ENHANCING ANALYTICAL PERFORMANCE WITH A BIO-INERT HPLC SYSTEM

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INTRODUCTION

In biopharmaceutical analysis, undesired secondary analyte/surface interactions have hindered performance in HPLC. Analytes that contain electron rich functional groups are susceptible to adsorb onto surfaces along the stainless-steel flow path causing reduced resolution and recovery. To overcome these challenges, hardware surfaces were modified with a chemically resistant hybrid organic/inorganic barrier called MaxPeak™ High Performance Surfaces (HPS) Technology. This technology was then used in the construction of a bio-inert system called the Alliance™ iS Bio HPLC System.

In this poster, the Alliance iS Bio HPLC System was evaluated by analysing oligonucleotides, monoclonal antibodies (mAbs), and glucagon-like-peptides (GLP-1) and compared to a legacy HPLC system for improved resolution and recovery.

METHODS

Established methods that were previously analyzed on legacy HPLC platforms and columns were scaled and transferred to the Alliance iS Bio HPLC System. Ion-pairing reversed phase chromatography (IP-RPLC), size exclusion chromatograph (SEC), and RPLC were used as the prevailing techniques for analyzing the analytes on both systems.

Oligonucleotide Analysis[1]

Waters MassPREP™ Oligonucleotide Standard (OST) containing 15 - 35 mer oligodeoxythymidines (4 pmol/µL) and GEM91, a 25 mer fully thiolated phosphorothioate oligonucleotide (0.5 mg/mL) were injected onto both systems.

Legacy HPLC System Method Conditions:

Column:	XBridge™ BEH™ C_{18} Column 5 µm,130Å, 4.6 x 100 mm (p/n: 186003115) XBridge BEH C_{18} Column 2.5 µm,130Å, 4.6 x 100 mm (p/n: 186006039)

Alliance iS Bio HPLC System Method Conditions:

Column:	XBridge Premier Oligonucleotide BEH C_{18} Column 2.5 µm, 130Å, 4.6 x 100 mm (p/n:186009902) XBridge™ BEH™ C_{18} Column 5 µm,130Å, 4.6 x 100 mm (p/n: 186003115)

Shared Conditions:

Mobile Phase A:	25 mM Hexylammonium acetate (HAA) in water, pH 7.0
Mobile Phase B:	25 mM HAA in water/acetonitrile (ACN) 40/60, pH 7.0
Mobile Phase C:	Water
Mobile Phase D:	ACN
Flow Rate:	0.650 mL/min for OST, 0.500 mL/min for GEM91
Injection volume:	10 µL
Column temp.:	60 °C
Wavelength:	260 nm
MassPREP OST:	1.73% B/min gradient
GEM91:	0.5% B/min gradient

Monoclonal Antibody Analysis[2]

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

Legacy HPLC System Method Conditions:[3]

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

Alliance iS Bio HPLC System Method Conditions:

Column:	XBridge Premier Protein SEC Column 250Å, 2.5 µm, 7.8 x 150 mm (p/n: 186009961)
Injection volume:	3.5 µL
Flow Rate:	1.000 mL/min
Run Time:	7.5 minutes, isocratic

Shared Conditions:[3]

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

GLP-1 Analysis

Dulaglutide and glucagon stock were prepared with DMSO at 1 mg/mL. Liraglutide and tirzepatide stock were prepared with DMSO at 0.5 mg/mL. Exenatide and semaglutide stock were prepared with 10 mM ammonium formate buffer, pH 8.5 at 0.5 mg/mL. The GLP-1 panel mixture was prepared at 0.05 mg/mL for each peptide in 0.5% trifluoracetic acid, 1% formic acid in ACN, and 98.5% water.

