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Application Note

Modernizing Compendial SEC Methods for Biotherapeutics Using the Alliance™ iS Bio HPLC System

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Abstract

The process of method modernization, which involves integrating advancements in system and column technology for routine analysis, is a challenge many regulated laboratories face due to a larger focus on method life cycle management from regulators. Regulated laboratories often rely on methods that were validated several years ago and while still robust and effective, improvement opportunities could be identified, evaluated, and replaced with faster, more robust, or more sensitive technology. To address these demands, Waters™ has introduced the next generation of HPLC for biotherapeutics, the Alliance iS Bio HPLC System. This next-generation HPLC was designed with a bio-inert flow path and MaxPeak™ High Performance Surfaces (HPS) Technology for robust analysis of biopharmaceutical applications. While the Alliance iS Bio HPLC System maintains the identity of legacy HPLC systems as a rugged and reliable "workhorse" for daily operation, it is also designed with several new key features such as an intuitive touchscreen control, pre-run checks, and method modernization tools within Empower™ Chromatography Data System (CDS) to reduce common errors. In this application note, we'll assess the benefits of the Alliance iS Bio HPLC System by migrating and modernizing a compendial SEC method outlined in the United States Pharmacopeia (USP) General Chapter <129>. By deploying this next-generation bio-inert HPLC system, a significant decrease in solvent consumption and a

shorter runtime was observed. Additionally, the Alliance iS Bio HPLC System exhibited an increase in resolution and sensitivity towards size variant impurities in comparison to a legacy HPLC system.

Benefits

- Modernization of a compendial SEC method provides significant reduction in analysis time and mobile phase consumption
- The biocompatible and bio-inert construction of the Alliance iS Bio HPLC System is well suited for high ionic strength mobile phases used in SEC protein analysis

Introduction

In HPLC analysis, compendial methods are frequently implemented as part of routine quality control (QC) strategies. However, these methods, which were established during an era when columns with larger particle sizes and systems with higher system dispersion were the norm, are increasingly viewed as outdated in terms of resolving power and efficiency. This is relevant for many USP general chapters, which consequently resulted in an update to USP General Chapter <621> titled “Chromatography”. This update aimed at harmonizing with the European Pharmacopoeia and Japanese Pharmacopeia, permitting modifications in flow rate, column dimensions, and particle size for USP monographs.[1] By capitalizing on these allowable changes, QC scientists can utilize modern HPLC technologies to enhance method performance without needing a revalidation of the adjusted method.

Parallel to this, the Alliance iS Bio HPLC System (Figure 1) is a next generation HPLC platform designed for biopharmaceutical applications. The Alliance iS Bio HPLC System is both biocompatible and bio-inert, engineered specifically for QC environments in the biopharmaceutical industry. This instrument is constructed with biocompatible materials including titanium, PEEK, MP35N®, and other non-ferrous materials that are resistant to corrosion when exposed to high ionic strength and acidic mobile phases. The design also incorporates MaxPeak HPS Technology within the sample flow path to eliminate non-specific adsorption of analytes to metal surfaces. In tandem, these design elements provide a holistic solution by eliminating the unpredictability of analyzing metal-sensitive analytes in routine biopharmaceutical workflows.

This study aims to assess the benefits of migrating and modernizing size-exclusion chromatography (SEC)

methods with the Alliance iS Bio HPLC System. USP General Chapter <129> was chosen as a compendial method and encompasses a variety of standardized analytical procedures for mAb analysis. This chapter specifies the use of a 5-micron particle size column to run a 30-minute isocratic method for SEC analysis.[2] To evaluate the advantages of the Alliance iS Bio HPLC System, the compendial method was migrated without any alterations and compared to a legacy HPLC system. The compendial method was also scaled and modified to suit modern column hardware dimensions following guidance in USP General Chapter <621>. A comparative analysis was then performed to evaluate the enhancement in performance and throughput when compared to legacy systems.

Figure 1. The Alliance iS Bio HPLC System with a Tunable UV Detector.

Experimental

Potassium chloride (CAS 7447–40–7) was purchased from Sigma Aldrich. Monobasic potassium phosphate (CAS 7778–77–0) was purchased from Acros Organics. Dibasic potassium phosphate (CAS 7758–11–4) was purchased from J. T. Baker. Monoclonal IgG system suitability and USP mAb reference standards were purchased from USP. All USP mAbs were reconstituted with 200 µL of mobile phase and injected at a concentration of 10 mg/mL.

