Interlaboratory study of an optimised peptide mapping workflow using automated trypsin digestion for monitoring product quality attributes

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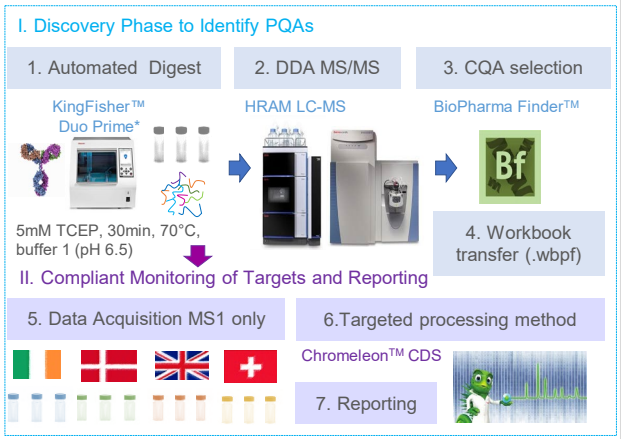
INTRODUCTION

This work describes a fast and reproducible automated trypsin digestion protocol which has been incorporated into an optimised, regulatory compliant peptide mapping workflow to show method transferability across laboratories. The complete workflow has the potential for the use within a Multi-attribute Method (MAM) approach in drug development, production and QC laboratories.



*Swiss Lab used magnetic beads manually

MATERIALS and METHODS

Samples (2 mg mL^{-1}), buffer and 1-5mM TCEP were incubated for 5 to 40 minutes at 70°C. Following digestion, 1 µL of 10% TFA was added (final concentration 1% TFA). ICH stability samples were prepared based on temporal stress (40°C) for 0, 3 and 6 months.

Table 1. HRAM LC-MS method parameters

Column	Acclaim Vanquish™ C18 2.1 x 250mm		
Column Temp:	80°C	Flow rate:	300 µL/min
Solvent A:	Water/0.1% FA	Solvent B:	ACN/0.1% FA
Gradient:	0min-2%B; 105min-40%B; 111min-80%B; 115min-80%B; 115.5min-2%B; 120min-2%B		
General	**Setting**	**ddTop 5 HCD**	**Setting**
Runtime:	0-120 min	Resolution:	17,500
Polarity:	Positive	AGC:	1.0 x 10^5
Full MS range:	200-2000 m/z	Isolation Width:	2.0 m/z
Resolution:	70,000	Threshold:	1.0 x 10^4
AGC:	3.0 x 10^6	Collision energy:	28
Max injection time	100 ms	Max injection time	200 ms
Microscans:	1	Dynamic exclusion	7 s

Table 2. Data processing parameters settings

Biopharma Finder™3.1		Chromeleon™ CDS 7.2.9	
Protease:	High specificity	MS algorithm:	ICIS
Modifications:	PyroGlu; Lys; N-glycans deamidation; oxidation; glycation; succinimide	Mass precision:	5 decimal places
		Mass tolerance:	8 ppm
		Smoothing	None
Max Pep Mass:	7,000	Peptide table	BPF .wbpf file
Mass Accuracy:	5 ppm	Pass score if ≥	2 criteria passed
Threshold	1.0 x 10^4	Fail score if <	1 criteria passed

RESULTS

Intact protein analysis to evaluate digestion completeness

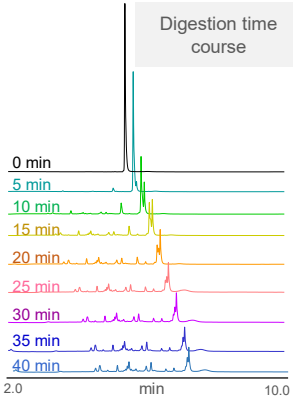


Fig 1. UV traces (280nm) for trastuzumab intact analysis during digestion time course study using buffer 1 (pH 6.5)