Legacy HPLC System Method Conditions:

Column:	XSelect™ Peptide CSH™ C_{18} Column 130Å, 2.5 µm, 4.6 x 150 mm (p/n: 186007038)

Alliance iS Bio HPLC System Method Conditions:

Column:	XSelect Premier Peptide CSH C_{18} Column 130Å, 2.5 µm, 4.6 x 150 mm (p/n: 186009909)

Shared Conditions:

Mobile Phase A:	0.1% formic acid in water
Mobile Phase B:	0.1% formic acid in ACN
Column temp.:	60 °C
Wavelength:	214 nm
Injection volume:	10 µL
Flow Rate:	0.960 mL/min
Gradient:	2.725% ACN/min

RESULTS AND DISCUSSION

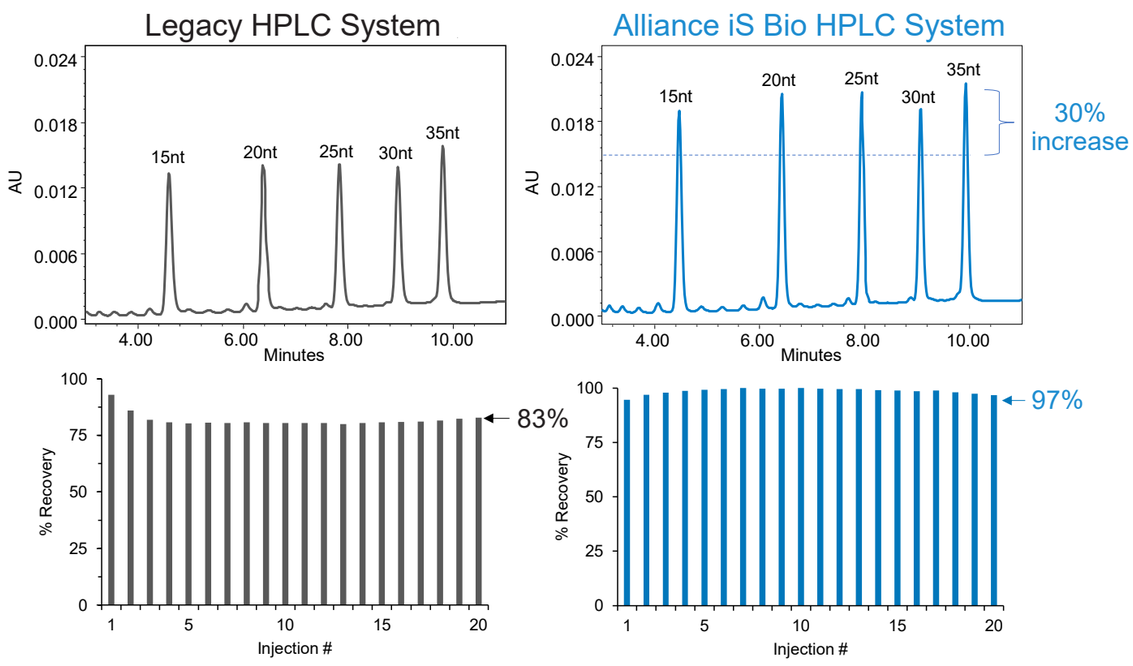

Figure 1: Oligonucleotides are notorious for low recovery in LC separations due to the chelating nature of the phosphate backbone. This chelation results in adsorption along the flow path which impacts recovery and reproducibility. With MaxPeak HPS Technology, the Alliance iS Bio HPLC System with a XBridge Premier Oligonucleotide BEH Column showed a 30% increase in peak height of 15 - 35 mer oligodeoxythymidines when compared to a legacy HPLC system with a stainless-steel column. Additionally, the Alliance iS Bio HPLC System achieved maximum recovery in a shorter time span, thereby helping eliminate the need for lengthy passivation procedures when analyzing oligonucleotides.

