METHOD TRANSFER AND ROUTINE ANALYSIS OF PROTEIN AND PEPTIDE-BASED DRUG PRODUCTS USING A BIOCOMPATIBLE UHPLC SYSTEM

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INTRODUCTION

Many of the top-selling pharmaceuticals currently on the market and in the pipeline are biologics. To date, many of the methods used in development and quality control laboratories are HPLC-based. While this may be sufficient in some cases, there are noted advantages of updating legacy systems and methods with more modern instrumentation. By updating from an HPLC platform, better resolution, shorter run time, and greater peak capacity can be achieved.

The product lifecycle includes development and manufacturing activities as well as technology transfers. When adopting new technology, it is of critical importance that instrumentation be robust and easily deployed. In this work, a biocompatible UHPLC platform will be used to replicate legacy HPLC methods and demonstrate system equivalency across HPLC, UHPLC, and UPLC platforms. Furthermore, the benefits of laboratory modernization are shown through new column chemistries with smaller particle size and lower dispersion LC systems. Bio-inert flow paths aid in system robustness and performance, which is especially important when using the high salt or extreme pH conditions that are required for many of the mobile phases used in bioseparations. To this point, intra system repeatability is demonstrated for SEC, IEX, and HIC separations. Additional features of the UHPLC system including dual flow paths and a tool for modifying the gradient start time (without making changes to the gradient table) are employed to ensure consistent results are maintained across LC platforms. System features for aiding in the method development process are also highlighted in an IEX workflow for charge variant analysis in method transfer across different vendor platforms. Through purposeful LC system design, method transfer and development as well as routine bioseparations are streamlined to add confidence to a dynamic biotherapeutic environment.

CONCLUSION

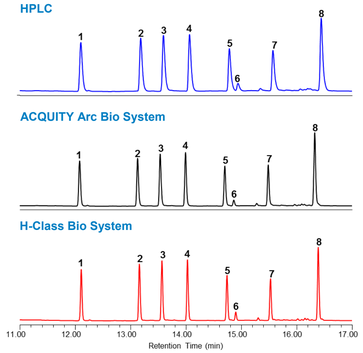
- **Seamless IEX method transfer from a different LC platform without requiring changes to method**
- **Resolution and peak capacity improvements through modernization of HPLC instrumentation**
- **Repeatability demonstrated in high salt mobile phase**
- **A single particle size used across LC platforms for mAb subunit analysis**
- **Complementary orthogonal mass detection increases confidence in results compared to optical-based assays**

References

1. ICH, ICH Q10 Pharmaceutical Quality System. 2008.

Performance gains achieved through modernization of equipment

Updating legacy HPLC methods and instrumentation to more modern instrumentation can offer performance benefits such as improved resolution and peak capacity. In the example below, the same RPLC method was run on an HPLC, an ACQUITY Arc Bio System, and an H-Class Bio System. As the same column was used on all three systems, peak broadening can largely be attributed to differences in the physical components within the flow path of each of the respective instruments. This can have important implications when working with complex samples, realizing better resolution between critical peak pairs or increased sensitivity for detection and monitoring of impurities.



	HPLC	Arc Bio	H-Class Bio
Resolution (Peaks 5 and 6)	1.7	2.7	3.1
Peak Capacity, 4σ (Ave)	118	170	196

Table 1. Resolution and peptide peak capacity comparison across LC platforms. By updating legacy HPLC systems to more modern instrumentation such as the Arc Bio System and the H-Class Bio System, greater performance gains were achieved, which could ultimately provide greater insights in to product quality.

Figure 2. Performance assessment using a preserved method transferred across LC systems. The ACQUITY Arc System and the H-Class Bio System (top performer) generated narrower peak widths, resulting in higher peak capacity was achieved. (Standard mix in order of elution: 1. angiotensin (frag. 1-7), 2. bradykinin, 3. angiotensin II, 4. angiotensin I, 5. renin substrate, 6.—, 7. enolase T35, 8. enolase 37.)

Conditions. Sample: MassPREP Peptide Standards; Column: XBridge BEH C18, 130 Å, 2.5 µm, 4.6 mm x 100 mm; Mobile phase: MPA: water with 0.1% (v/v) TFA, MPB: acetonitrile with 0.1% (v/v) TFA; Flow rate: 0.5 mL/min; Column temperature: 60 °C; Sample temperature: 10 °C; Absorption wavelength: 215 nm; Injection volume: 10 µL; Gradient: 5% B to 50% B in 10 minutes.

