IMPROVING ANALYSIS OF QUALITY INDICATING ATTRIBUTES FOR BETTER LIFECYCLE MANAGEMENT

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INTRODUCTION

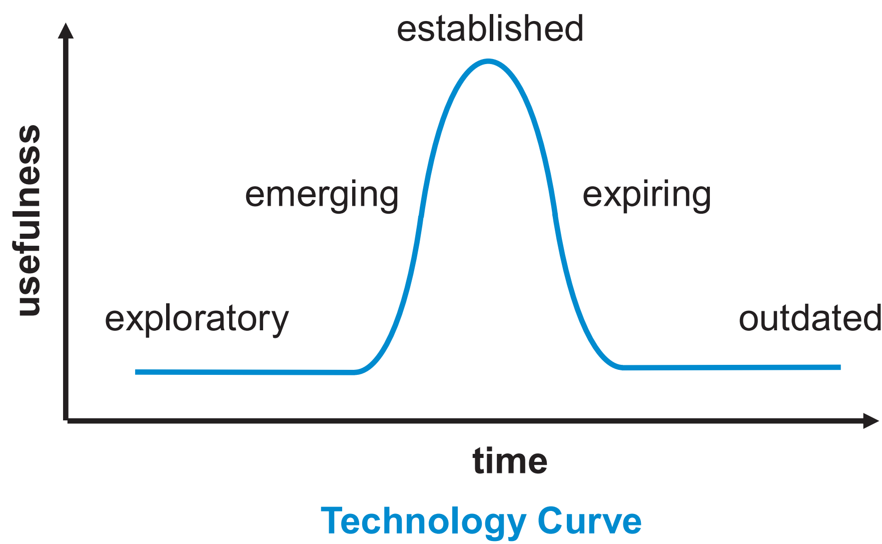
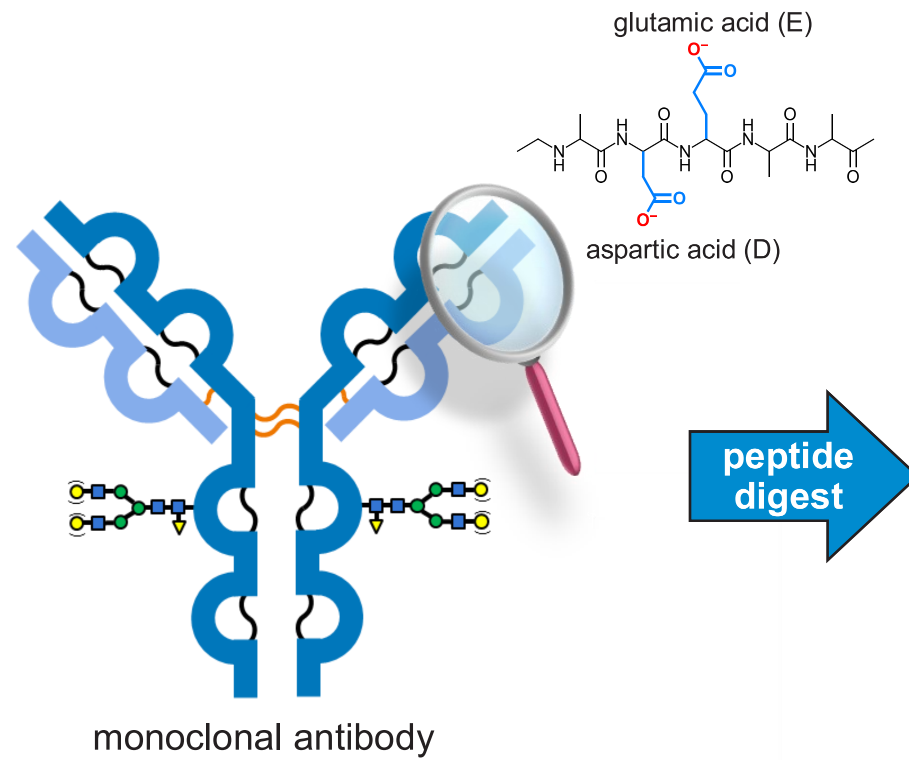


Figure 1. As part of lifecycle management, ICH guidance recommends identifying and evaluating opportunities to improve drug safety and continually expand the body of knowledge in the manufacturing of drug products. This process can be applied to analysis methods as well instruments as they become outdated with faster, more robust, or more sensitive technology that directly impacts consumer safety.



