

Next-Generation Bio-Inert HPLC System for Enhanced Analysis of Biotherapeutics

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PURPOSE

For routine analysis during the manufacturing of biopharmaceuticals, HPLC systems are widely used because of their reliability and robustness. However, with regulatory bodies placing greater emphasis on method life cycle management, there is a need to explore ways to enhance the performance of HPLC instruments in terms of speed and sensitivity. In response to these regulatory pressures and to mitigate the challenges inherent in biotherapeutic analysis, a next-generation HPLC instrument platform, called the Alliance™ iS Bio HPLC System, has been developed and designed to keep pace with the progress made in biotherapeutics.

In this poster, the Alliance iS Bio HPLC System was used for the analysis of oligonucleotides, monoclonal antibodies, and GLP-1 peptides. This next-generation HPLC system is both biocompatible and bio-inert, constructed with biocompatible materials that are resistant to corrosion from high ionic strength and acidic mobile phases and engineered with MaxPeak™ High Performance Surfaces (HPS) Technology to eliminate non-specific adsorption of analytes to metal surfaces. To evaluate the advantages that the Alliance iS Bio HPLC System provides for new modalities and traditional biotherapeutics, data was compared to a legacy HPLC system and evaluated on speed and recovery.

OBJECTIVE(S)

Demonstrate that the Alliance iS Bio HPLC System is an advancement in HPLC platform technology designed to address challenges of non-specific adsorption in quality control labs. The next-generation HPLC incorporates MaxPeak HPS Technology to provide a bio-inert solution, delivering increased resolution, sensitivity, and speed to yield more accurate and consistent results for new modalities and traditional biotherapeutics.

METHOD(S)

Oligonucleotide Analysis¹

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™ C₁₈ Column, 5 μm,130Å, 4.6 x 100 mm (p/n: 186003115)
XBridge BEH C₁₈ 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 C₁₈ Column, 2.5 μm, 130Å, 4.6 x 100 mm (p/n:186009902)
XBridge BEH C₁₈ Column, 5 μm,130Å, 4.6 x 100 mm (p/n: 186003115)

Shared Conditions:

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

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% trifluoroacetic acid, 1% formic acid in ACN, and 98.5% water.

Monoclonal Antibody Analysis²

USP mAb reference standards were injected at 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 vol.: 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 Column 2.5 μm, 7.8 x 150 mm (p/n: 186009961)
Injection vol.: 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

Legacy HPLC System Method Conditions:

Column: XSelect™ Peptide CSH™ C₁₈ Column, 130Å, 2.5 μm, 4.6 x 150 mm (p/n: 186007038)

