Easy Multi-Attribute Method (MAM): A Robust Kit-Based Workflow for Biotherapeutic Quality Monitoring

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INTRODUCTION & MOTIVATION

Multi-Attribute Method (MAM) workflows enable simultaneous assessment of biotherapeutic identity, purity, and post-translational modifications (PTMs). They have become essentail for peptide mapping and monitoring of critical quality attributes (CQAs). In such workflows, sample preparation is often the largest source of variability where a wide variety of contaminants must by reproducibly removed and enzymatic processing enabled without introducing artifactual changes in samples.

ProtiFi's Multi-Attribute Method Kit standardizes MAM sample preparation for samples from development through final quality control (QC). Its general-purpose approach eliminates the need for product-specific method development and is designed for use across multiple products and laboratories. It efficiently removes a broad range of contaminants including detergents, salts, buffers, stabilizers, excipients, and related interferents, and minimizes artifactual deamidation and oxidation.

Here, we compare the published National Institute for Bioprocessing Research and Training (NIBRT) MAM[1] sample preparation workflow with the ProtiFi Multi-Attribute Method Kit workflow using NISTmAb 8671. We systematically evaluated both workflows for robustness, contaminant removal, peptide coverage, PTM preservation, reproducibility, and overall suitability for peptide mapping-based MAM under contaminated sample conditions.

Schematic 1

A graphical summary of the NIBRT in-solution workflow in comaprison to the ProtiFi S-trap MAM kit workflow.

METHODS

Matched NISTmAb 8671 samples were prepared using either the published NIBRT MAM workflow or the ProtiFi Multi-Attribute Method Kit protocol, with or without 41 foulants commonly encountered in biologics manufacturing, including salts, buffers, surfactants, stabilizers, and other process-related contaminants (**Table 1**). The workflows differ in denaturation, cleanup, and digestion strategy. The NIBRT workflow uses chaotropic denaturation, size-exclusion desalting, and in-solution digestion, whereas the ProtiFi workflow uses detergent-based denaturation, optional reduction and alkylation, protein trapping and washing, and in-plate digestion in TruPROT™ Solution to reduce chemical artifacts such as deamidation and oxidation. Workflow performance was evaluated by LC-MS for foulant removal, sample cleanliness, sequence coverage, reproducibility, robustness, and product quality attribute characterization.

Group combination	Foulant	Challenge concentration	Dilution for LC-MS
DMSO	DMSO	50 %v/v	n/a
NaOH	NaOH	500 mM	n/a
HEPES	NaCl	500 mM	n/a
	HEPES	1000 mM	1 in 2,000
PEG	Phosphate	250 mM	1 in 20,000
	EDTA-Na2	250 mM	1 in 2,000
	Imidazole	500 mM	1 in 2,000
	PEG 8000	20 %w/v	1 in 2,000
TCEP	Guanidine-HCl	3000 mM	1 in 20,000
	TCEP-HCl	100 mM	1 in 2,000
Plurionic	Chitosan	0.5 %w/v	1 in 2,000
	Linear PEI 25 kDa	1 %w/v	1 in 2,000
	pDADMAC	1 % v/v	1 in 2,000
	Plurionic F-68	2 %w/v	1 in 2,000
qs21	L-Histidine-HCl	500 mM	1 in 2,000
	Polysorbate 80	1 %w/v	1 in 20,000
	AS03	5 %v/v	1 in 2,000
	QS-21	1 mM	1 in 2,000
Dextran	D-Glucose	10 %w/v	1 in 20,000
	Sucrose	10 %w/v	1 in 20,000
	Trehalose	10 %w/v	1 in 20,000
	Dextran 40 kDa	4 %w/v	1 in 2,000
ZnCl2	L-Arginine-HCl	500 mM	1 in 2,000
	CaCl2	100 mM	1 in 2,000
	MgCl2	100 mM	1 in 20,000
	ZnCl2	100 mM	1 in 10,000
Brij	Urea	4000 mM	1 in 20,000
	Brij-35	10 % w/v	1 in 20,000
Silicone	DEHP	50 %v/v	1 in 2,000
	Caprylic Acid	2 %v/v	1 in 2,000
	Triton X-100	5 %v/v	1 in 2,000
	Dimethyl-polysiolxane	1 %v/v	1 in 2,000
	Silicone Oil	0.05 %v/v	1 in 2,000
Benzyl	D5	50 %v/v	1 in 2,000
	Irgafos 168	5 %v/v	1 in 10,000
	Benzyl alcohol	4 %v/v	1 in 1,000
Triethlamine	NaHCO3	250 mM	1 in 1,000
	Sodium citrate	500 mM	1 in 1,000
	L-Methionine	50 mM	1 in 1,000
	Triethylamine	1000 mM	1 in 1,000
SDS	Deviron 13-S9	10 % w/v	1 in 20,000
	SDS	10 %w/v	1 in 2,000

Table 1

List of foulants added to NISTmAb at the indicated concentrations prior to cleanup using either the published NIBRT method or the ProtiFi MAM workflow.