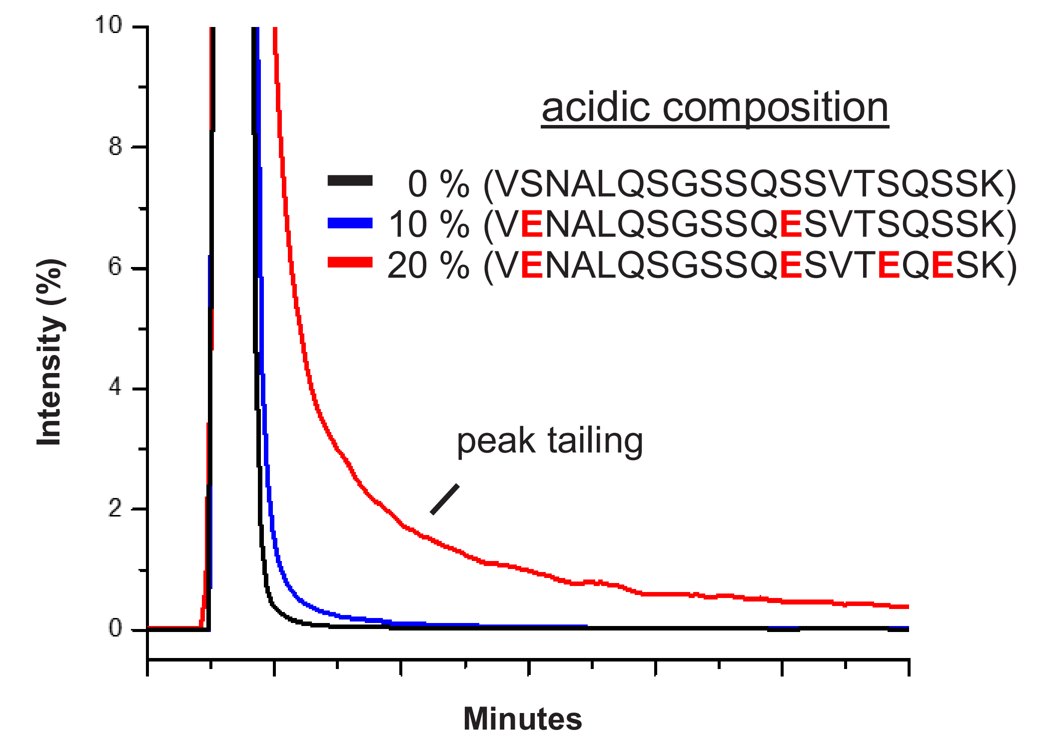


Figure 2. As the building blocks of protein-based therapeutics, peptide mapping is an essential assay for characterization and monitoring of biopharmaceutical products to ensure they are safe and efficacious. Analysis of proteins at the peptide level enables scientists to establish protein identity, investigate site-specific modifications, and monitor impurities. Non-specific adsorption is a phenomenon that has historically hindered performance in separation sciences. Analytes containing electron rich functional groups, such as phosphate and carboxylate groups, are susceptible to adsorb onto metal surfaces which results in peak tailing, diminished recovery, and increased variability.

