# ADVANCING ANALYTICAL PERFORMANCE FOR BIOTHERAPEUTICS 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 Technology. This technology was then used in the construction of a bio-inert system called the Alliance™ iS Bio HPLC System.

This poster will demonstrate that the Alliance iS Bio HPLC System can be broadly deployed to analyse new modalities and traditional biotherapeutics for routine analysis during manufacturing. We showcase that improved resolution and recovery can be achieved when compared to legacy HPLC Systems.

## 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 chromatography (SEC), and RPLC were used as the prevailing techniques for analyzing biotherapeutics on these HPLC platforms.

### Oligonucleotide Analysis (IP-RPLC)[1]

GEM91, a 25 mer fully thiolated phosphorothioate oligonucleotide (0.5 mg/mL) were injected onto the legacy HPLC system and the Alliance iS Bio HPLC System. Both systems used the XBridge™ BEH™ C18 Column, 5 µm,130Å, 4.6 x 100 mm and 25 mM hexylammonium acetate as the ion-pairing agent.

### Monoclonal Antibody Analysis (SEC)[2]

NISTmAb RM 8671 was injected at a concentration of 10 mg/mL in formulation buffer. The legacy HPLC system used a BioSuite™ Diol (OH) Column, 250Å, 5 µm, 7.8 mm x 300 mm and the Alliance iS Bio HPLC System used an XBridge Premier Protein SEC Column, 250Å, 2.5 µm, 7.8 x 300 mm. Different concentrations of potassium phosphate and potassium chloride were evaluated on both systems.

### GLP-1 Analysis (RPLC)[3]

The GLP-1 panel mixture was prepared at 0.05 mg/mL for each peptide in 1% trifluoracetic acid, 0.5% formic acid, and 98.5% water. The legacy HPLC system used an XSelect™ Peptide CSH™ C18 Column, 130Å, 2.5 µm, 4.6 x 150 mm and the Alliance iS Bio HPLC System used an XSelect Premier Peptide CSH C18 Column, 130Å, 2.5 µm, 4.6 x 150 mm. A 2.725% ACN/min gradient with 0.1% formic acid was used to separate the GLP-1 panel mixture.

## RESULTS AND DISCUSSION

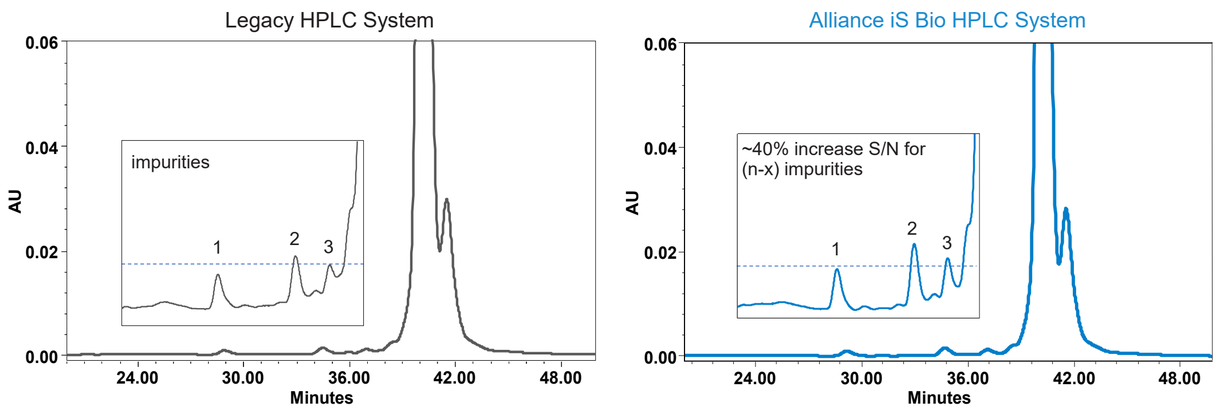



Figure 1: Despite its refinement as a drug substance, GEM91 contains impurities that necessitate monitoring. Utilizing the 5 µm XBridge BEH $C_{18}$ Column on both systems, the Alliance iS Bio HPLC System showed an ~40% increase in the signal-to-noise ratio for the trace impurities versus the legacy HPLC system. This improvement translates to enhanced recovery and increased accuracy when analyzing critical species for novel therapies.