Compendial Method Conditions

LC system:	Legacy HPLC System
Detection:	λ = 280 nm
Column:	BioSuite™ Diol (OH) Column, 250 Å, 5 µm, 7.8 mm x 300 mm, (p/n: 186002165)
Column temperature:	30 °C
Sample temperature:	8 °C
Injection volume:	20 µL
Flow rate:	0.50 mL/min
Mobile phase:	10.5 g dibasic potassium phosphate, 19.1 g monobasic potassium phosphate, and 18.6 g potassium chloride per liter of water, (0.20 M potassium phosphate and 0.25 M potassium chloride) pH 6.2
Run time:	30 minutes, isocratic
Chromatography software:	Empower 3, FR4

Modernized Method #1

LC system:	Alliance iS Bio HPLC System
Detection:	TUV, λ = 280 nm
Column:	XBridge™ Premier Protein SEC Column, 250 Å, 2.5 µm, 7.8 x 150 mm, (p/n: 186009961)
Column temperature:	30 °C
Sample temperature:	8 °C
Injection volume:	10 µL
Flow rate:	1.00 mL/min
Mobile phase:	10.5 g dibasic potassium phosphate, 19.1 g monobasic potassium phosphate, and 18.6 g potassium chloride per liter of water, (0.20 M potassium phosphate and 0.25 M potassium chloride) pH 6.2
Run time:	7.5 minutes, isocratic
Chromatography software:	Empower 3.8.0

Modernized Method #2

LC system:	Alliance iS Bio HPLC System
Detection:	TUV, λ = 280 nm

Column:	XBridge Premier Protein SEC Column, 250 Å, 2.5 µm, 4.6 x 150 mm +eConnect, (p/n: 186009959RF)
Column temperature:	30 °C
Sample temperature:	8 °C
Injection volume:	3.5 µL
Flow rate:	0.35 mL/min
Mobile phase:	10.5 g dibasic potassium phosphate, 19.1 g monobasic potassium phosphate, and 18.6 g potassium chloride per liter of water, (0.20 M potassium phosphate and 0.25 M potassium chloride) pH 6.2
Run time:	7.5 minutes, isocratic
Chromatography software:	Empower 3.8.0

Results and Discussion

Method Migration

In QC environments, the priority is to maintain consistent results of validated methods across different systems. These labs expect methods to be seamlessly migrated and equivalency to be demonstrated on newer technology platforms. The most straight forward instrument migration involves transferring all method parameters to the newer platform and evaluating the instruments performance versus the legacy system. To streamline the process of method migration, Waters has developed the Intelligent Method Translator app (iMTA) that can be accessed through the Empower Apps menu. As shown in Figure 2, iMTA can translate methods from various systems and

automatically converts the key parameters for the pump, sample manager, column compartment, and detector into an Alliance iS Bio HPLC System instrument method. The laborious process of copying old method parameters into a new system has been simplified to a few clicks, eliminating the risk of user transcription errors.