METHODS

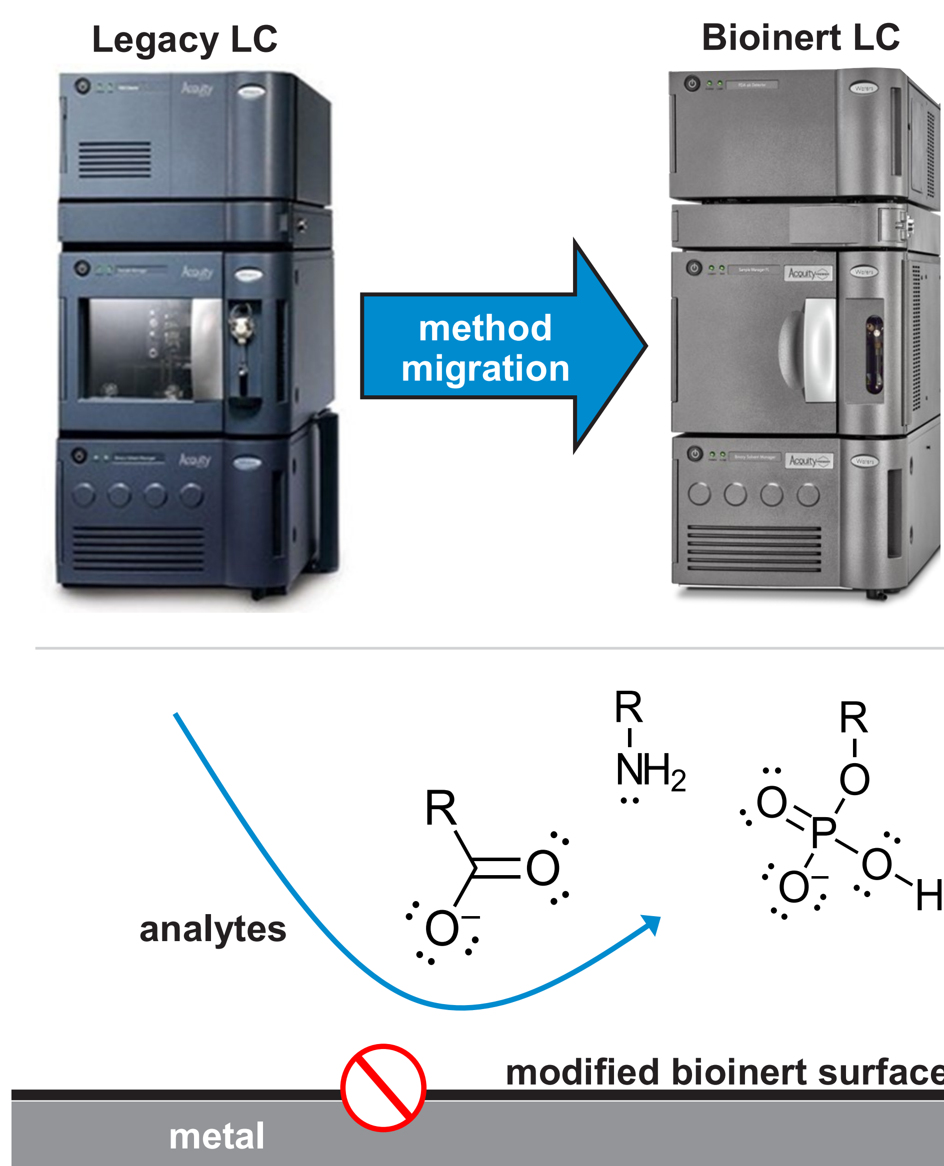


Figure 3. The aim of this study is to migrate a RPLC LC/MS peptide mapping assay method from an existing "Legacy" LC to a LC modified with bioinert hardware "Bioinert LC". Method equivalency will be evaluated in terms of retention time and peak area. Peak tailing and recovery will be used as metrics to determine benefits the bioinert system has towards quality indicating attributes.