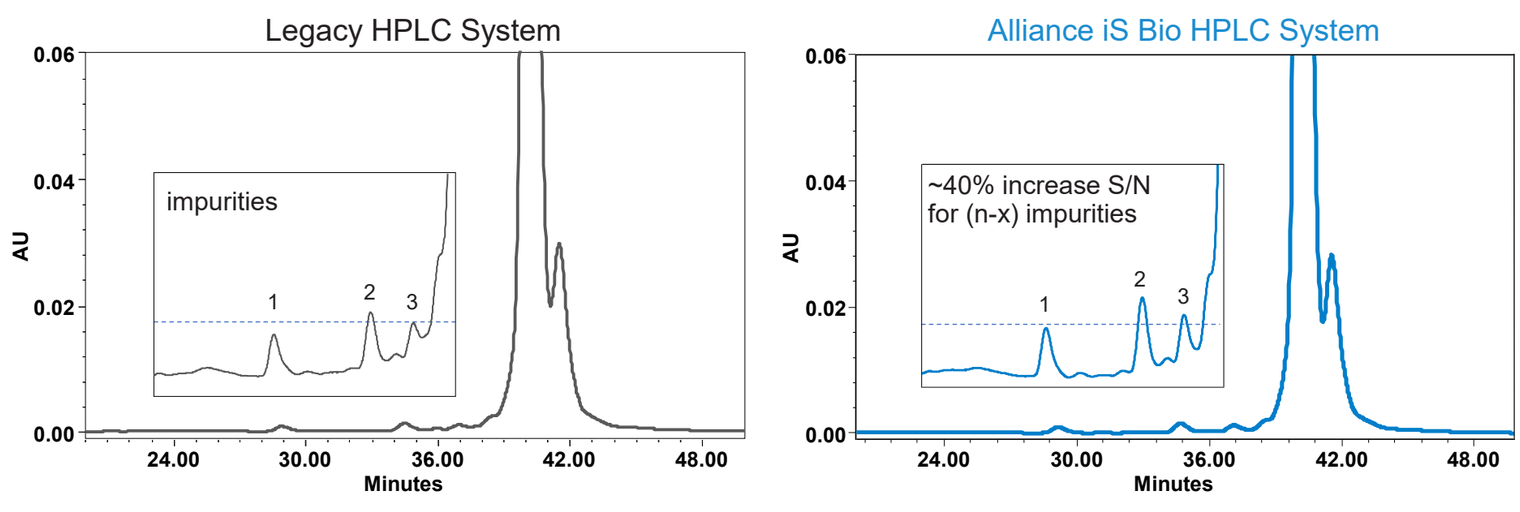

Figure 2: GEM91 was analyzed to further investigate the performance differences across systems. Despite its refinement as a drug substance, GEM91 contains impurities that necessitate monitoring. Utilizing the 5 µm XBridge BEH C_{18} Column on both systems, the Alliance iS Bio HPLC System showed an ~40% increase in the signal-to-noise ratio for the trace impurities versus the legacy HPLC system. This improvement translates to enhanced recovery and increased accuracy when analyzing critical species for novel therapies.

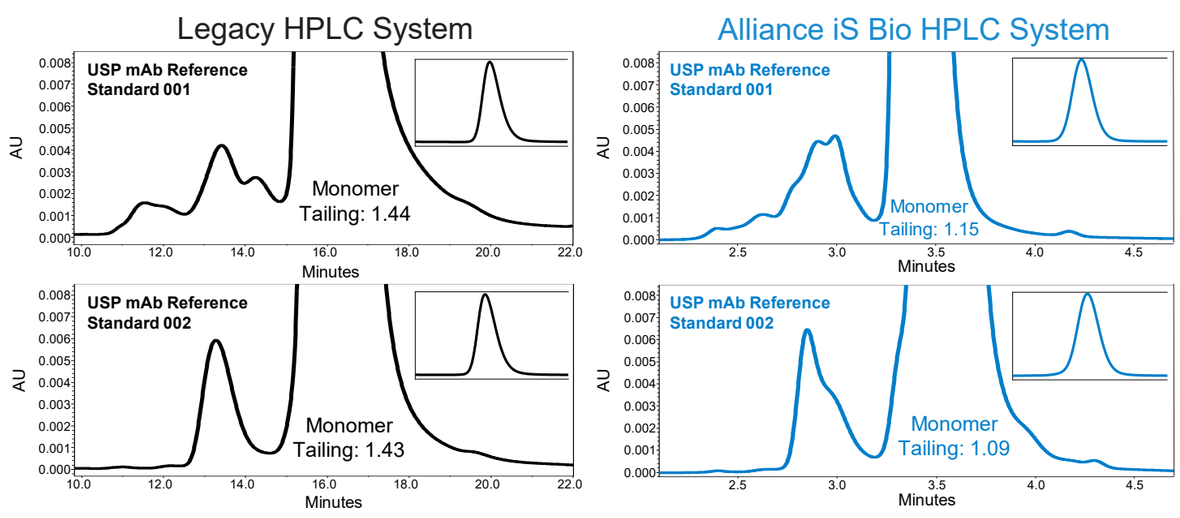

Figure 3: mAb reference standards were analyzed using a stainless-steel SEC column on a legacy HPLC system and a XBridge Premier Protein SEC column on the Alliance iS Bio HPLC System. By taking advantage of MaxPeak HPS Technology coupled with the lower system dispersion on the Alliance iS Bio HPLC System, monomer tailing significantly improved which consequently resulted in improved resolution of the low molecular weight species (LMWS).