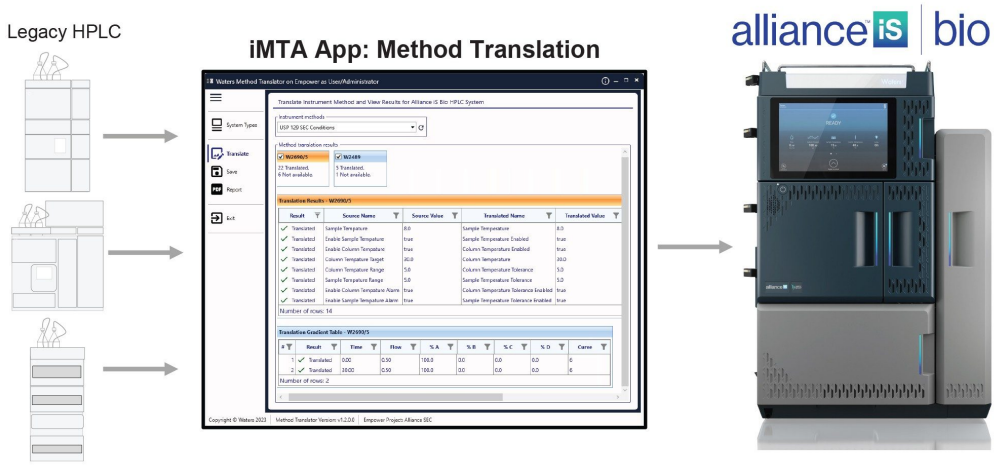
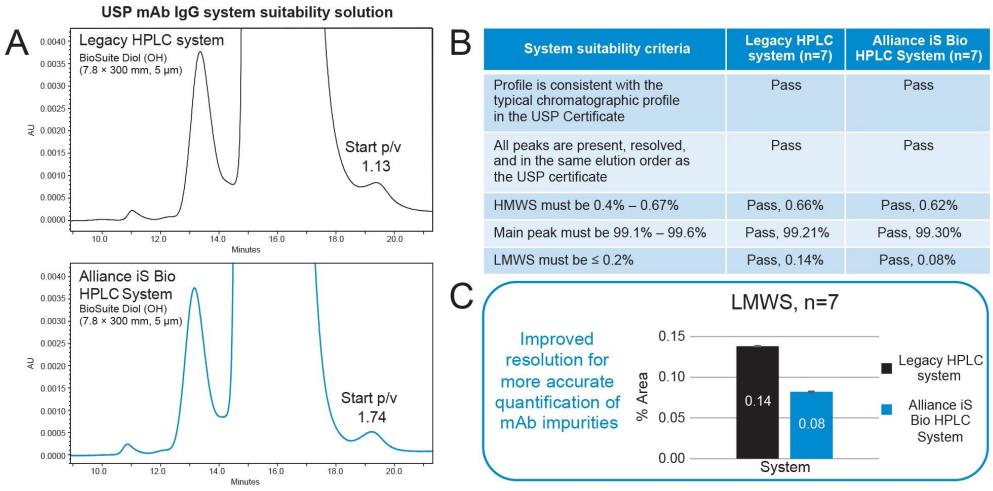


Figure 2. The iMTA application can seamlessly translate method conditions from several legacy HPLC systems and convert the parameters to an Alliance iS Bio HPLC System method.

To evaluate the Alliance iS Bio HPLC System in its ability to migrate methods, a SEC compendial method, as outlined in USP General Chapter <129>, was selected due to it's listed system suitability criteria which could be compared across systems. As a benchmark, the method was run using a L59, 5-micron, 7.8 mm x 300 mm column on a legacy HPLC system and migrated using the iMTA app to the Alliance iS Bio HPLC System. Figure 3 depicts the chromatograms from both systems as well as the system suitability criteria for the compendial method. In the tabular data, both systems pass the system suitability criteria, and the chromatographic profiles are consistent across systems. One immediate benefit of the migration was the increased resolution between the monomer and impurities observed in the Alliance iS Bio HPLC System; highlighting the benefit of modern LCs with lower system dispersion for SEC-based methods. This increase in resolution was most pronounced in the low molecular weight species (LMWS), enabling more accurate quantitation of the impurities, as shown in the bar plot. These results demonstrate that the Alliance iS Bio HPLC System can analyze compendial SEC methods and yield comparable results without needing further method optimization.



System suitability criteria	Legacy HPLC system (n=7)	Alliance iS Bio HPLC System (n=7)
Profile is consistent with the typical chromatographic profile in the USP Certificate	Pass	Pass
All peaks are present, resolved, and in the same elution order as the USP certificate	Pass	Pass
HMWS must be 0.4% – 0.67%	Pass, 0.66%	Pass, 0.62%
Main peak must be 99.1% – 99.6%	Pass, 99.21%	Pass, 99.30%
LMWS must be ≤ 0.2%	Pass, 0.14%	Pass, 0.08%

Figure 3. A) SEC separation of a USP mAb System Suitability Solution on a legacy HPLC system and the Alliance iS Bio HPLC System with a BioSuite Diol SEC column. B) System suitability results for n=7 injections on both instruments. C) Bar plot comparison of the LMWS recovery on both systems.