ACQUITY Arc Bio System supports chemistries from method development to QC

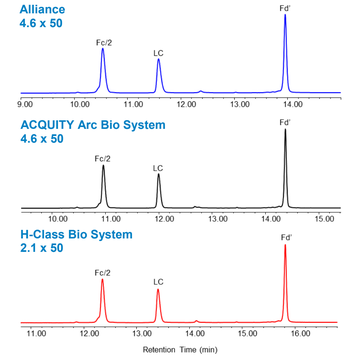


Figure 3. NIST mAb Subunit Standard analyzed by RPLC. Waters BioResolve RP mAb columns were designed specifically for intact and subunit based mAb analysis, using a 2.7 µm superficially porous particle that can be deployed across HPLC, UHPLC, and UPLC instrument platforms. A method for subunit analysis was run on an H-Class Bio System (UPLC) using a column having dimensions 2.1 mm x 50 mm. This method was then scaled to a column having dimensions 4.6 mm x 50 mm and run on an Alliance (HPLC) or an Arc Bio System (UHPLC). Peak area is conserved across all systems (Table 2).

Conditions. Alliance and Arc Bio conditions are shown in parentheses when scaled conditions differ from H-Class Bio method. Sample: Waters Subunit Standard; Column: BioResolve RP mAb, 2.7 µm, 2.1 mm x 50 mm (4.6 mm x 50 mm); Mobile phase: MPA: water with 0.1% (v/v) TFA, MPB: acetonitrile with 0.1% (v/v) TFA; Flow rate: 0.20 mL/min (0.96 mL/min); Column temperature: 60 °C; Sample temperature: 10 °C; Absorption wavelength: 280 nm; Injection volume: 4 µL (19.2 µL); Gradient: 15% B to 55% B in 20 minutes.

	Fc/2 (%)	LC (%)	Fd' (%)
Alliance	34.52	24.43	41.05
Arc Bio	34.23	24.47	41.30
H-Class Bio	34.50	24.31	41.19

Table 2: Peak area (%) across systems.

RESULTS AND DISCUSSION

Figure 1. ACQUITY Arc Bio System. The ACQUITY Arc Bio System is a quaternary UHPLC system engineered with bio-inert materials, which are resistant to system corrosion that can be accelerated by the use of high salt mobile phases and pH extremes, while minimizing carryover from undesirable protein system-interactions.

Repeatability of gradient and isocratic separations

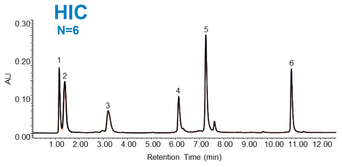


Figure 4. HIC of protein standard mix. For six replicate injections, the retention time standard deviation was <0.007. (Standard mix in order : cytochrome C, myoglobin, ribonuclease A, lysozyme, enolase, α-chymotrypsinogen A.)

Conditions. Sample: HIC Protein Standard Mix; Column: Protein-Pak Hi Res HIC, 2.5 µm, 4.6 mm x 100 mm Column; Mobile phase: MPA: 2 M $(NH_4)_2SO_4$ in 50 mM NaH_2PO_4/Na_2HPO_4 at pH 7, MPB: 50 mM NaH_2PO_4/Na_2HPO_4 at pH 7; Flow rate: 0.60 mL/min; Column temperature: 30 °C; Sample temperature: 4 °C; Absorption wavelength: 220 nm; Injection volume: 2 µL; Gradient: 0% B to 100% B in 15 minutes.

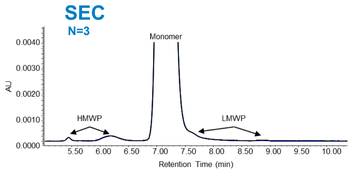
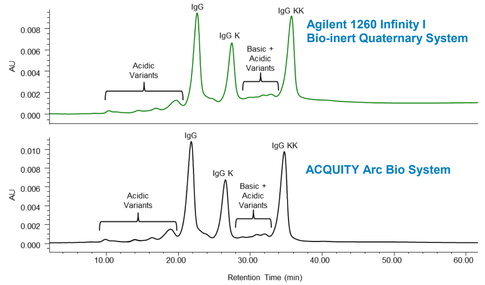


Figure 5. SEC of trastuzumab. Retention time standard deviation was <0.004 for each of the identified peaks.

