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Application Note 187

Discovery BIO GFC Separation Volume and Peak Capacity

Gel filtration chromatography (GFC) is widely used to separate biomolecules. Retention is based on the size and shape of the analytes relative to the pore size of the particles. Small analytes can diffuse into the pores, while relatively large molecules are hindered from entering the pores and thus elute earlier. Discovery BIO GFC columns excel in two measures of the effectiveness of a GFC column: separation volume and peak capacity.

Separation volume (V_s) is the difference in the retention volume between a fully retained analyte and a completely excluded analyte. It is, in effect, the total accessible pore volume of the column. A larger separation volume provides a greater volume in which chromatographic separation takes place and potentially greater peak resolution. Separation volume is calculated using the following equation:

$$V_s = V_t - V_0 \quad [1]$$

Where:

V_s = separation volume

V_t = total column volume; V_t = elution volume (V_e) of fully retained analyte (analyte not adsorbed)

V_0 = void volume of column; $V_0 = V_e$ of excluded analyte

Peak capacity (P_c) is the number of peaks that are resolved within a given retention time window. There are both isocratic and gradient forms of the equation. Isocratic peak capacity is determined using this equation:

$$P_c = (t_{rn} - t_{r1}) / W \quad [2]$$

Where:

P_c = peak capacity

t_{rn} and t_{r1} = retention time of last and first eluting peaks, respectively

W = average peak width at baseline

Peak capacity is directly proportional to $t_{rn} - t_{r1}$, which is related to $V_t - V_0$ in equation [1] by the flow rate, and inversely proportional to peak width (W). Peak width is, in turn, related to column efficiency in that the higher the efficiency, the narrower the peaks. Therefore, the ideal GFC column exhibits high separation volume and high efficiency, which provide high peak capacity for maximum analyte resolution.

These properties are demonstrated on Discovery BIO GFC columns in Figures 1 and 2. In this study, we compared Discovery BIO GFC columns to competitive silica-based GFC columns of comparable dimensions in the isocratic separation of two sets of peptides. In both examples, Discovery BIO GFC was shown to have ~0.5 mL higher separation volume than the competitive column. In Figure 2, Discovery BIO GFC provided much narrower peaks and significantly higher resolution of the peptides than the competitive GFC column. Both of these attributes, combined with excellent column stability, lifetime and the wide separation range provided by individual columns and the entire line, make Discovery BIO GFC the columns of choice for GFC separation of biomolecules.

For more information on Discovery BIO GFC, please visit our website: *sigma-aldrich.com/discovery*

Discovery BIO GFC Properties

Particle:	spherical, porous silica, 5 µm
Surface:	inert, hydrophilic bonded phase
pH Range:	pH 2 - 8.5
Salt Concentration:	20 mM - 2 M
Maximum Temperature:	80 °C

Conditions for Figures 1 and 2

column:	30 cm x 7.8 mm ID., 5 µm particles
mobile phase:	150 mM phosphate buffered saline, pH 7
flow rate:	1 mL/min.
temp.:	ambient (~23° C)
det.:	214 nm
injection:	10 µL
samples:	**Figure 1:** thyroglobulin, BSA dimer, BSA monomer, ribonuclease A, poly-DL-alanine, uracil
	Figure 2: thyroglobulin aggregate, thyroglobulin, γ-globulin dimer, γ-globulin, ovalbumin, myoglobin, poly-DL-alanine, uracil

Figure 1. Peptide Mix 1 - Separation Volume and Peak Capacity on Discovery BIO GFC and Competitive GFC Column