A distinctive feature that sets the Alliance iS Bio HPLC System apart from legacy HPLC systems is the suite of error reduction tools designed to mitigate risk in regulated laboratories. Among these tools, the most significant are the pre-run checks that can be configured via the touchscreen control kiosk. Figure 4 shows a screen grab from the kiosk's interface, allowing users to select customizable items for verification before data acquisition begins. Some of these checks include verifying the column matches the instrument method, assessing the solvent levels and expiration dates of mobile phase, and confirming the presence of all vials in the sample set are present in the autosampler. If any of the configurable pre-run checks fail, the system will pause acquisition and stop flow to reduce consumable costs. These pre-run checks ensure that even without any modifications to a method, the Alliance iS Bio HPLC System will improve productivity compared to legacy systems by reducing common human errors that result in out of specification results and repeat analysis.

Figure 4. The pre-run configurable checklist can be found in "setup" on the Alliance iS Bio HPLC System touchscreen interface.

Method Modernization

Method modernization is another challenge many QC laboratories face due to added pressures for method life cycle management from regulatory bodies. Similarly, USP has also endorsed method modernization while complying to USP <621> guidance through their application notes.[3] As a next-generation HPLC system, the Alliance iS Bio HPLC System has been designed to be compatible with modern column hardware dimensions for increased throughput and efficiency. To modernize a method, several parameters must be scaled to preserve separation across systems and column formats. The allowable changes are listed in USP General Chapter <621> but to efficiently scale method parameters between columns, the Waters Columns Calculator was used. To modernize the compendial USP General Chapter <129> method, we selected two modern SEC Columns that maintained the same resolving power, or length-to-particle size ratio, as the original method. Figure 5 highlights the changed method parameters that were scaled for both modernized methods utilizing the Waters Columns Calculator.

Method variable	USP method	Modernized method #1	Modernized method #2
Column	BioSuite Diol (OH) Column, 250Å, 5 µm, 7.8 mm × 300 mm	XBridge Premier Protein SEC Column, 250Å, 2.5 µm, 7.8 × 150 mm	XBridge Premier Protein SEC Column, 250Å, 2.5 µm, 4.6 × 150 mm
L/dp	60,000	60,000	60,000
Flow rate	0.5 mL/min	1.0 mL/min	0.35 mL/min
Injection volume	20 µL	10 µL	3.5 µL
Run time	30 min	7.5 min	

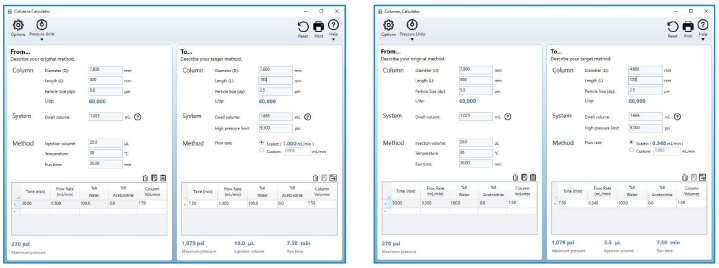


Figure 5. Waters Columns Calculator is used for scaling method parameters of two modern columns that maintained the same length to particle size ratio (L/dp) as the compendial USP method.

Both of the modernized columns are XBridge Premier Protein SEC Columns, identical in particle size and length, with the distinction of internal diameters (ID), with one measuring 7.8 mm and the other 4.6 mm. These columns, while still classified as L59, incorporate a polyethylene oxide polymer bonded to a BEH™ particle surface (BEH-PEO) coupled with MaxPeak HPS to provide a new level of inertness to SEC.[4] We examined both scaled methods using the Alliance iS Bio HPLC System and compared the results with data produced by the legacy HPLC system under compendial method conditions. Two USP mAb reference standards were analyzed for the three methods and the results shown in Figures 6 and 7. Each column format presents unique advantages that can be selected based on specific user needs.

