MONITORING PRODUCT ATTRIBUTES IN BIOPHARMACEUTICAL DEVELOPMENT AND QC WITH LC/MS

Scott J. Berger, Liuxi Chen, Henry Shion, Min Du, Ying Qing Yu

Waters Corp, Milford, MA, United States

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INTRODUCTION

- LC/MS peptide mapping acquisition workflows used for biotherapeutic characterization can be reapplied to development monitoring product attributes, and again for QC/lot release.
- Peptide map acquisition methods can be maintained, but informatics workflows should change between characterization exercise the more targeted monitoring assays.
- LCMS^E HRMS peptide mapping data for Infliximab was acquired on a compliant UNIFI based UPLC/UV/ Xevo QTof MS platform.
- Characterization results from a reference sample were parsed to select peptides for targeted monitoring of product attributes.
- This targeted monitoring list was applied to screen across a multibatch sample set, using common acquisition conditions.
- The rationale for component review, quantification, and handling of new or differential peaks is described.

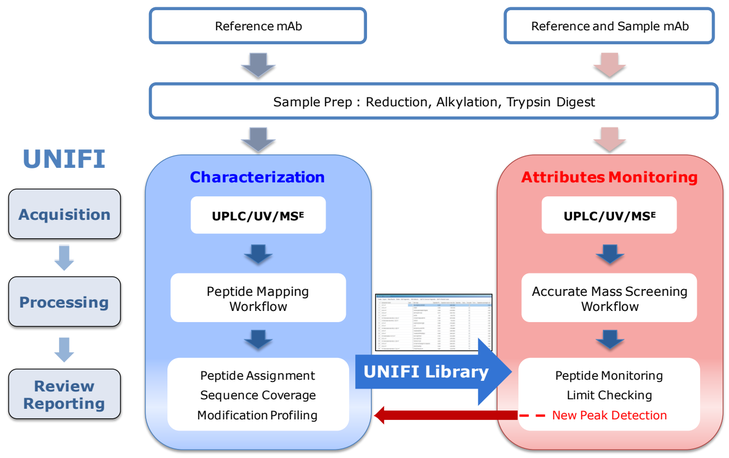

Figure 1: Transitioning from characterization to attribute monitoring workflows within the UNIFI Platform Solution.

METHODS

Sample Preparation:

Peptide map analysis: 18 Samples (3 batches x 2 process x triplicate) of Infliximab were denatured, alkylated and digested by trypsin.

LC/MS:

LC System: ACQUTIY UPLC H-Class Bio System
Column: ACQUITY UPLC BEH300 C18, 1.7 µm, 2.1 x 100 mm
Column temperature: 65 °C
Mobile phase: A. 0.02% Trifluoroacetic acid in water,
B. 0.02% Trifluoroacetic acid in acetonitrile
Gradient: 1-35 %B over 60 min
TUV Detection: 214 nm

MS System: *Xevo G2-S QTof MS*
Data Acquisition: MS^E Mode: ESI positive mode
Capillary Voltage: 3.0 kV Cone Voltage: 30 V
Source Temperature:100 °C Desolvation Temperature: 250 °C
Mass Range (*m/z*): 100-2000
Lock mass used: Glu-Fibrinopeptide B ([M+2H]$^{2+}$, 785.8426)

MS^E settings:
Scan rate for alternating low/high Energy: 0.5 sec
Low energy: 6 V
High energy ramp: 20-45 V

Informatics:

UNIFI Scientific Information System v1.8
- Peptide mapping (MS^E) workflow
- Accurate mass screening workflow
- UNIFI scientific library

CONCLUSIONS

- Product attribute characterization and monitoring analyses were executed using a common peptide mapping data acquisition methodology (UPLC/UV/MS^E), but using unique UNIFI post-acquisition processing workflows optimized for each analysis.
- This approach enables efficient transfer of peptide mapping platforms and analytical methods between groups in early and late development, and multi-attribute monitoring in QC.
- Such transferability is facilitated by:
  - ✓ A compliant-ready UNIFI platform
  - ✓ System suitability and limit check capabilities
  - ✓ Robust Multi-Channel Quantification (UV and MS)
  - ✓ New/differential peak detection
  - ✓ Ability to rapidly update the multi-attribute screening method as new product knowledge is acquired.

