MODERNIZING BIOTHERAPEUTIC COMPENDIAL METHODS WITH A NEXT-GENERATION HPLC SYSTEM

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INTRODUCTION

As the field of analytics continues to evolve, methods guided by regulatory general chapters are increasingly seen as outdated due to their limited resolving power and speed. These compendial methods were originally written when larger particle sizes and systems with larger system dispersion were the norm. In response, regulatory agencies have lifted restrictions on method parameters such as flow rate, column dimensions, and particle size. This allows scientists using compendial methods to leverage modern instruments and particle sizes for a significant enhancement in performance.

In this poster, an Alliance™ iS Bio HPLC System with several columns containing MaxPeak™ High Performance Surfaces (HPS) Technology were used to modernize compendial biotherapeutic methods. To assess the benefits of method modernization by utilizing this technology, the modernized methods were compared to the regulatory general chapter methods analysed on a legacy HPLC system, evaluating them based on speed and recovery.

METHODS

The compendial methods evaluated on both systems are from USP General Chapters <129> and <121.1>. For USP <129>, size exclusion chromatography (SEC) was used for size variant analysis of USP System Suitability and USP mAb reference standards.[1] For USP <121.1>, a reversed phase chromatography (RPLC) gradient was used to evaluate a peptide map of insulin.[2]

Monoclonal Antibody Analysis[3]

USP mAb reference standards were injected at a concentration of 10 mg/mL in formulation buffer onto both systems.

Compendial Method Conditions:[1]

System:	Legacy HPLC system
Column:	BioSuite™ Diol (OH) Column, 250Å, 5 µm, 7.8 mm x 300 mm (p/n: 186002165)
Injection volume:	20 µL
Flow Rate:	0.500 mL/min
Run Time:	30 minutes, isocratic

Modernized Method Conditions:

System:	Alliance iS Bio HPLC System
Column:	XBridge™ Premier Protein SEC Column 250Å, 2.5 µm, 4.6 x 150 mm (p/n: 186009959RF)
Injection volume:	3.5 µL
Flow Rate:	0.350 mL/min
Run Time:	7.5 minutes, isocratic
Column:	XBridge Premier Protein SEC Column 250Å, 2.5 µm, 7.8 x 150 mm (p/n: 186009961)
Injection volume:	10 µL
Flow Rate:	1.000 mL/min
Run Time:	7.5 minutes, isocratic

Shared Conditions:[1]

Mobile Phase:	0.20 M potassium phosphate and 0.25 M potassium chloride, pH 6.2
Column temp.:	30 °C
Wavelength:	280 nm

Insulin Peptide Analysis

Insulin analogs were digested with Glu-C following the digestion procedure outlined in USP <121.1>.[2] Digesting samples were incubated at 25°C for 6 hours and quenched with sulfate buffer prior to injection.

Compendial Method Conditions:[2]

System:	Legacy HPLC system
Column:	XSelect™ Peptide CSH™ C_{18} Column 130Å, 5 µm, 4.6 x 100 mm (p/n: 186005289)
Injection volume:	50 µL
Flow Rate:	1.000 mL/min
Run time:	50 minutes, gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	1.000	95.0	5.0	Initial
3.00	1.000	95.0	5.0	6
30.00	1.000	41.0	59.0	6
35.00	1.000	20.0	80.0	6
40.00	1.000	95.0	5.0	6
50.00	1.000	95.0	5.0	6

Modernized Method Conditions:

System:	Alliance iS Bio HPLC System
Column:	XSelect Premier Peptide CSH C_{18} Column 130Å, 2.5 µm, 4.6 x 50 mm (p/n: 186009907)
Injection volume:	25 µL
Flow Rate:	2.000 mL/min
Run Time:	12.5 minutes, gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	2.000	95.0	5.0	Initial
0.75	2.000	95.0	5.0	6
7.50	2.000	41.0	59.0	6
8.75	2.000	20.0	80.0	6
10.00	2.000	95.0	5.0	6
12.50	2.000	95.0	5.0	6

Shared Conditions:[2]