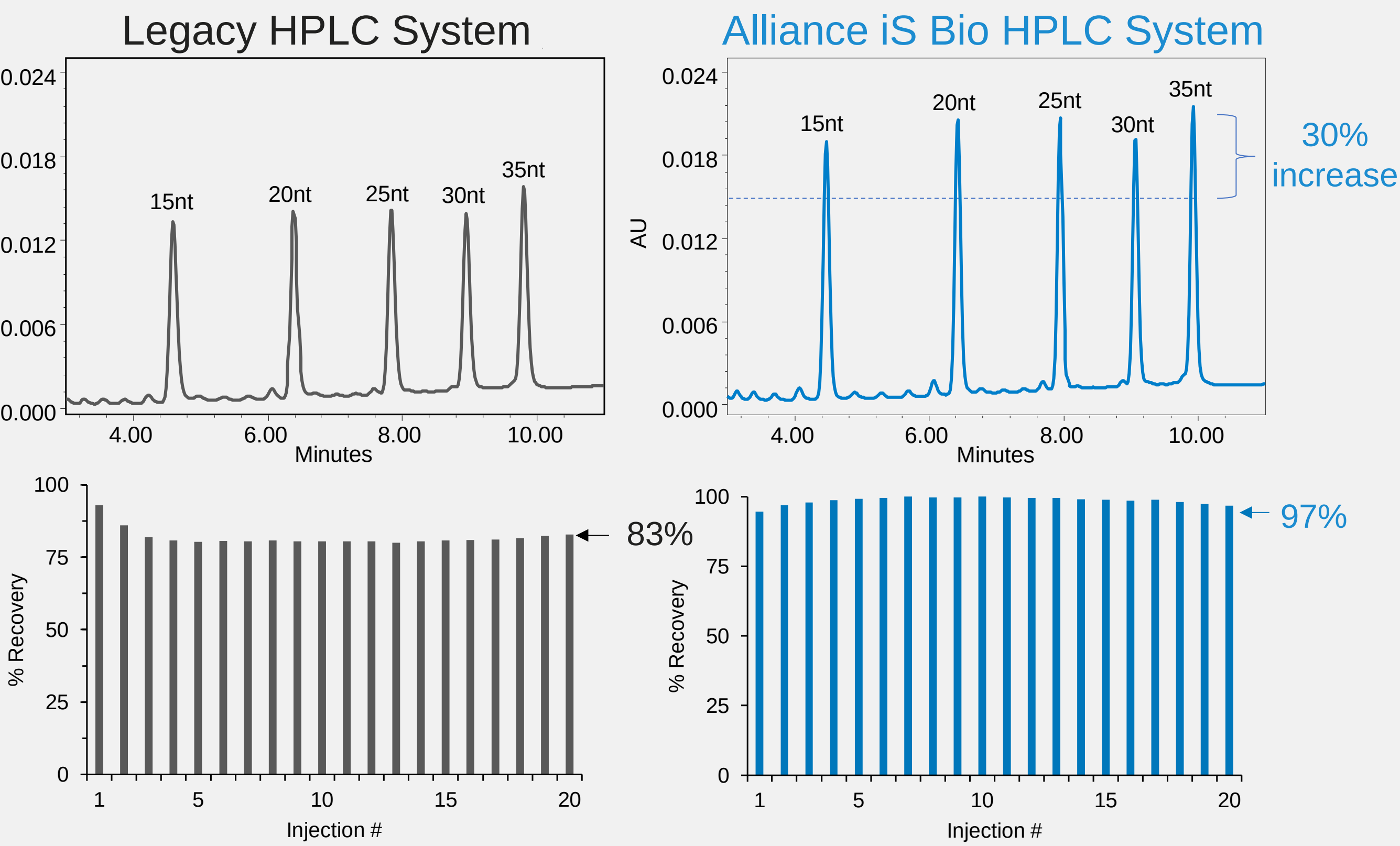
Alliance iS Bio HPLC System Method Conditions:

Column: XSelect Premier Peptide CSH C₁₈ Column, 130Å, 2.5 μm, 4.6 x 150 mm (p/n: 186009909)