METHODS (CONT.)

NISTmAb tryptic digests were analyzed by nano-flow LC-MS using a Dionex UltiMate 3000 coupled to a Q Exactive HF Orbitrap mass spectrometer. Peptides were separated on a Reprosil Saphir C18 column (Dr Maisch) using 0.1% formic acid in water and acetonitrile with 0.1% formic acid. Data-dependent MS/MS was performed in positive ion mode. Full MS scans were acquired at 60,000 resolution over m/z 200–2,000 with an AGC target of 3×10^6.

Peptide identification and CQA characterization were performed in Byos by Pretein Metrics using a either a PTM or HCP workflows. Fixed modifications were carboxymethylation of cysteine for NIBRT samples and methyl thiolation for MAM Kit samples. Variable modifications included deamidation, oxidation, succinimide formation, N-terminal pyroglutamate formation, C-terminal lysine clipping, and lysine glycation. Results were filtered to include peptides with up to two missed cleavages while excluding adducts and gas phase-generated ions.

Finally, to test the activity of Tryp-N, a proteolytic NISTmAb peptide ladder was generated using the ProtiFi MAM Kit with either trypsin or Tryp-N at protease-to-protein ratios of 1:200, 1:100, 1:50, 1:25, and 1:10. Samples were incubated for 1 hour at 37°C for trypsin or 55°C for Tryp-N™ , then pooled and analyzed by LC-MS.

RESULTS

Peptide recovery using the NIBRT method was at ~50% of protein input as determined by BCA assay, and peptide recovery for S-trap MAM was at ~90% recovery. Sequence coverage was at 100% for both heavy and light chains of NistMab using the NIBRT and S-trap MAM protocols, when peptide concentrations for injecrtions were matched. Compared with the NIBRT workflow, the kit removed surfactant and polymer contaminants (Fig.1), Produced less deamidation and oxidation (Fig. 2), produced similar monoclonal antibody (mAb) protein ion current, but 4.5-fold more host-cell protein (HCP) peptide identifications (Fig. 3). In addition, under the conditions tested Tryp-N produced more peptides of NistMab than trypsin as illustrated in figure 4.

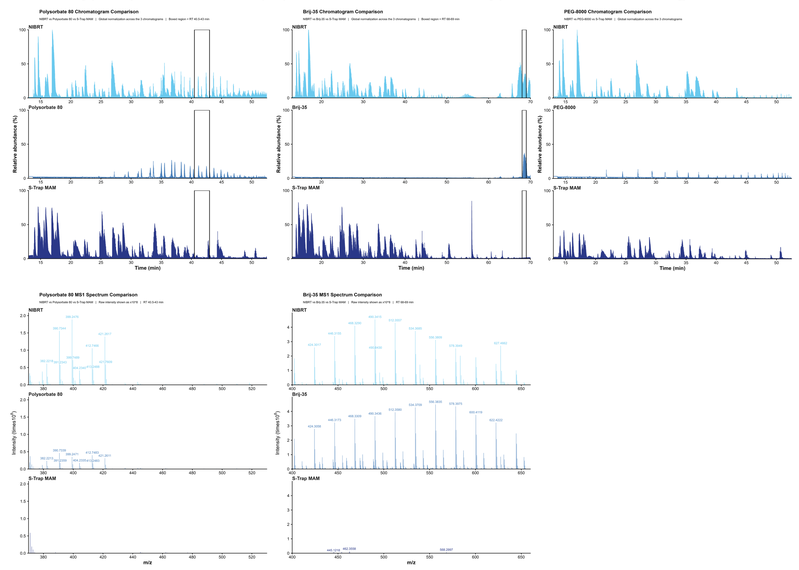

Fig. 1

Removal of Polysorbate-80, Brij-35 and PEG-8000 from NISTmAb samples. Globally normalized base peak intensity (BPI) chromatograms are shown for Polysorbate-80 (A), Brij-35 (B), and PEG-8000 (C), comparing NIBRT-prepared NISTmAb, excipient-only reference, and S-Trap™ MAM Kit-prepared NISTmAb samples. Corresponding spectra are shown for Polysorbate-80 (D) and Brij-35 (E); Polysorbate-80 and Brij-35 are averaged full-scan MS1 spectra. Spectral intensities are shown as raw intensity ($\times 10^8$), with selected prominent m/z values labeled. Reduced corresponding signal in S-Trap™ MAM Kit-prepared samples is consistent with enhanced foulant removal. Chromatograms and spectra were visualized and exported using Thermo Fisher Scientific FreeStyle™ 1.8 Software.