Mobile Phase A:	700:100:200 H_2O:ACN:$(NH_4)_2SO_4$ buffer
Mobile Phase B:	400:400:200 H_2O:ACN:$(NH_4)_2SO_4$ buffer
Column temp.:	40 °C
Wavelength:	214 nm

RESULTS AND DISCUSSION

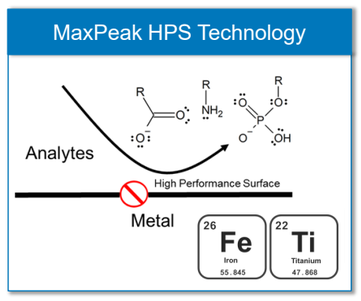

Figure 1: The Alliance iS Bio HPLC System is a bio-inert system with MaxPeak HPS Technology that was designed for separating biomolecules. This advanced surface technology eliminates risk when analyzing biotherapeutics which may have the potential to adsorb to metal surfaces.

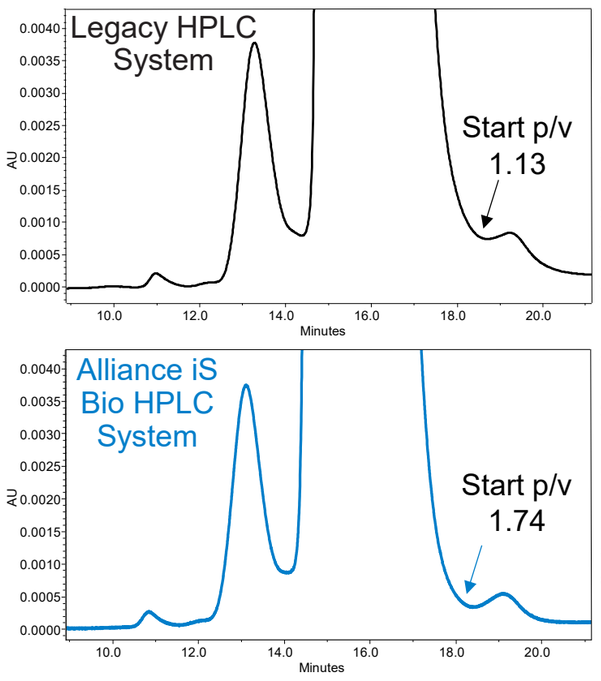

Figure 2: The USP <129> compendial SEC method was analyzed on a legacy HPLC system and the Alliance iS Bio HPLC System. Without any modifications, the Alliance iS Bio HPLC System showed improvements in resolution (peak height to valley ratio) for more accurate quantification of mAb impurities.