RESULTS AND DISCUSSION

Characterizing Product Attributes using the UNIFI Peptide Mapping Workflow

Figure 2: The UNIFI MS^E peptide mapping workflow was used to define Infliximab reference sample attributes, confirm protein identity/sequence, and determine product modification variants.

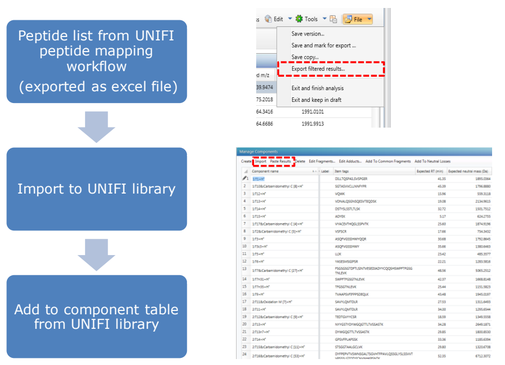

Figure 3: The UNIFI Scientific Library is a repository where information from multiple characterization runs can be aggregated and managed to produce target lists for subsequent screening based analyses.

Monitoring Product Attributes using the UNIFI Accurate Mass Screening Workflow

Figure 4: The UNIFI accurate mass screening workflow enabled targeted multiattribute monitoring of 6 batches (3 replicates injection) of Infliximab samples, providing rapid qualitative assessment (A) of each sample, and for robust quantification and comparison across this larger multi-batch data set (B).

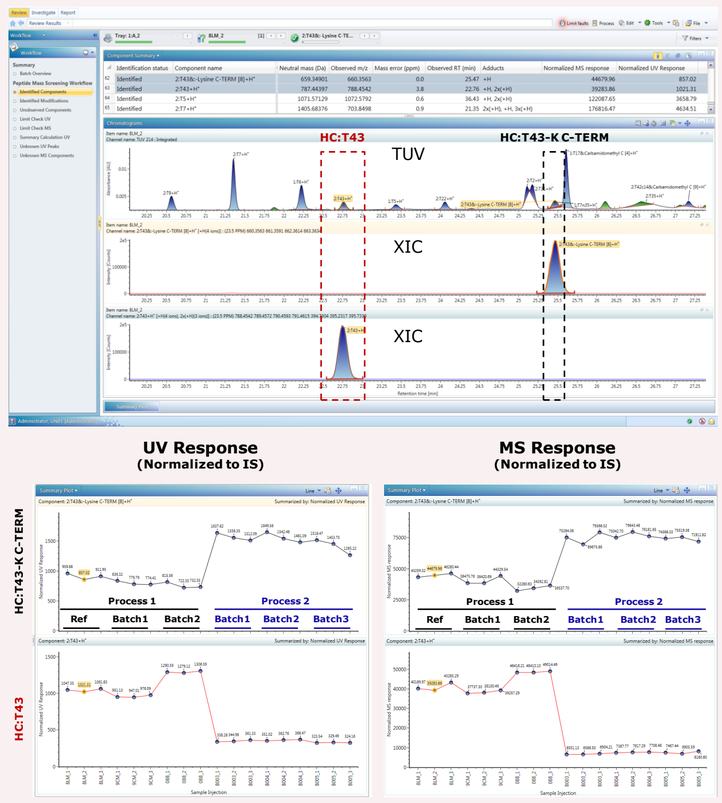

Figure 5: Variation in C-terminal Lysine processing across Infliximab batches. A) Reviewing processed and integrated results in UNIFI can be obtained for both optical and MS data channels, including eXtracted Ion Chromatograms (XIC) for each targeted component. B) Summary plots demonstrate normalized UV and MS response for C-terminal peptides with and without the terminal lysine residue.