Legacy LC: ACQUITY ™ UPLC ™ "Classic" System
Bioinert LC: ACQUITY Premier System (fixed-loop)

Sample: NIST mAb RM 8671 (Trypsin digest)
Injection: 10 µL loop, 5µL injection, PLUNO mode
Sample Temp.: 10 °C
Weak wash: 50:50 H_2O:ACN, 600 µL
Strong wash: 50:50 H_2O:ACN, 200 µL

Column(s):
ACQUITY UPLC CSH ™ C18 Column (1.7µL, 2.1×100mm)
ACQUITY Premier Peptide CSH C18 Column (1.7µL, 2.1×100mm)
Column Temp.: 60 °C

UV settings: 5mm Ti FC, l=214 nm, 10Hz

MS settings (ACQUITY QDa ™ Mass Detector):
Scan mode: Positive
Scan range: 350-1250 m/z
Sampling rate: 5 Hz
Capillary Voltage: 1.5V
Probe temp.: 600 °C
Cone Voltage: 15V

MP A: H_2O, 0.1% FA
MP B: ACN, 0.1% FA

Seal wash:
80:20 H_2O:ACN

Gradient:

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.200	99.0	1.0	Initial
1.00	0.200	99.0	1.0	6
51.00	0.200	65.0	35.0	6
57.00	0.200	15.0	85.0	6
61.00	0.200	15.0	85.0	6
67.00	0.200	99.0	1.0	6
80.00	0.200	99.0	1.0	6