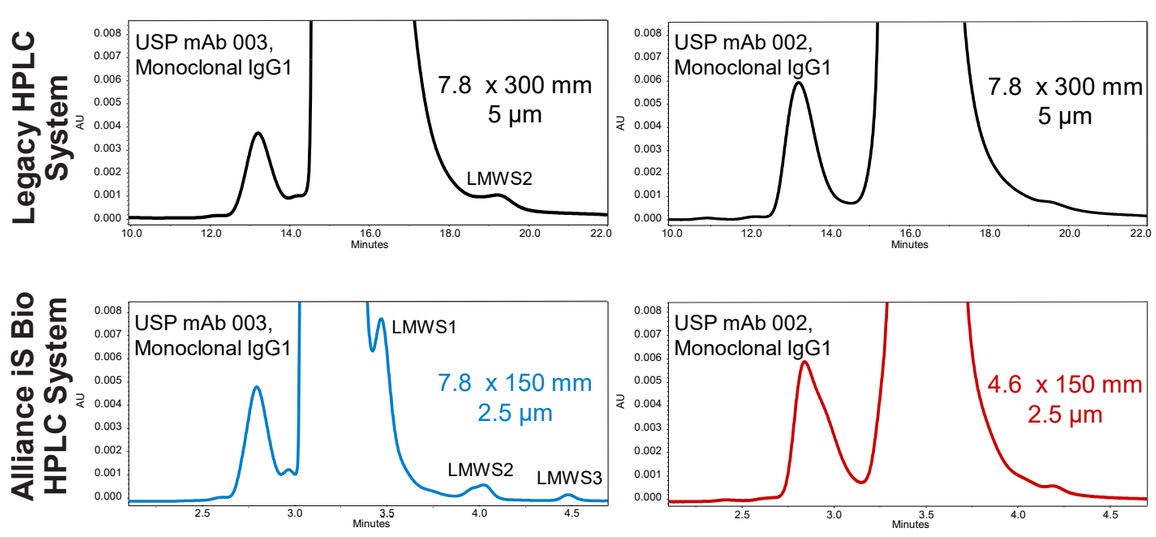

Figure 3: Following the scaling guidance in USP <621>, the compendial SEC method was modernized using XBridge Premier Protein SEC columns. Two columns were selected that maintained the same resolving power, or length-to-particle size ratio, as the original method. The 7.8 mm ID column reduced the method runtime 4-fold and provided the best resolution for the low molecular weight species (LMWS) whereas the 4.6 mm ID column provided benefits in reducing laboratory consumption by reducing solvent and sample use 6-fold.

XBridge Premier Protein SEC Column 250Å, 2.5 µm, 7.8 x 150 mm

4X Reduced runtime:
30 min → 7.5 min

Improved LMWS resolution:

Chromatogram	LMWS1 (Start p/v)	LMWS2
▬	N.D.	1.13
▬	1.22	3.59

XBridge Premier Protein SEC Column 250Å, 2.5 µm, 4.6 x 150 mm

6-fold reduction

in solvent and sample use per injection

Chromatogram	MP	Sample
▬	15 mL	20 µL
▬	2.625 mL	3.5 µL

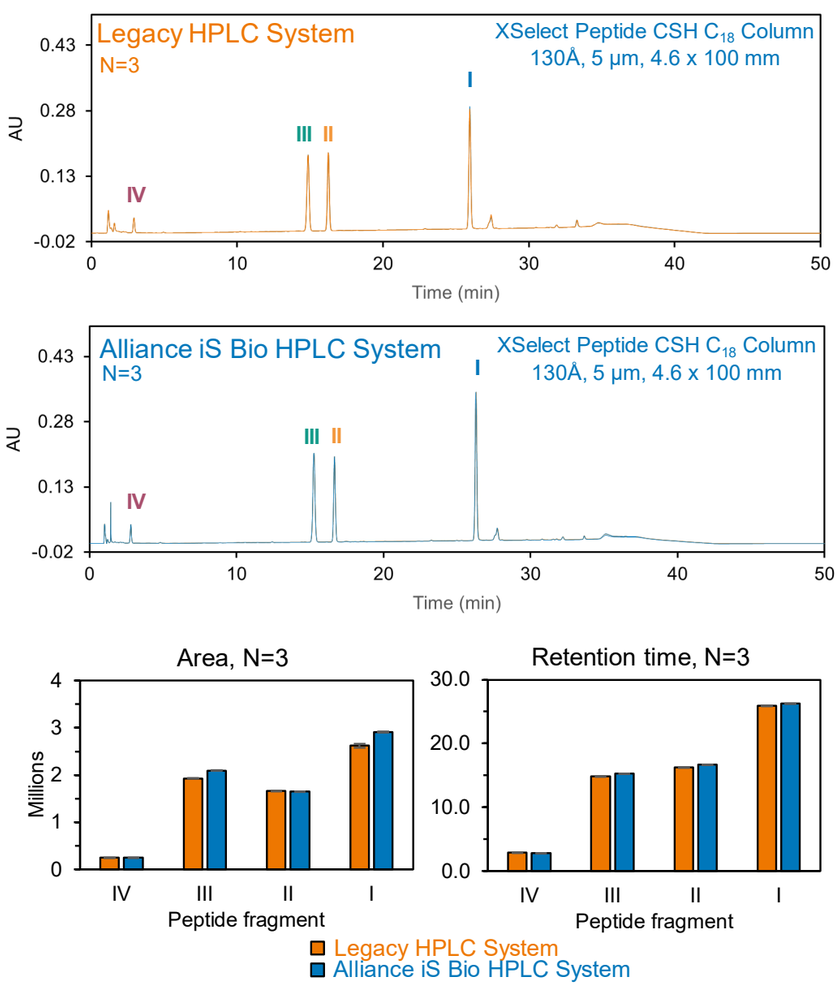

Figure 4: Following digestion with Glu-C, insulin human produces four peptide fragments that can be used in identification assays. The digested product was analyzed on both the legacy HPLC system and the Alliance is Bio HPLC System and provided comparable performance for area and retention time.

