DEPLOYING EFFICIENT WORKFLOWS FOR PEPTIDE MAPPING WITH LC-UV/MS PLATFORMS

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INTRODUCTION

Note: ACQUITY QDa Detector colorized for presentation

Peptide mapping is widely used in biopharmaceutical laboratories across workflows from attribute identification and early-stage process development to quality control and manufacturing. Methods and tools that can automate these workflows are advantageous, as they save time and enable easy transfer across labs. The demonstrated inter– and intra-system reproducibility of the ACQUITY™ Premier LC System with integrated Empower™ Software enables confident, streamlined data acquisition and processing to support both process development and manufacturing workflows.

METHODS

Digested mAb samples were analyzed on an ACQUITY Premier BSM LC System with an ACQUITY Premier CSH C18 Column coupled to both a TUV detector and an ACQUITY QDa™ Mass Analyzer.

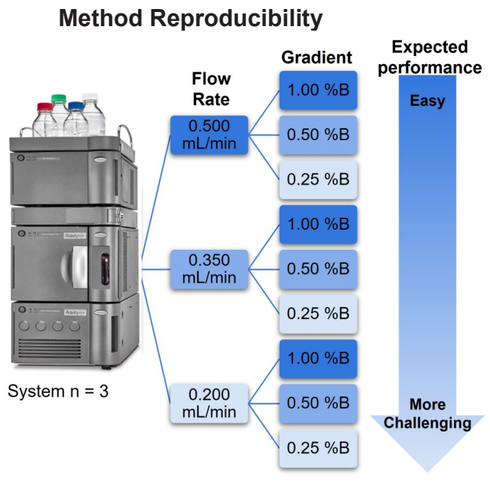

Figure 1. Experimental design for evaluation of intersystem performance across LC conditions.

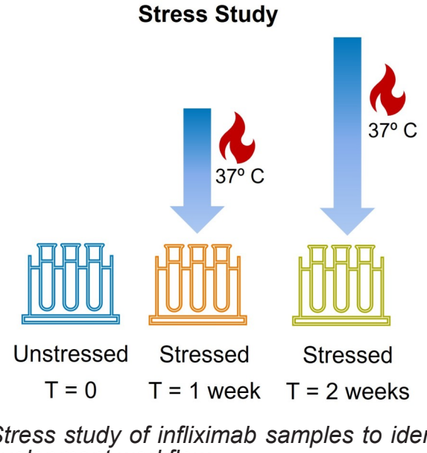

Figure 2. Stress study of infliximab samples to identify attributes in a process development workflow

RESULTS

Method Reproducibility. The retention time reproducibility of peaks in a mAb digest were systematically measured across three distinct systems and a set of nine conditions representing varying degrees of challenge for the binary pump system.

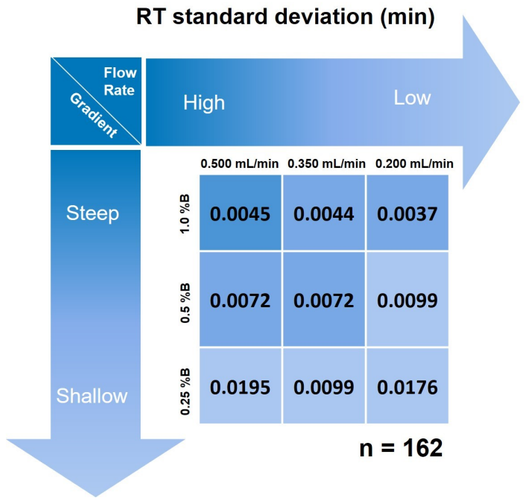

Figure 3. Intersystem retention time standard deviation at each of nine conditions evaluated. Retention times were calculated from 18 peaks measured in triplicate injections on three distinct systems (n = 162).

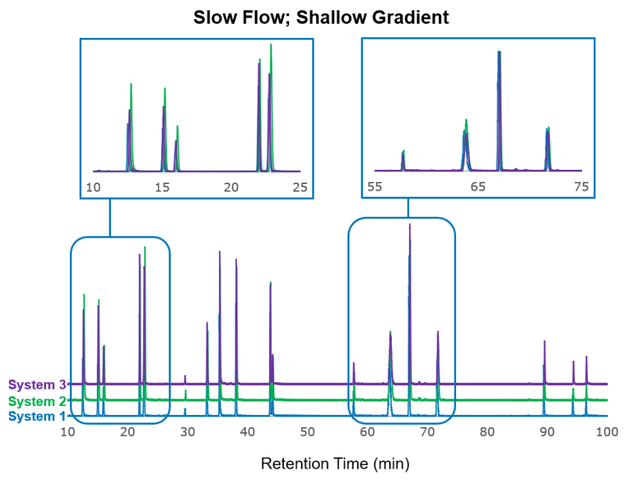

Figure 4. Overlay of NIST mAb digest run on three ACQUITY Premier BSM Systems under the most demanding separation condition. Chromatograms are offset by 10% intensity for visualization.