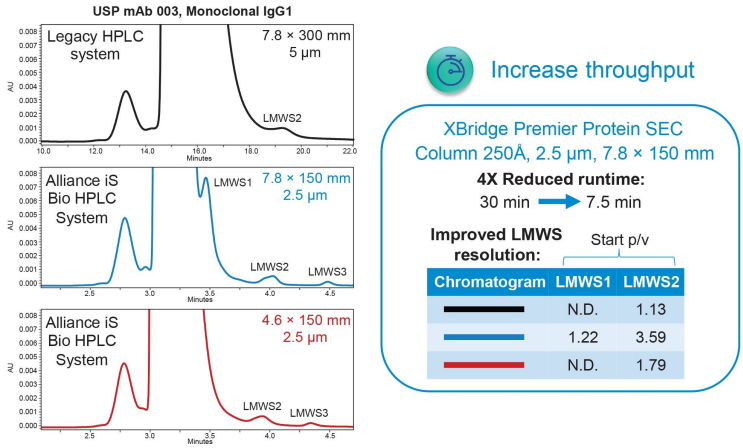


Figure 6. SEC separation of USP mAb 003 reference standard on the legacy system and Alliance iS Bio HPLC System with the XBridge Premier Protein SEC Column, 250 Å, 2.5 µm, 7.8 x 150 mm (blue trace) and the 2.5 µm, 4.6 x 150 mm (red trace).

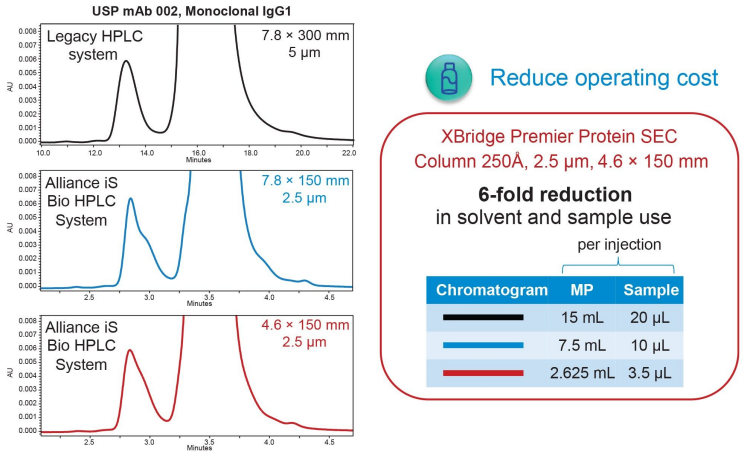


Figure 7. SEC separation of USP mAb 002 reference standard on the legacy system and Alliance iS Bio HPLC System with the XBridge Premier Protein SEC Column, 250 Å, 2.5 µm, 7.8 x 150 mm (blue trace) and the 2.5 µm, 4.6 x 150 mm (red trace).

In figure 6, focusing on the 7.8 mm ID XBridge Premier Protein SEC Column (blue trace), the column was able to reduce the compendial method runtime 4-fold, from 30 minutes to 7.5 minutes due to the reduction in particle size and length of the column. The 7.8 mm ID column also offers the highest resolution of the three methods and was able to resolve a LMWS peak which was not detected on the legacy HPLC system. These results indicate that the 7.8 mm ID column would be ideal for users whose primary objective is to increase the resolution of impurities for accurate quantification of size variants. In Figure 7, using a different USP reference mAb standard we again see preserved selectivity of the two scaled methods with the compendial method. Focusing on the 4.6 mm ID XBridge Premier Protein SEC Column (red trace), we see a 6-fold decrease in mobile phase and sample consumption when compared to the compendial method. This column would be ideal for users concerned with sample and mobile phase costs and offers the lowest cost of operation for HPLC analysis.

Conclusion

Regulatory agencies are encouraging laboratories to explore modernization opportunities in their analytical workflows by substituting ageing analytical instrumentation with modern technology. The experimental results demonstrated that the Alliance iS Bio HPLC System is capable of migrating and modernizing compendial SEC methods to accommodate both present and future biopharmaceutical workflows in QC environments. The Alliance iS Bio HPLC System when coupled with XBridge Premier Protein SEC Columns can improve resolution while providing 75% reduction in analysis time and 82.5% reduction in mobile phase consumption. The Alliance iS Bio HPLC System also has been developed with a host of new software features, making workflows easier to operate and alerting users of errors before data acquisition starts. Overall, the system empowers scientists to match the pace of advancements made in biotherapeutics, utilizing a similarly advanced HPLC instrument to yield accurate and consistent results.

References

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