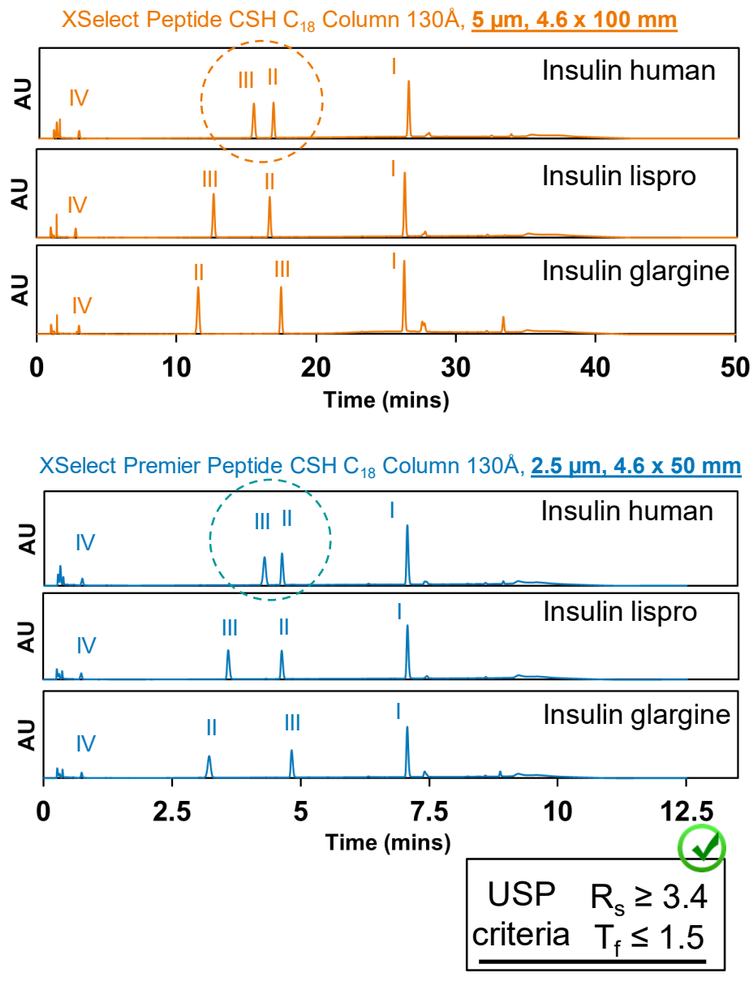

Figure 5: Taking advantage of the separation efficiency gains of smaller particles, the compendial method was scaled to a 50 mm column packed with 2.5 µm sized particles as the stationary phase on the Alliance iS Bio HPLC System. The selectivity of the separation was preserved and the compendial method requirements for resolution (≥ 3.4) and peak tailing (≤ 1.5) of the encircled critical pair were met. The Alliance iS Bio HPLC System supports 2.5 µm column technology due to higher system pressure tolerance and lower system dispersion, thereby significantly reducing operating costs such as analysis time, solvent, and sample use.

CONCLUSION

- **Compendial SEC and insulin peptide mapping methods were scaled to 2.5 µm columns and provided a significant reduction in analysis time and mobile phase consumption.**
- **The Alliance iS Bio HPLC System is capable of migrating and modernizing compendial methods to accommodate both present and future biopharmaceutical workflows in QC environments.**

References

1. USP. Chromatography <621>. In: USP-NF. Rockville, MD: USP; Dec 1, 2022.
2. USP. Physicochemical Analytical Procedures for Insulins <121.1>. In: USP-NF. Rockville, MD: USP; Dec 1, 2016.
3. Bigos P, Birdsall RE, Nyholm K. Modernizing Compendial SEC Methods for Biotherapeutics Using the Alliance™ iS Bio HPLC System. Waters Application Note. April 2024. 720008290EN

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