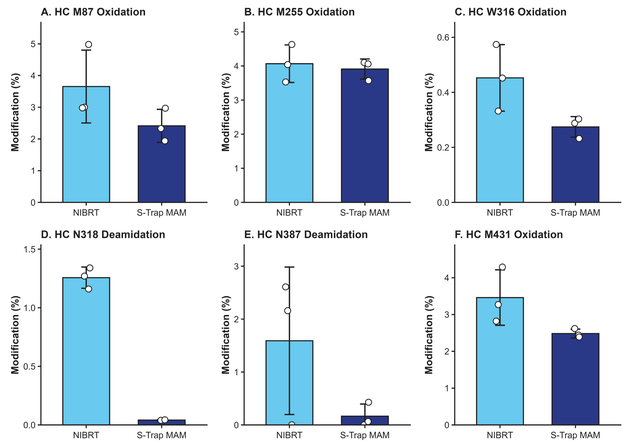

Fig. 2

MAM Kit Minimizes Artificial Deamidation Across NISTmAb Peptides. Selected peptide-level critical quality attributes measured following NIBRT and S-Trap™ MAM sample preparation. Representative heavy-chain modifications identified from the broader using Byos® (Protein Metrics) peptide-level PTM analysis are shown for M87 oxidation (A), M255 oxidation (B), W316 oxidation (C), N318 deamidation (D), N387 deamidation (E), and M431 oxidation (F). NIBRT and S-Trap™ MAM values were measured in triplicate. These selected CQAs illustrate site-specific differences observed within the broader peptide-level modification profile.

RESULTS (CONT.)

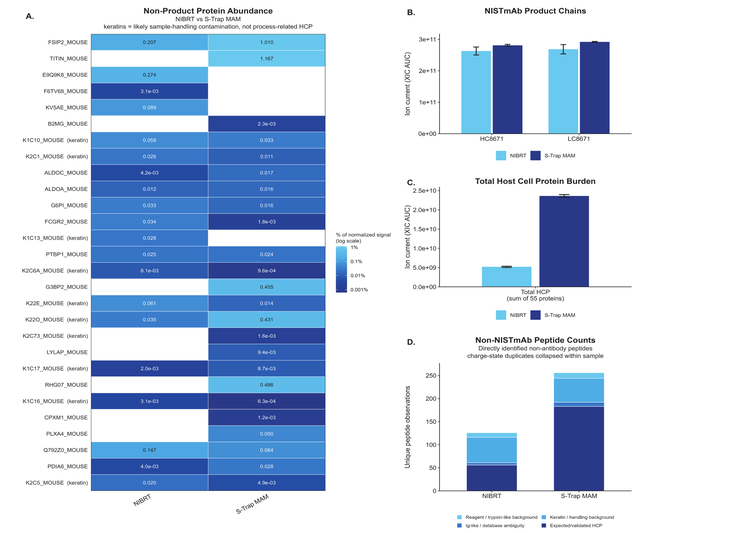

Fig. 3

Comparison of NISTmAb product and non-product protein recovery following S-Trap™ MAM and NIBRT sample preparation. (A) Relative abundance of detected non-product proteins shown as percent normalized signal on a logarithmic scale. (B) NISTmAb heavy- and light-chain ion current. (C) Total host cell protein ion current. (D) Non-NISTmAb peptide observations grouped as expected/validated HCPs, Ig-like/database-ambiguous proteins, keratin/handling background, or reagent/trypsin-like background.

Fig. 4

NISTmAb ladder peptides from trypsin and Tryp-N™. NISTmAb Reference Material 8671 was digested in solution with either Tryp-N™ or trypsin for 1 hour, analyzed by LC-MS/MS, and mapped using Byos® from Protein Metrics, with digestion specificity being set to nonspecific. Peptide coverage for the heavy and light chains is shown, with MS2-identified peptide assignments indicated for each protease. Tryp-N™ digestion resulted in 95.1% sequence coverage for the heavy chain and 85.9% for the light chain (dark blue lines), while trypsin digestion yielded 75.6% coverage for the heavy chain and 74.6% for the light chain (light blue lines).

CONCLUSION

The Multi-Attribute Method (MAM) Kit improved NISTmAb 8671 peptide mapping relative to the National Institute for Bioprocessing Research and Training (NIBRT) workflow by increasing sequence coverage, enhancing protein recovery, and providing more effective contaminant removal. Under foulant-challenged conditions, the kit removed all tested sample preparation interferents, including even large PEG-8000, Brij-35 and extremely common polysorbate-80, which can otherwise easily compromise LC-MS performance and data quality. The MAM Kit showed significantly increased sensitivity in detection of low-abundance non-monoclonal signals like host cell proteins (HCPs) as well as enhanced NISTmAb signal. Incorporation of Tryp-N™ ladder digestion enabled extensive heavy- and light-chain sequence coverage, complementing trypsin-based peptide mapping. Together, these results support the ProtiFi MAM Kit is a robust, standardized workflow for biotherapeutic characterization, including sequence coverage, HCP assessment, foulant removal, and peptide-level product quality attribute (PQA) monitoring.

REFERENCES

1. Millán-Martín, S., Jakes, C., Carillo, S., Rogers, R., Ren, D., & Bones, J. (2023). Comprehensive multi-attribute method workflow for biotherapeutic characterization and current good manufacturing practices testing. Nature Protocols, 18(4), 1056-1089.