RESULTS

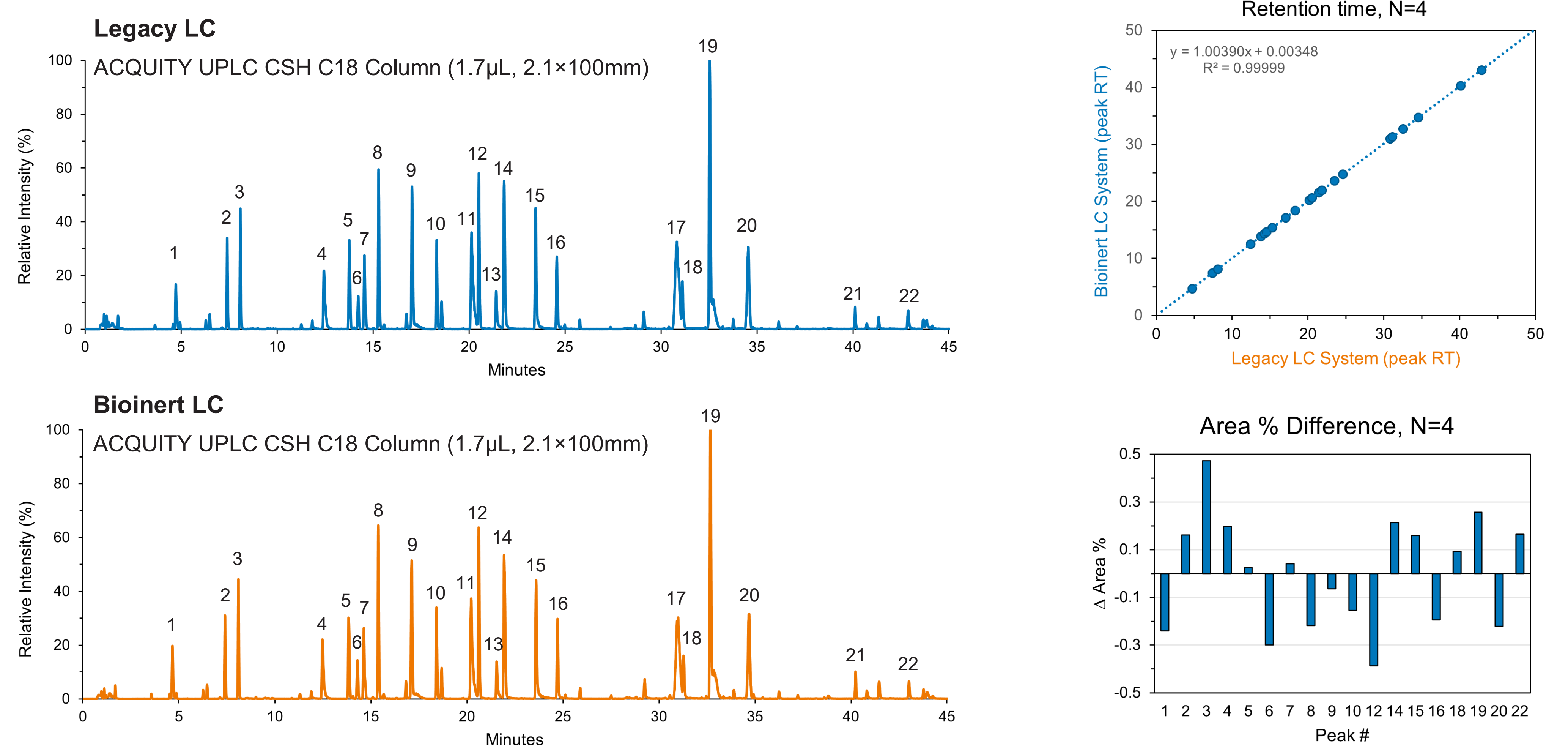
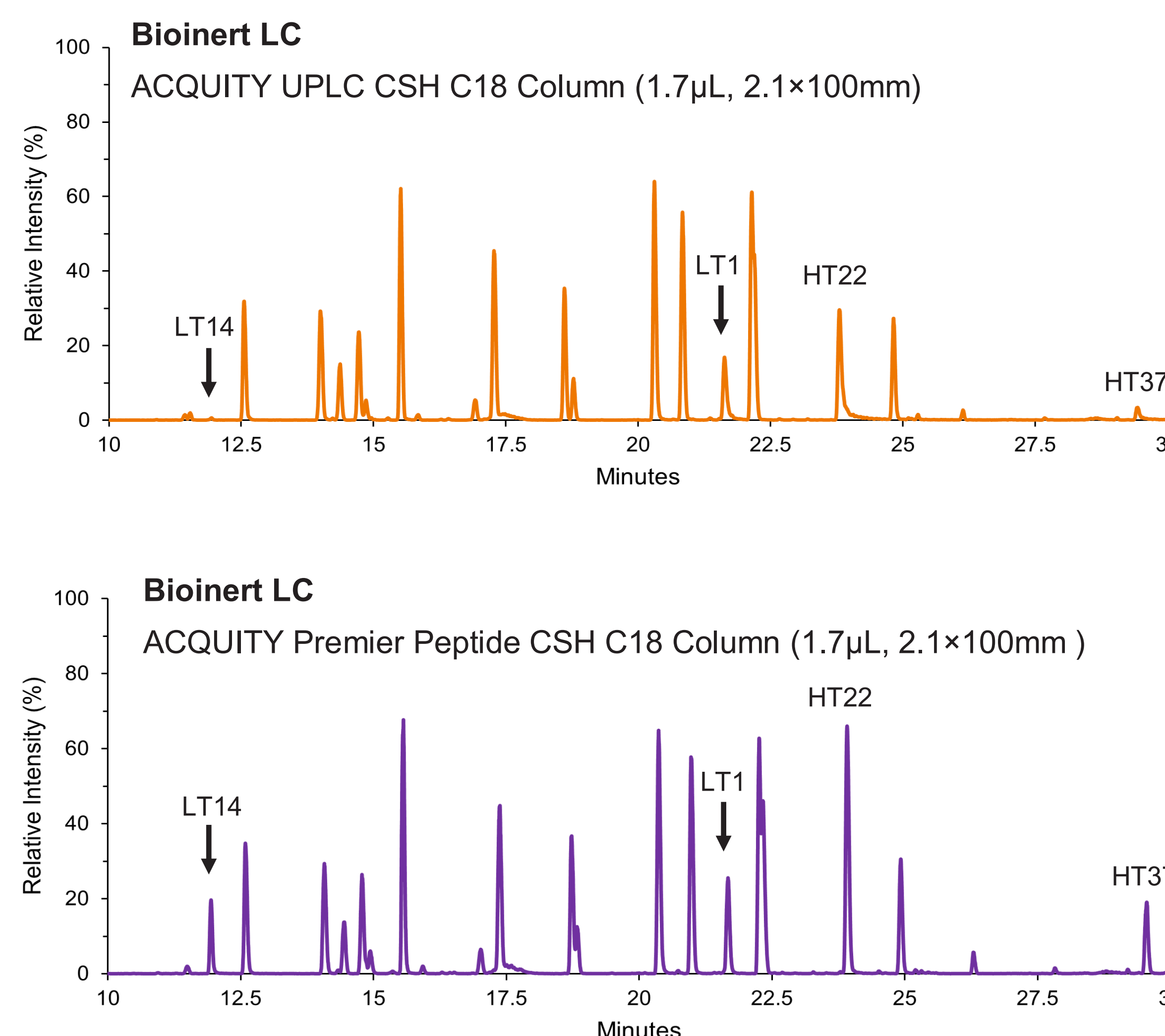


