

Excellent Protein Separations from Viva™ HPLC Columns

Best Performance Among Five Tested Wide Pore Columns

By Bruce Albright, HPLC Chemist; Vernon Bartlett, HPLC Manager; Julie Kowalski, Foods, Flavors, and Fragrances Innovations Chemist; and Becky Wittrig, Ph.D., HPLC Product Marketing Manager

- Best overall performance among five columns evaluated.
- Best resolution and peak symmetry for test proteins.
- C18, C8, C4, and silica columns available; other phases on request.

Reversed phase HPLC is an important technique for separating large biomolecules, such as proteins and peptides. Analysts generally employ C18 stationary phases, because these typically provide the best separations of related compounds, such as genetic variants of a protein or complex tryptic digests. However, limitations often are encountered when analyzing samples containing complex mixtures of closely related analytes. Columns containing wide pore silica (e.g., 300Å) are designed specifically for large molecule analyses, addressing this need for more resolving power.

Developed on Viva™ wide pore silica, Viva™ HPLC columns have ideal performance characteristics for separating large molecules and biomolecules. Using a reversed phase test mix, we compared column efficiency, peak asymmetry, and retention for Viva™ C18 columns and four other C18 wide pore HPLC columns. The Viva™ C18 column ranked highest in retention and selectivity and produced the best peak symmetry measurements (Table I).

To determine overall separating power, retention, and peak shape, we evaluated each column with a protein test mix. The Viva™ C18 column provided excellent resolution and peak shapes, as Figure 1 shows.

300Å silicas enhance resolution of similar or related analytes for several reasons. Large pore materials can provide greater retention because higher molecular weight analytes can enter more of the pores and access more surface area. Theoretically, the more surface to which an analyte has access, the longer the retention. For analytes with molecular weights greater than 3000, silica materials with pore diameters in the 250-350Å range yield the needed retention. Further, the mean pore diameter within the distribution (e.g., 250Å vs 350Å) can define the selectivity in some separations, by changing the elution order for certain analytes.

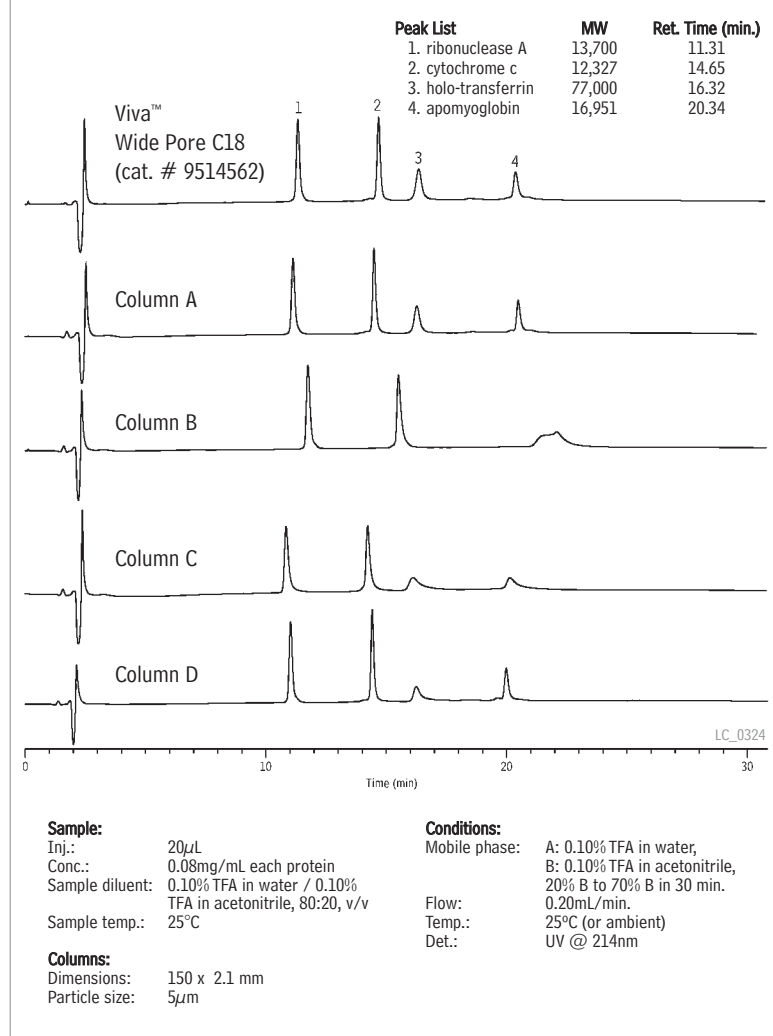
A 250-350Å mean pore diameter also is important because silicas with excessive numbers of pores smaller than 200Å can be more easily fouled by