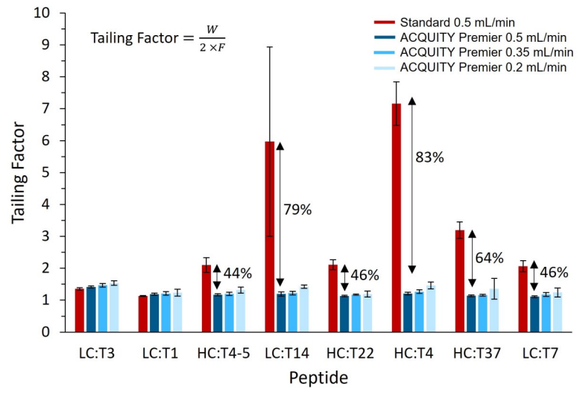

Figure 5. Evaluation of the performance of MaxPeak™ High Performance Surface (HPS) Technology. Tailing factor for one basic peptide (LC:T3) and seven acidic peptides on a standard and an ACQUITY Premier Column at three flow rates, showing reduction in tailing with the ACQUITY Premier Column. Error bars represent standard deviation across 3 systems with 3 replicate injections each (n=9), annotations indicate percent reduction in tailing between columns.

Automated Data Analysis. To streamline data analysis, a comparative processing method was evaluated as a "universal" processing method that can be deployed in both upstream and downstream settings using the same CDS. The results are easily visualized in a report format for rapid analysis of key information.

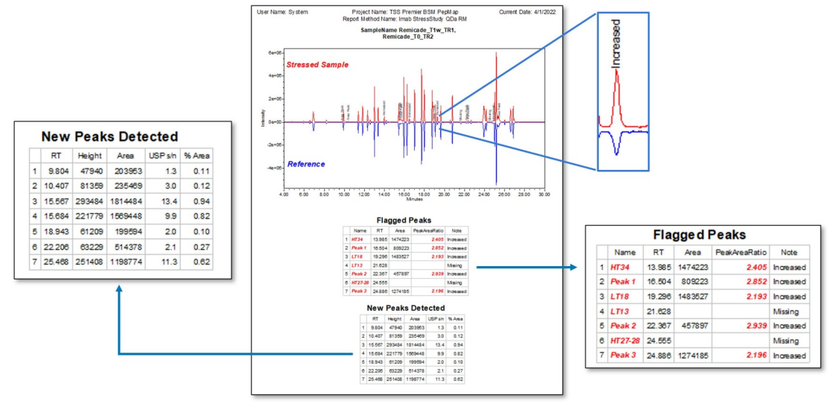

Figure 6. Custom report generated in Empower Software for Infliximab stress study showing 1 week stress compared to 0 week stress reference sample

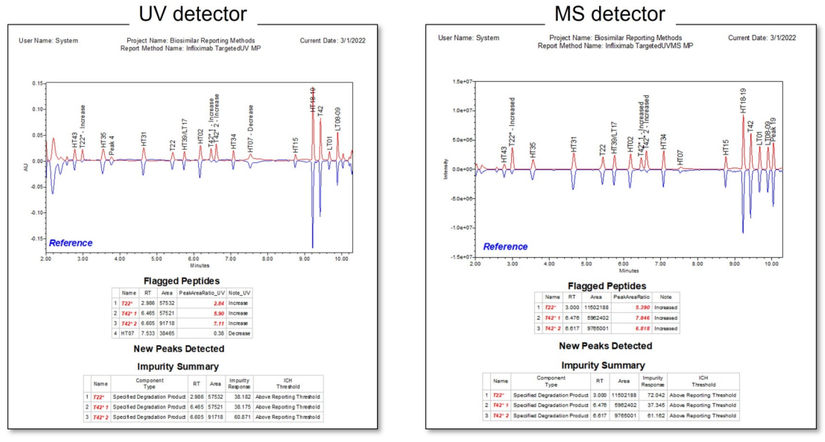

Figure 7. Custom report generated in Empower Software for monitoring levels of target peptides that exceeded defined ratio thresholds that works for both (left) UV and (right) MS detectors.

CONCLUSION

- **ACQUITY Premier UPLC System consistently delivers reproducible results even under challenging conditions.**
- **MaxPeak HPS Technology improves peak shape of acidic peptides.**
- **Empower Integrated Control and Data Analysis Software streamlines data processing with elegant report generation to support process development and manufacturing workflows.**

References

1. DeLaney K, Birdsall RE, Yu YQ. High Precision Performance of the ACQUITY Premier System (BSM Configuration) for Reproducible Peptide Mapping. Waters Application Note, 2022 April, 720007593.
2. DeLaney K, Birdsall RE, Yu YQ. Utilizing Empower Software to Streamline and Automate the Detection of Sample-to-Sample Differences within Peptide Maps of Biopharmaceuticals. Waters Application Note, 2022 May, 720007610.

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