Conditions. Sample: 2 mg/mL trastuzumab; Column: XBridge Protein BEH SEC, 200 Å, 3.5 µm, 7.8 mm x 300 mm; Mobile phase: 400 mM NaH_2PO_4 + 25 mM NaCl, pH 6.8; Flow rate: 1 mL/min; Column temperature: ambient; Sample temperature: 4 °C; Absorption wavelength: 280 nm; Injection volume: 10 µL.

Established methods can be replicated to show method equivalency



Conditions. Sample: 1 mg/mL infliximab in 20 mM sodium phosphate, pH 6.8; Column: Protein-Pak Hi Res SP, 7 µm, 4.6 mm x 100 mm; Mobile phase (pH 6.4): MPA: 100 mM sodium phosphate monobasic, MPB: 100 mM sodium phosphate dibasic, MPC: 500 mM sodium chloride, MPD: water; Flow rate: 0.5 mL/min; Column temperature: 30 °C; Sample temperature: 4 °C; Absorption wavelength: 280 nm; Injection volume: 20 µL.

Gradient table:

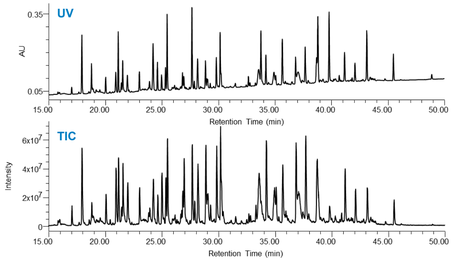
	%A	%B	%C	%D
Initial	14.3	5.7	5.0	75.0
60.00	14.3	5.7	15.0	65.0
65.00	14.3	5.7	70.0	10.0
70.00	14.3	5.7	70.0	10.0
70.10	14.3	5.7	5.0	75.0

Figure 6. IEX method transfer of infliximab. An IEX method for analysis of infliximab was run on an Agilent 1260 Infinity I Bio-inert Quaternary System (top) and transferred to an ACQUITY Arc Bio System (bottom). Because IgG, IgG K, and IgG KK all contain acidic and basic residues, a combination of basic and acidic variants result, as labelled above. Chromatograms are visually highly similar. Relative retention time differences between the two systems ranged from 0.0009 to 0.0478 for all identified peaks following method transfer.

	Acidic Variants			Basic Variants			IgG			IgG K			IgG KK		
	Mean	σ	% RSD	Mean	σ	% RSD	Mean	σ	% RSD	Mean	σ	% RSD	Mean	σ	% RSD
Agilent 1260 Infinity I	4.45	0.18	3.96	0.74	0.08	10.85	38.83	0.22	0.57	21.49	0.04	0.18	33.9	0.39	1.15
ACQUITY Arc Bio	4.67	0.03	0.61	0.67	0.02	3.17	38.88	0.06	0.15	21.4	0.01	0.06	33.7	0.06	0.16
Δ	-0.22			0.07			-0.05			0.09			0.20		

Table 3. IEX peak area percent comparison. Peak area percentage of triplicate injections on the Agilent 1260 Infinity I Bio-inert Quaternary System were compared to the ACQUITY Arc Bio System. All values are within generally acceptable tolerances both within a system and when compared between systems.

Mass detection improves confidence in the ability to transfer peptide maps



Conditions. Sample: trastuzumab digest; Column: XSelect CSH C18, 130 Å, 2.5 µm, 4.6 mm x 100 mm; Mobile phase: MPA: water with 0.1% (v/v) FA, MPB: acetonitrile with 0.1% (v/v) FA; Flow rate: 0.5 mL/min; Column temperature: 60 °C; Sample temperature: 10 °C; Absorption wavelength: 215 nm; Injection volume: 25 µL; Gradient: 1% B to 50% B in 60 minutes; QDa. Ionization mode: ES+, centroid; Mass range: 350 – 1250 m/z; Capillary voltage: 1.5 kV; Probe temperature: 500 ° C; Cone voltage: 10 V.

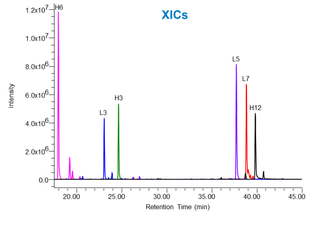


Figure 7 (Left). Peptide map of trastuzumab. An ACQUITY QDa mass detector was incorporated post optical detection to provide complementary mass data.

Figure 8 (Above). XICs of CDR peptides (those peptides unique to trastuzumab). Previous characterization was done using a high resolution MS technique.

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