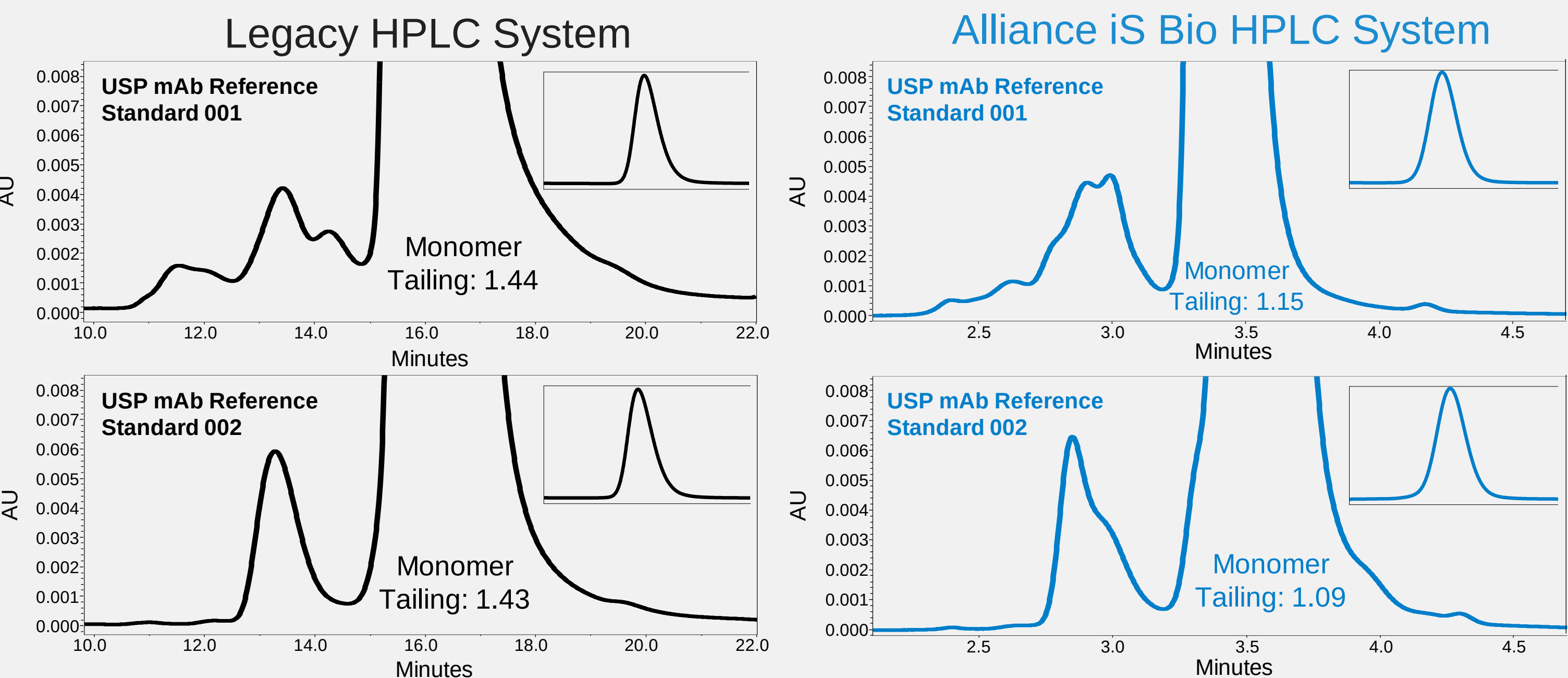
Shared Conditions:

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

RESULT(S)



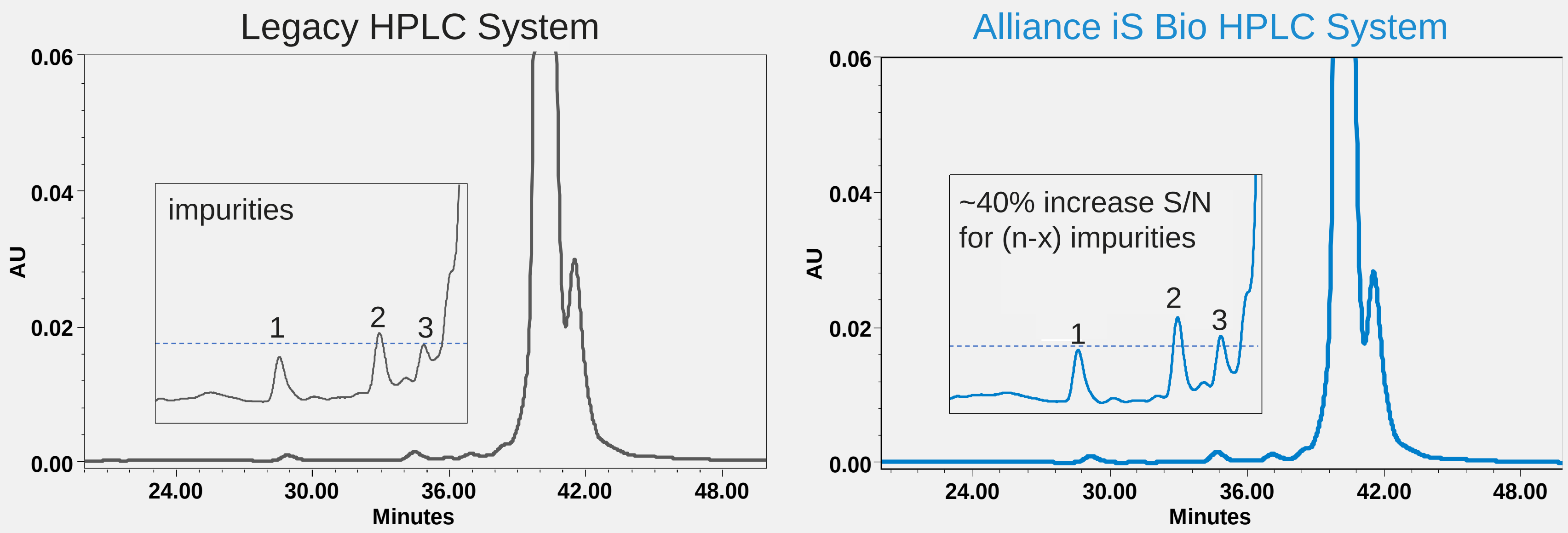
The Alliance iS Bio HPLC System with an XBridge Premier Oligonucleotide BEH Column showed a 30% increase in peak height when compared to a legacy HPLC system with a stainless-steel column when analysing the MassPREP OST. Additionally, the Alliance iS Bio HPLC System achieved maximum recovery in a shorter time span, thereby helping eliminate the need for lengthy passivation procedures.