Table I Viva™ wide pore C18 columns provide the best overall performance among five tested columns.

Column	Efficiency (plates/meter)	Asymmetry (biphenyl)	Retention Time (biphenyl)	Column Pressure (bar)
Viva™ 300 C18	>50,000	1.16	6.30	60
Column A C18	~50,000	1.46	5.77	72
Column B C18	>50,000	1.46	4.96	102
Column C C18	>50,000	1.30	5.89	66
Column D C18	<50,000	1.49	3.79	80

Reversed phase test mix; 150 x 2.1mm C18 phase columns, 5µm particles

Figure 1 Analysis of a four protein test mix shows the superior performance of the Viva™ C18 column.



larger molecular weight debris, and silicas with a high percentage of pores larger than 500Å can be impractically fragile for conventional HPLC applications. A narrow distribution around the mean pore diameter is advantageous; it better ensures that proper selectivity is maintained, and aids in separating closely related analytes that differ only slightly in hydrodynamic volume (molecular size in solution).

We developed and introduced Viva™ wide pore silica specifically to meet these challenging criteria. Among the materials we have tested, this new silica provides the greatest available surface area represented by 250-350 Å pores, with a highly desirable pore volume and pore diameter distribution (The Restek Advantage, 2005v1).

Superior physical characteristics and strong test performances show Viva™ HPLC columns are an excellent choice for analyzing proteins, peptides, or other large molecules or biomolecules. C18, C8, C4, and silica columns currently are available; other phases can be prepared on request. If you require a wide pore silica column for your analysis, we highly recommend new Viva™ columns.

Viva™ Wide Pore HPLC Columns

- Excellent for separating peptides or proteins.
- Rugged, spherical particles, with 300Å pore size.
- High proportion of pore/surface area available to large molecules.

Length	1.0mm ID		2.1mm ID		3.2mm ID		4.6mm ID	
	cat.#	cat.#	cat.#	cat.#	cat.#	cat.#	cat.#	cat.#
Viva™ Wide Pore C18 Columns, 5µm								
30mm	9514531		9514532		9514533		9514535	
50mm	9514551		9514552		9514553		9514555	
100mm	9514511		9514512		9514513		9514515	
150mm	9514561		9514562		9514563		9514565	
200mm	9514521		9514522		9514523		9514525	
250mm	9514571		9514572		9514573		9514575	
Viva™ Wide Pore Silica Columns, 5µm								
30mm	9510531		9510532		9510533		9510535	
50mm	9510551		9510552		9510553		9510555	
100mm	9510511		9510512		9510513		9510515	
150mm	9510561		9510562		9510563		9510565	
200mm	9510521		9510522		9510523		9510525	
250mm	9510571		9510572		9510573		9510575	

HPLC Reversed Phase Test Mix #1

Routine analysis using these products can assist in determining the need to perform column and/or system maintenance.

benzene	3.00mg/mL	naphthalene	0.50
uracil	0.02	biphenyl	0.06

In methanol:water (75:25), 1mL/ampul

cat. # 35005 (ea.)

No data pack available.