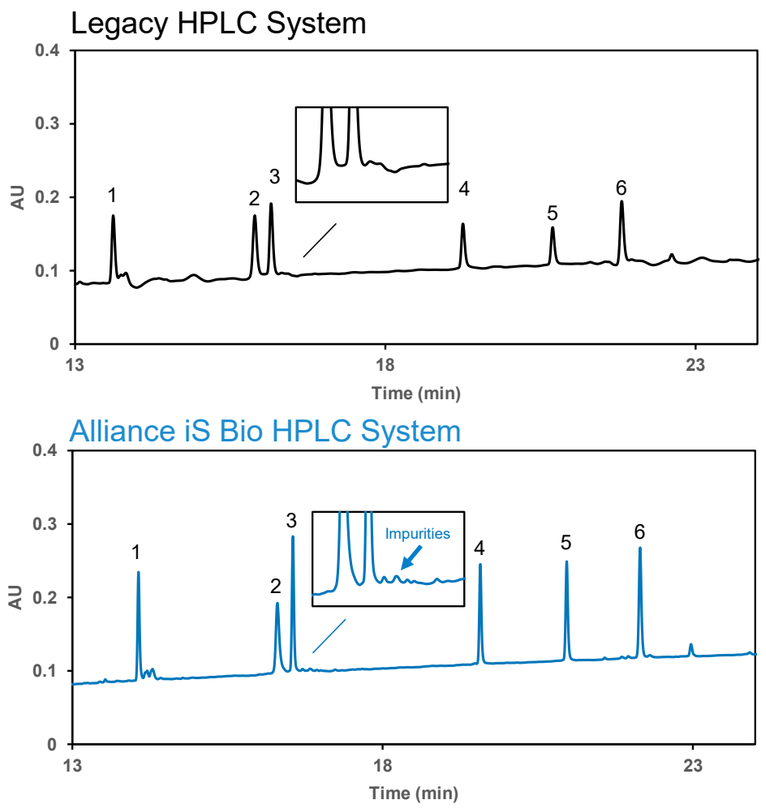

Figure 4: The larger mixing volume of the Alliance iS Bio HPLC System produces chromatograms with lower baseline noise and improved peak shape for the GLP-1 peptides when compared to the legacy HPLC system. This enables improved accuracy in the detection and integration of low abundant impurities and main peaks.

Peak	Analyte	Legacy HPLC system (n=6)		Alliance iS Bio HPLC System (n=6)	
		Area	RSD%	Area	RSD%
1	Glucagon	355246	7.06%	340303	0.28%
2	Exenatide	366927	6.43%	393247	0.70%
3	Dulaglutide	389359	6.60%	425136	0.66%
4	Semaglutide	253344	5.05%	339947	1.57%
5	Liraglutide	229023	6.77%	345345	2.04%
6	Tirzepatide	374355	1.45%	423525	1.63%

CONCLUSION

- **The biocompatible and bio-inert construction of the Alliance iS Bio HPLC System is well suited for biotherapeutic analysis of oligonucleotides, monoclonal antibodies, and small biologics such as GLP-1 analytes.**
- **The Alliance iS Bio HPLC System demonstrated increased resolution and recovery of biotherapeutics while delivering consistent performance with improved precision.**
- **The larger mixing volume enhances low-level impurities for improved accuracy in detection and integration.**

References

1. Du X, Birdsall RE, Bigos P, Han D, Nyholm K. Deploying the Alliance™ iS Bio HPLC System as a modern HPLC for biopharmaceutical analysis in QC Environments. Waters Application Note. April 2024. 720008288EN.
2. 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.
3. USP. Chromatography <621>. In: USP-NF. Rockville, MD: USP; Dec 1, 2022.

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