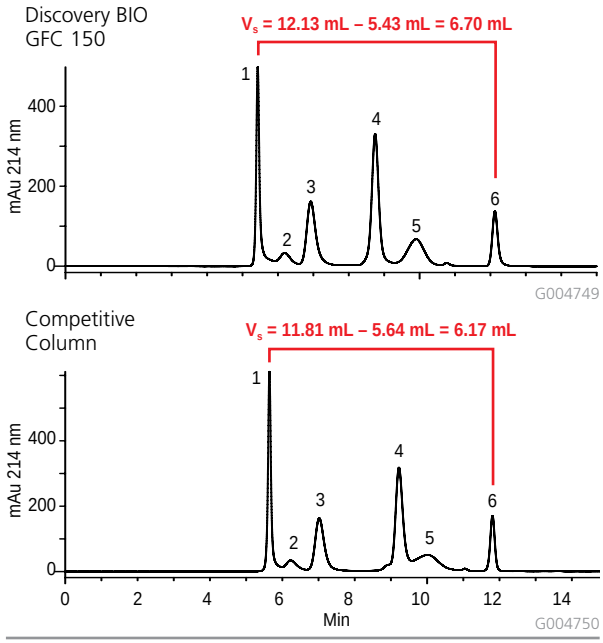
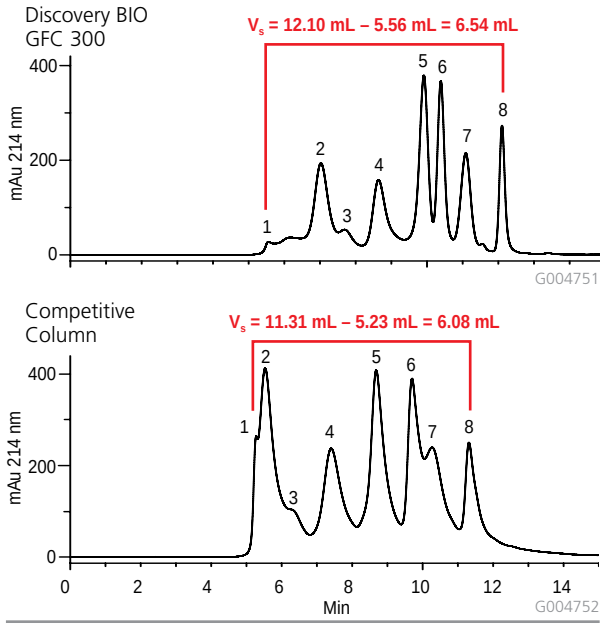


Figure 2. Peptide Mix 2 - Separation Volume and Peak Capacity on Discovery BIO GFC Compared to Competitive GFC Column



Ordering Information

Description*	Pore Diameter (Å)	mw (min.)	mw (max.)	Cat. No.
Discovery BIO GFC 100				
5 cm x 4.6 mm I.D.	100	100	100,000	567299-U
30 cm x 4.6 mm I.D.	100	100	100,000	567297-U
5 cm x 7.8 mm I.D.	100	100	100,000	567298-U
30 cm x 7.8 mm I.D.	100	100	100,000	567296-U
Discovery BIO GFC 150				
5 cm x 4.6 mm I.D.	150	500	5,000	567303-U
30 cm x 4.6 mm I.D.	150	500	5,000	567301-U
5 cm x 7.8 mm I.D.	150	500	5,000	567302-U
30 cm x 7.8 mm I.D.	150	500	5,000	567300-U
Discovery BIO GFC 300				
5 cm x 4.6 mm I.D.	150	300	5,000	567307-U
30 cm x 4.6 mm I.D.	150	300	5,000	567305-U
5 cm x 7.8 mm I.D.	150	300	5,000	567306-U
30 cm x 7.8 mm I.D.	150	300	5,000	567304-U
Discovery BIO GFC 500				
5 cm x 4.6 mm I.D.	500	15,000	5,000,000	567311-U
30 cm x 4.6 mm I.D.	500	15,000	5,000,000	567309-U
5 cm x 7.8 mm I.D.	500	15,000	5,000,000	567310-U
30 cm x 7.8 mm I.D.	500	15,000	5,000,000	567308-U
Discovery BIO GFC 1000				
5 cm x 4.6 mm I.D.	1000	50,000	7,500,000	567315-U
15 cm x 4.6 mm I.D.	1000	50,000	7,500,000	567313-U
5 cm x 7.8 mm I.D.	1000	50,000	7,500,000	567314-U
30 cm x 7.8 mm I.D.	1000	50,000	7,500,000	567312-U
30 cm x 4.6 mm I.D.	1000	50,000	7,500,000	567287-U
Discovery BIO GFC 2000				
5 cm x 4.6 mm I.D.	2000	10,000,000	--	567319-U
15 cm x 4.6 mm I.D.	2000	10,000,000	--	567317-U
5 cm x 7.8 mm I.D.	2000	10,000,000	--	567318-U
30 cm x 7.8 mm I.D.	2000	10,000,000	--	567316-U
30 cm x 4.6 mm I.D.	2000	10,000,000	--	567288-U

* Note: All particles 5 µm spherical. The 5 cm length columns are used to protect the 15 or 30 cm columns of the same I.D. and packing material.

Trademarks

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