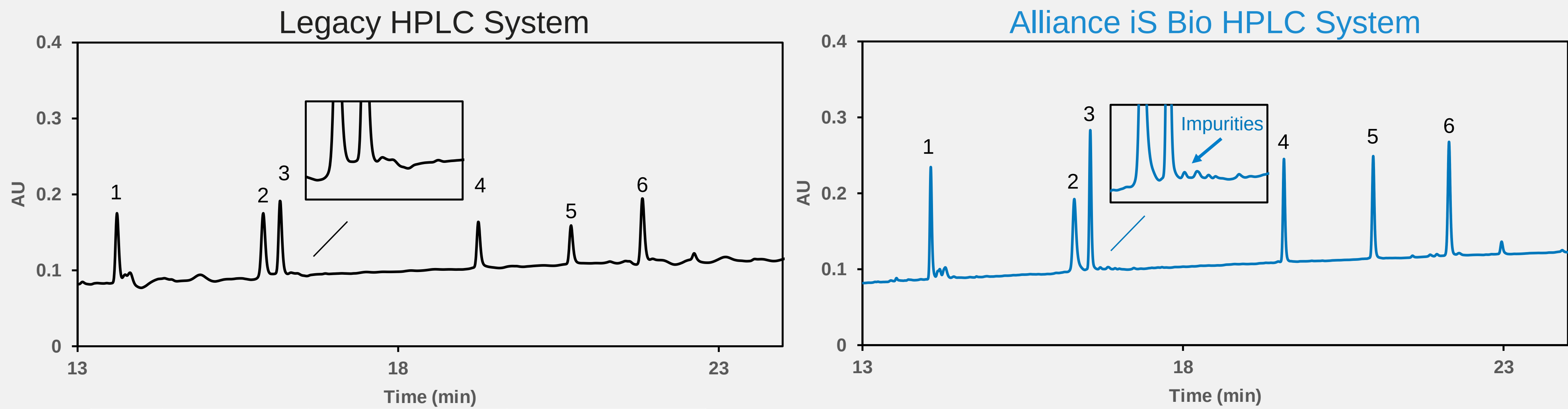
mAb reference standards were analyzed using a stainless-steel SEC column on a legacy HPLC system and an XBridge Premier Protein SEC Column on the Alliance iS Bio HPLC System. By using MaxPeak HPS Technology coupled with the lower system dispersion on the Alliance iS Bio HPLC System, monomer tailing significantly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

CONCLUSION(S)

- The biocompatible and bio-inert construction of the Alliance iS Bio HPLC System is designed for biotherapeutics and can be broadly deployed to analyze new and traditional modalities for routine analysis during manufacturing.
- The Alliance iS Bio HPLC System demonstrated increased resolution and recovery while delivering consistent performance with improved precision for oligonucleotides, mAbs, and GLP-1 analytes.
- With higher system pressure tolerance and lower system dispersion, the Alliance iS Bio HPLC System supports 2.5 μm columns which significantly reduces operating costs in analysis time, solvent, and sample use.



GEM91 was analyzed for impurities to further investigate the performance differences across systems. Utilizing the 5 μm XBridge BEH C₁₈ 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.



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%

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.

REFERENCE(S)

- 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.
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