METHOD MODERNIZATION OF COMPENDIAL METHODS USING USP <621> 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As the field of analytics continues to evolve, methods guided by regulatory general chapters are increasingly seen as outdated due to their limited resolving power and speed.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]

Parameters	Compendial Method	Modernized Method
System	Legacy HPLC system	Alliance iS Bio HPLC System
Column	BioSuite™ Diol (OH) Column, 250Å, 5 µm, 7.8 x 300 mm (p/n: 186002165)	XBridge™ Premier Protein SEC Column 250Å, 2.5 µm, 4.6 x 150 mm (p/n: 186009959) and 2.5 µm, 7.8 x 150 mm (p/n: 186009961)
Inj. Vol.	20 µL	3.5 µL
Flow Rate	0.500 mL/min	0.350 mL/min
Run Time	30 minutes	7.5 minutes
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[4]

Parameters	Compendial Method	Modernized Method
System	Legacy HPLC system	Alliance iS Bio HPLC System
Column	XSelect™ Peptide CSH™ C18 Column 130Å, 5 µm, 4.6 x 100 mm (p/n: 186005289)	XSelect Premier Peptide CSH C18 Column 130Å, 2.5 µm, 4.6 x 50 mm (p/n: 186009907)
Inj. Vol.	50 µL	25 µL
Flow Rate	1.000 mL/min	2.000 mL/min
Run Time	50 min, 0.5% MP B/min	7.5 min, 8% MP B/min
Mobile Phase	A: 700:100:200 H_2O:ACN:$(NH_4)_2SO_4$ buffer B: 400:400:200 H_2O:ACN:$(NH_4)_2SO_4$ buffer	
Column Temp.	40 °C	
Wavelength	214 nm	

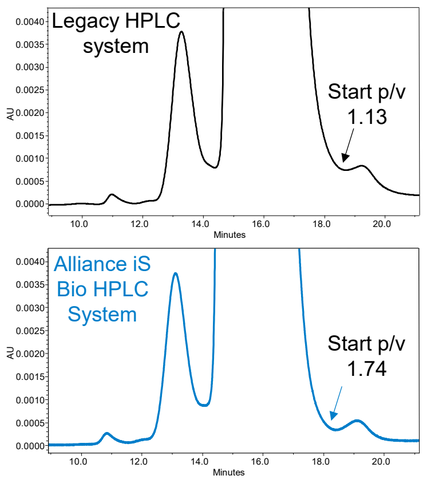

Figure 1: The USP <129> compendial SEC method was analyzed on a legacy HPLC system and the Alliance iS Bio HPLC System. Without any modifications, the Alliance iS Bio HPLC System showed improvements in resolution (peak height to valley ratio) for more accurate quantification of mAb impurities.

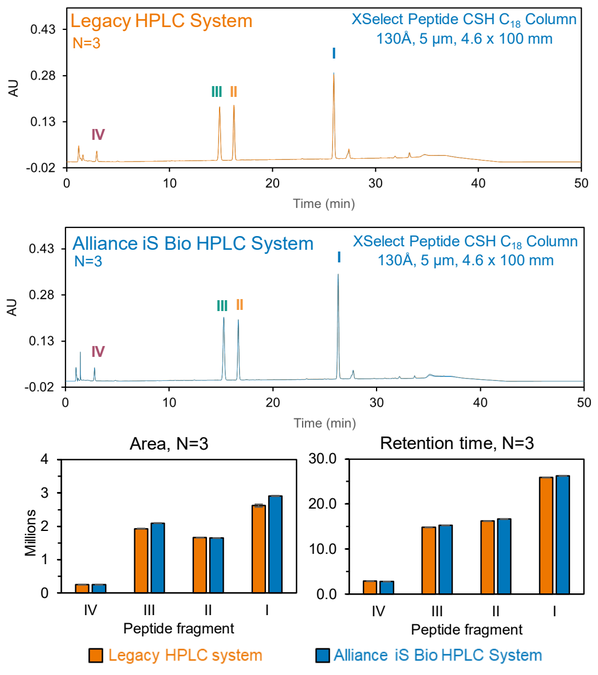

Figure 3: Following digestion with Glu-C, insulin human produces four peptide fragments that can be used in identification assays. The digested product was analyzed on both the legacy HPLC system and the Alliance iS Bio HPLC System and provided comparable performance for area and retention time.

RESULTS AND DISCUSSION

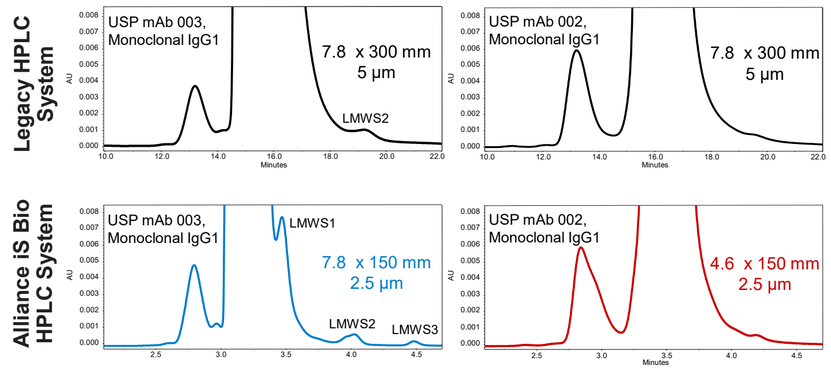

Figure 2: Following the scaling guidance in USP <621>, the compendial SEC method was modernized using XBridge Premier Protein SEC columns. Two columns were selected that maintained the same resolving power, or length-to-particle size ratio, as the original method. The 7.8 mm ID column reduced the method runtime 4-fold and provided the best resolution for the low molecular weight species (LMWS) whereas the 4.6 mm ID column reduced 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 (Start p/v)
▬	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

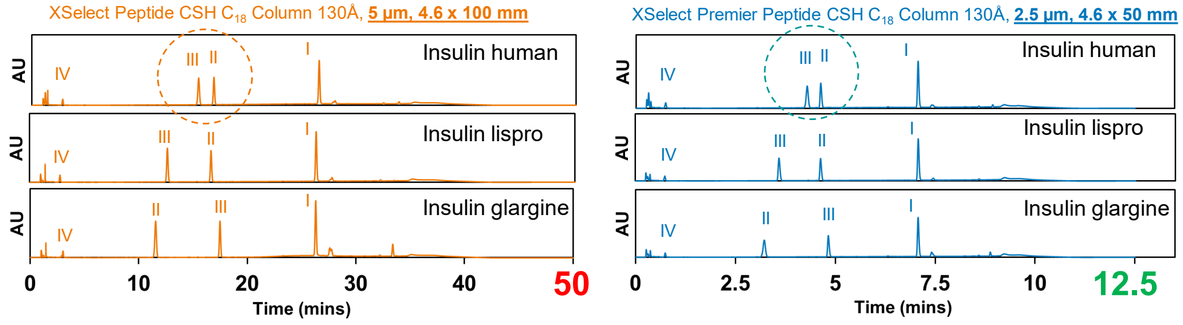

Figure 4: Taking advantage of the separation efficiency gains of smaller particles, the compendial method was scaled to a 50 mm column packed with 2.5 µm sized particles as the stationary phase on the Alliance iS Bio HPLC System. The selectivity of the separation was preserved and the compendial method 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
4. Han D, Birdsall RE, Nyholm K. Leveraging the Alliance™ iS Bio HPLC System as a Modern HPLC for Peptide Drug Substances Analysis in QC Environments. Waters Application Note. May 2024. 720008345EN

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