Figure 4. The closely matched dwell volume of the bioinert LC system to the legacy LC system (158.8 µL vs. 149.4 µL) allows for the same method to be run on each system without the need for scaling or dwell volume adjustments to achieve the same separation. An orthogonal plot of retention time exhibited a linear response with a y-intercept of 0.003 indicating selectivity and absolute retention time were preserved across systems. Using the same fixed-loop design between systems allows for consistent injection volume and mass load on the columns as indicated by less than 0.5% difference in peak area between systems enabling easier migration of legacy methods.



"Acidic" Peptides

Peptide	Sequence	Tailing factor	
		Legacy LC	Bioinert LC
LT14	VDNALQSGNSQESVTEQDSK	n.d.	1.3
LT1	DIQMTQSPSTLSASVGDR	1.9	1.2
HT22	TPEVTCVVVDVSHEDPEVK	2.3	1.3
HT37	GFYPSDIAVEWESNGQPENNYK	3.5	1.1

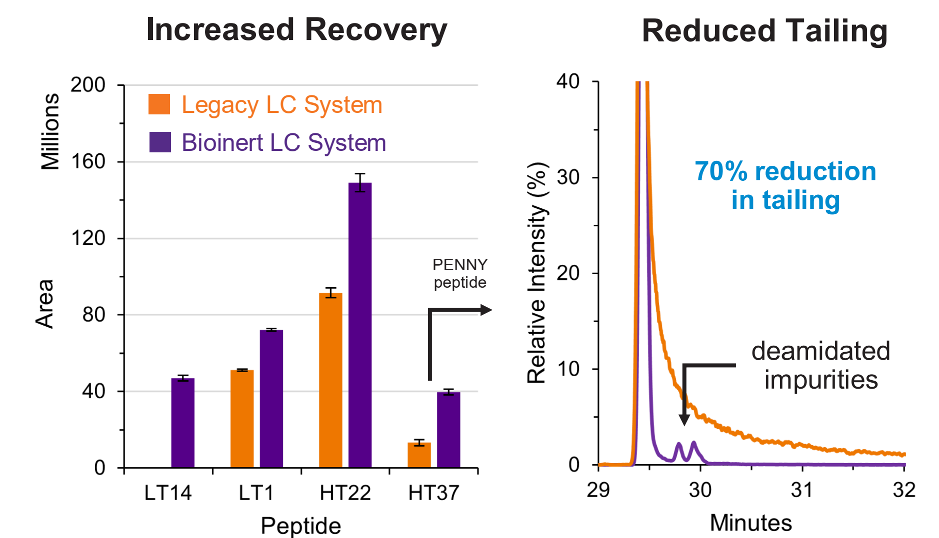


Figure 5. Four peptide that contained 2-4 acidic amino acid residues exhibited up to a 70% reduction (HT37) in peak tailing and 3-fold increase in peak area when separated on the bioinert LC system using a bioinert column. The higher recovery observed with the bioinert LC not only allowed for recovery of "acidic" peptides not previously observed (LT14), but also provided the ability to see critical impurities such as the deamidated species associated with the critical quality attribute related "penny" peptide (HT37), a peptide known to play a role in therapeutic efficacy of mAb-based drug products.

CONCLUSION

- **Closely matched dwell volume and needle design enable easier migration of legacy methods**
- **Bioinert surfaces reduce peak tailing and increase recovery of metal-sensitive analytes for better assay performance and drug safety**

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