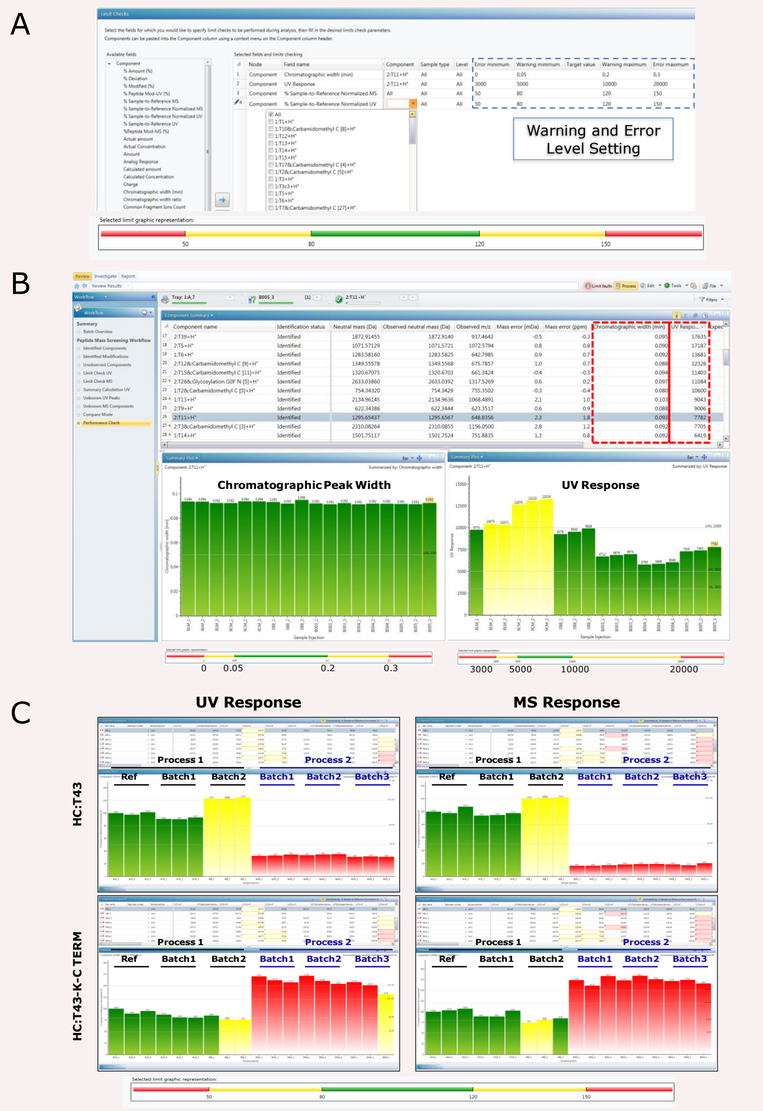

Figure 6: Setting limits and system suitability criteria (A) enables color coded highlights for samples or batches that exceed data quality criteria (B), or breach expected limits for component ranges (C).

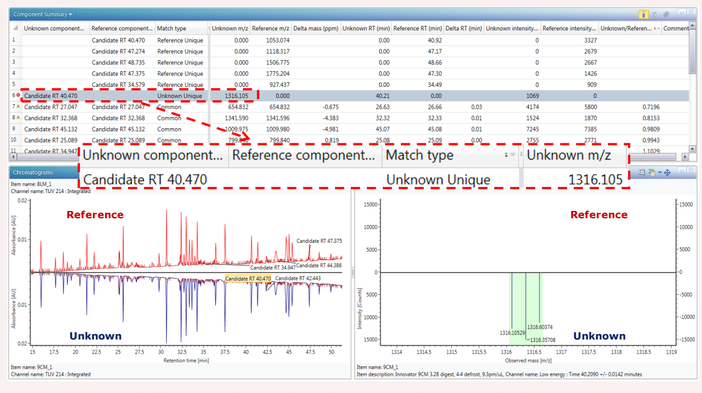

Figure 7: New/differential peaks that are detected when comparing against the reference sample can be reprocessed for identification using the peptide mapping workflow without acquiring new mapping data.

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