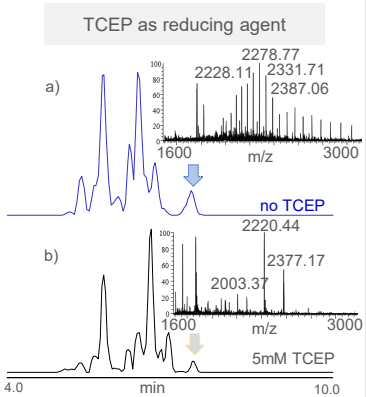


Fig 2. BPCs for trastuzumab HRAMS Intact analysis using 5mM TCEP as reducing agent (a), and without TCEP addition (b). Digestion for 35min with buffer 2 (pH 7.2)

Peptide mapping analysis for digestion time courses study

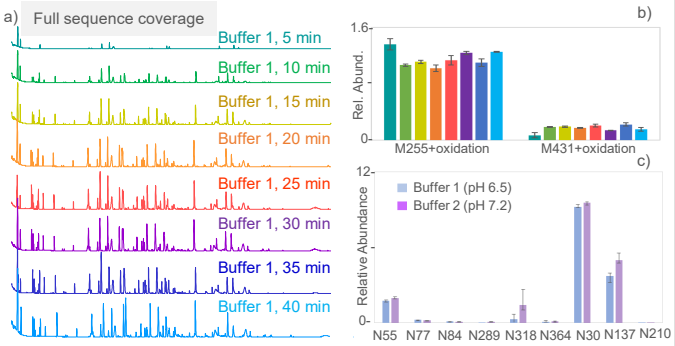


Fig 3. (a) Stacked BPCs of trastuzumab for the automated digestion time course study and detected oxidation levels (b) with buffer 1 (pH 6.5); (c) buffer effect on deamidation

Interlaboratory peptide mapping study of NISTmAb

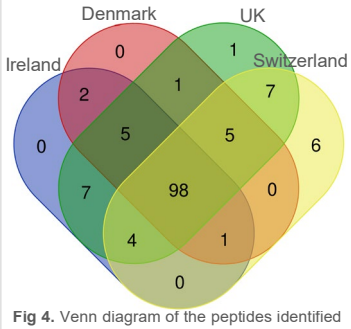


Fig 4. Venn diagram of the peptides identified from automated digestions performed in four sites (peptides with up 1 missed cleavage were selected by user, excluding adducts and nonspecific modifications). Full sequence coverage was attained

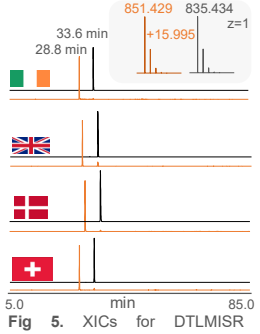


Fig 5. XICs for DTLMISR oxidized peptide (orange trace) and unmodified (black trace), obtained in four sites

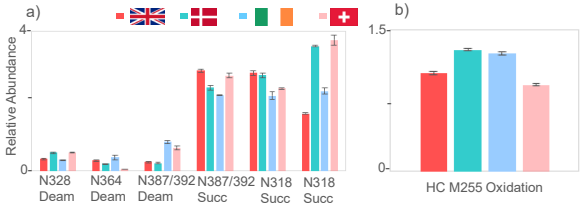


Fig 6. PTMs levels comparison from each site using described peptide mapping workflow for (a) deamidation and succinimide formation and (b) oxidation

Interlaboratory stability study of degraded mAb mixture

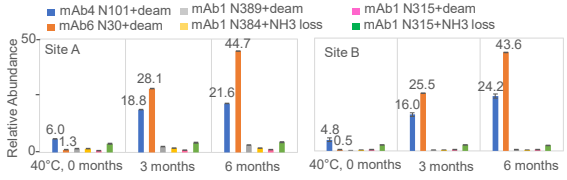


Fig 7. PTMs quantified in two different sites using developed peptide mapping protocol and compliant CDS data processing (overall RSD <10%)

CONCLUSIONS

We demonstrated robustness and accuracy of a complete peptide mapping workflow and to be considered as a preliminary study for the implementation of MAM approach in QC laboratories.

Acknowledgements

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