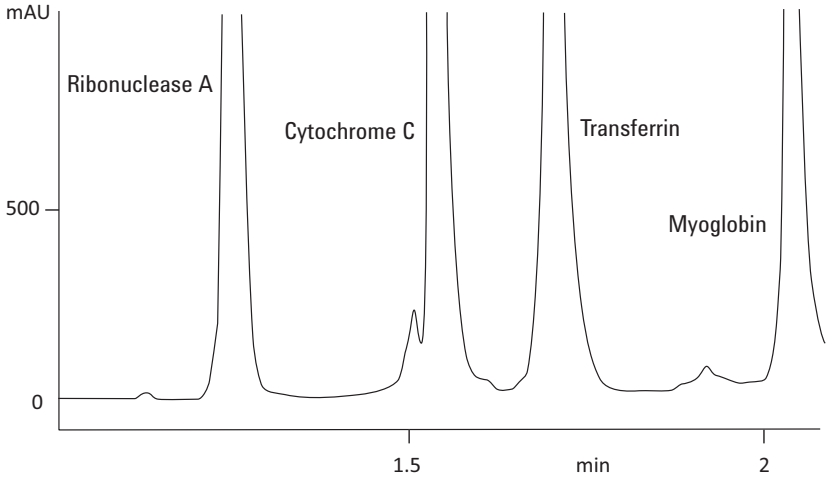

Figure 8. Base line expansion of Figure 7

Protein digest analysis

The same ZORBAX packing is used in longer 100 mm columns, for the analysis of peptide components and enzymatically digested proteins to identify changes in the primary amino acid sequence and amino acid modifications (Figure 9). Reproducibility of the column after 30 runs is shown in Figure 10.

Conditions

Column	Agilent ZORBAX RRHD 300SB-C18, 2.1 x 100 mm,1.8 µm (p/n 858750-902)
Sample	Protein digest
Sample conc	1 mg/mL
Inj vol	5 µL
Eluent	A, 0.1% TFA in water; B, 0.085% TFA in ACN
Gradient	20% B 1 min, 2-45% B 8.8 min, 45-95% B 0.2 min, 95% B 2 min, 98-2% B 0.2 min, 210% B 1.8 min
Temp	50 °C
Flow rate	0.5 mL/min
Pressure	~640 bar
System	Agilent 1290 Infinity LC

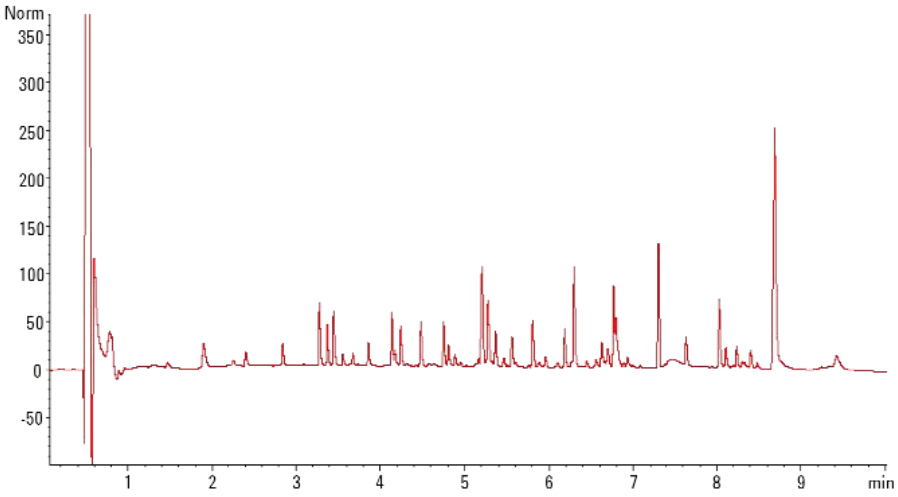

Figure 9. Peptide digest separation on an Agilent ZORBAX RRHD 300SB-C18, 2.1 x 100 mm, 1.8 µm column.

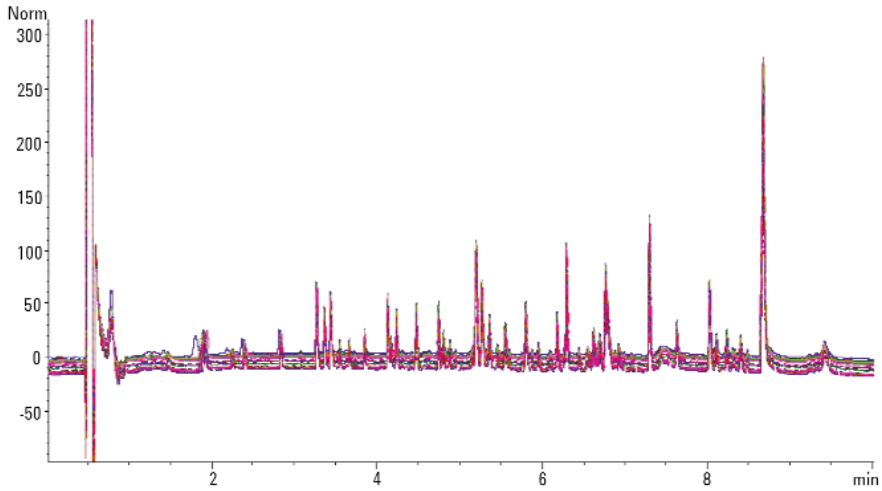

Figure 10. Overlaid chromatograms of 30 runs of a protein digest on an Agilent ZORBAX RRHD 300SB-C18, 2.1 x 100 mm, 1.8 µm column.

Agilent ZORBAX columns for proteins

Analyzing intact biotherapeutic proteins and peptide aliquots is fast and straightforward with Agilent ZORBAX RRHD 300SB-C18 1.8 µm columns. The column's rapid resolution high definition technology permits high pressure UHPLC, while the StableBond 300Å pore-sized particles are robust when analysis requires acidic conditions. Reproducibility is excellent, with good resolution, asymmetry and efficiency. The columns are ideal for protein primary sequence analysis.

Look at the Agilent Literature Library on www.agilent.com for a comprehensive range of application notes and technical overviews to help you get the best from your Agilent HPLC and UHPLC columns and instruments.

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Printed in the USA
June 8, 2011
5990-7989EN

Agilent Technologies