

Application News

Liquid Chromatography

Bridging Column Qualification and Process Development for Bioprocess Chromatography

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User Benefits

- ◆ Evaluate Bioprocess Chromatography Columns with Analytical Precision
- ◆ Support resin screening and process development on a single platform
- ◆ Accelerate development with scalable chromatography workflows

■ Introduction

Biopharmaceutical researchers and process development scientists face unique challenges when working with protein, antibodies, enzymes, vaccines and other large biomolecules. Unlike small molecules, these products are often sensitive to high pressure, temperature change and harsh chemical conditions. Maintaining biological activity while achieving high purity and recovery is critical throughout the purification process.

A FPLC (Fast Protein Liquid Chromatography) system is specifically designed to address these challenges. It enables gentle purification of biomolecules using chromatography techniques such as Affinity, Ion Exchange (IEX), Hydrophobic interaction chromatography (HIC) and Size Exclusion Chromatography (SEC).

Shimadzu Bioseparation LC combines the purification capabilities of an FPLC with the precision and flexible injection options and advanced detector technologies of HPLC chromatography on a single integrated platform. Supporting both analytical and bioprocessing columns, it enables resin screening, column qualification, method development and small scale purification within one workflow. A unique feature of the Shimadzu Bioseparation LC is the availability of a low pressure PDA flow cell, enabling full spectral analysis and peak identification capabilities traditionally associated with HPLC systems while maintaining the low pressure operating conditions required for bioprocess chromatography to protect sensitive biological molecules from denaturing and shear force.

■ Experimental

Shimadzu Bioseparation LC was used to evaluate the performance of a 5 mL prepacked affinity axial column and a 20 mL custom packed affinity radial flow column (RFC). Both columns contained the same affinity resin, allowing chromatographic performance to be compared across two column formats while minimising variability associated with resin chemistry.

Sample

Vitamin B12 in water

Analytical Conditions

LC Conditions

Column	5 mL Prepacked Affinity Column and 20 mL Custom Packed Affinity Sepragen RFC.
Oven Temp	Room Temperature
Mobile phases	25 mM Sodium Phosphate Buffer at 7.5 pH with 1.0 M NaCl
Flow	Isocratic 1 and 5 mL/min respectively
PDA	360 nm

LC Equipment

Instrument	Shimadzu Bioseparation LC Nexera Lite Inert
Pump	LC-40i
Autosampler	SIL-20A (with inert kit)
Column Oven	CTO-40S
Detector	SPD-M40 (Inert low-pressure cell)
Fraction Collector	FRC-10A



Fig 1: Shimadzu Bioseparation LC. Image is for illustrative purposes only

Analysis

Injected 1 % column volume of Vitamin B12 and recorded data using 360 nm wavelength.

■ Results

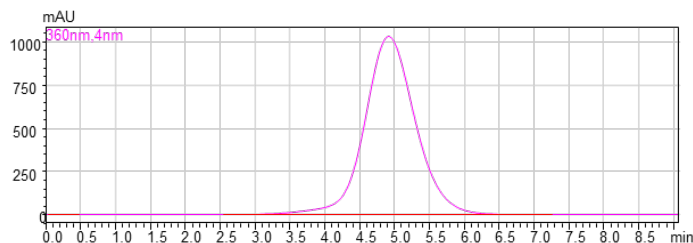


Fig. 2: Prepacked 5 mL Affinity Column Chromatogram

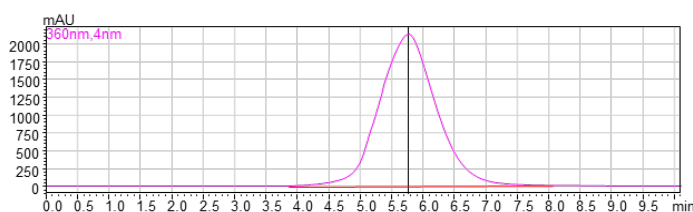


Fig. 3: Custom Packed 20 mL Affinity Sepragen Radial Flow Column Chromatogram

Table 1 Vitamin B12 sample results

Column	Flow Rate (mL/min)	Injection Volume (μL)	Retention Time tR (min)	Peak Height (mAU)	Peak Area	W _{0.5} (min)	Asymmetry	Tailing Factor (TF)	Pressure
5 mL Prepacked Column	1	50	4.917	1029108	51939653	1.772	~1.15	1.064	<10 psi
20 mL Custom Packed RFC	5	200	5.768	2123115	132997417	2.153	~1.20	1.047	<45 psi

Table 2 Column Performance Comparison
Plate count and HETP values are presented for comparative column evaluation purposes and are not intended to be compared directly with analytical HPLC columns

Metric	5 mL Prepacked Column	20 mL Custom Packed RFC
Asymmetry	~1.15	~1.20
Tailing Factor	1.064	1.047
Plate Count (N)	237	201
HETP	633.6	745.6

■ Discussion

The Vitamin B12 tracer study demonstrates that Shimadzu Bioseparation LC can effectively evaluate both 5 mL and 20 mL bioprocess chromatography columns. Both columns exhibited excellent peak shape, minimal tailing, and no evidence of major channeling or void formation, indicating effective packing and flow distribution. While the 5 mL prepacked column showed slightly higher calculated efficiency, both columns demonstrated comparable chromatographic performance based on peak symmetry and tailing characteristics.

■ Conclusion

The evaluation of 5 mL and 20 mL affinity columns packed with the same resin demonstrates how the Shimadzu Bioseparation LC bridges column qualification and process development. Comparable peak symmetry, minimal tailing, and low operating pressures were observed across both column formats, indicating effective packing quality and consistent chromatographic performance across scales.

These results highlight the suitability of the system for resin screening, column evaluation and small scale purification process development, enabling analytical characterisation and process development workflows to be performed on a single platform.

Acknowledgements

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