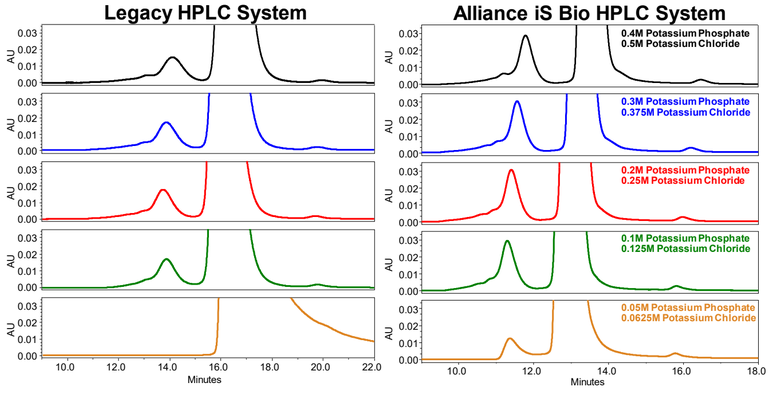
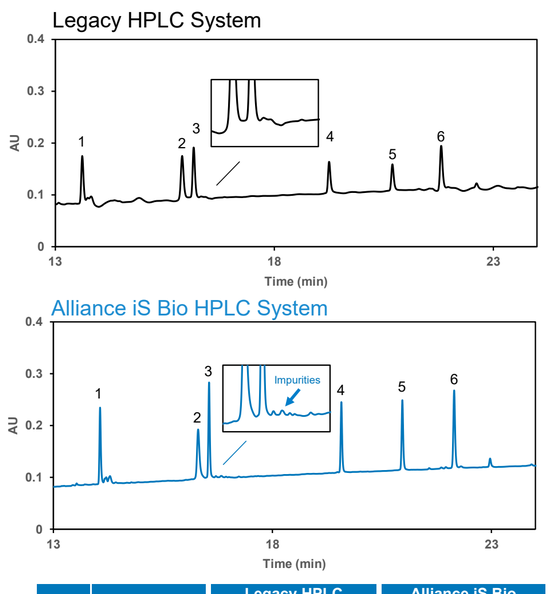



Figure 2: Salt concentration in mobile phase is a critical parameter for achieving robust and reliable SEC separations. The Alliance iS Bio HPLC System with an XBridge Premier Protein SEC Column demonstrated a broader range of salt tolerance which gives more flexibility in method development and a greater degree of adaptability in the analysis of biotherapeutics when compared to a legacy HPLC system with a stainless steel column.

| System | Salt conc. | Main peak (monomer) | | | | | HMWS | LMWS |
|---|---|---|---|---|---|---|---|---|
| | | RT | % Area | HH Res. | Width @ 4.4% | USP T | % Area | % Area |
| Legacy HPLC System | 2X | 16.30 | 96.69 | 1.64 | 1.59 | 1.45 | 3.11 | 0.20 |
| | 1.5X | 16.07 | 96.65 | 1.77 | 1.55 | 1.49 | 3.14 | 0.20 |
| | 1X | 15.96 | 96.65 | 1.89 | 1.51 | 1.49 | 3.16 | 0.20 |
| | 0.5X | 16.01 | 96.95 | 1.88 | 1.55 | 1.54 | 2.84 | 0.20 |
| | 0.25X | 16.33 | 100.00 | N/A | 3.02 | 3.24 | N.D. | N.D. |
| Alliance iS Bio HPLC System | 2X | 13.39 | 96.67 | 2.40 | 0.74 | 1.69 | 3.10 | 0.23 |
| | 1.5X | 13.11 | 96.53 | 2.32 | 0.74 | 1.78 | 3.24 | 0.23 |
| | 1X | 12.92 | 96.50 | 2.24 | 0.75 | 1.84 | 3.27 | 0.23 |
| | 0.5X | 12.78 | 96.61 | 2.29 | 0.77 | 1.88 | 3.16 | 0.22 |
| | 0.25X | 12.75 | 98.50 | 2.21 | 0.81 | 1.97 | 1.14 | 0.36 |




| 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% |

Figure 3: 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.

## 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 and lower system dispersion improves accuracy in detection and integration of biotherapeutics.**

## 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. 720008288EN.
2. Bigos P, Birdsall RE, Nyholm K. Evaluating the Ruggedness of the Alliance™ iS Bio HPLC System for SEC Separations. Waters Application Note. 720008427EN.
3. 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. 720008345EN.

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