Multi-Attribute Method for batch-to-batch comparison of Etanercept fusion protein

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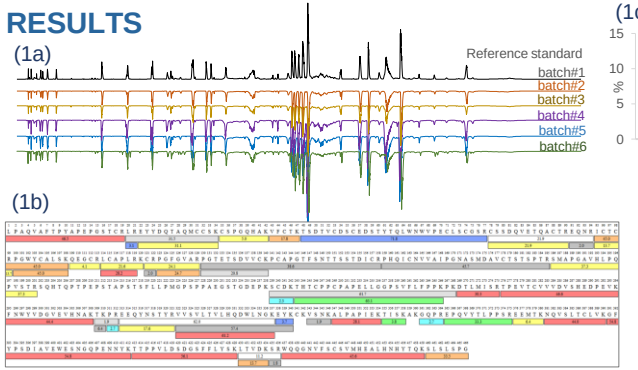
INTRODUCTION

The multi-attribute method (MAM) is an LC-MS-based method that is used to directly characterize and monitor many product quality attributes and impurities on biotherapeutics, most commonly at the peptide level. It has been shown it could replace conventional methods to support process development, product characterization and it is a promising technique due to quantitative and monitoring capabilities with potential for wide use in QC environments. This study describes the use of MAM for etanercept fusion protein batch-to-batch comparison, and it shows the benefits of a New Peak Detection (NPD) tool for the evaluation of new peaks or impurities compared to the reference standard. It also evaluates the performance of the new Thermo Scientific™ Orbitrap Exploris™ MX mass spectrometer with full MS capabilities for PQA/CQA assessment.

MATERIALS & METHODS

Etanercept is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human tumor necrosis factor receptor (TNFR) linked to the Fc portion of human IgG1. Digestion conditions were optimized in terms of sequence coverage, missed cleavages and non-specific generated peptides. Different batches and a reference material were digested in triplicate with trypsin for 4 hours at 37 °C, after reduction and alkylation steps. 10 µg were injected on a Thermo Scientific™ Acclaim Vanquish™ C18 column (2.1 x 250 mm) for peptide mapping analysis. Data were processed using Thermo Scientific™ BioPharma Finder™ and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software.

RESULTS



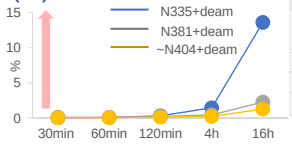


Figure 1. (a) Overlaid mirror TIC plots for the six analysed etanercept batches; (b) sequence coverage map after 4 h digestion and (c) study of deamidation levels for increasing digestion times.

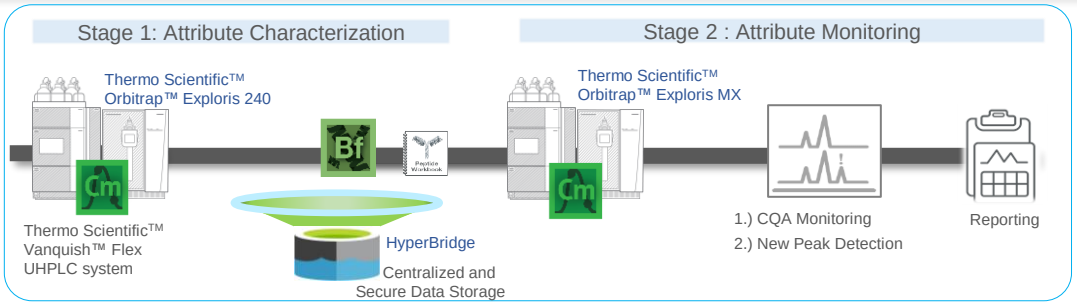


Table 1. Summary results for the digestion time course study

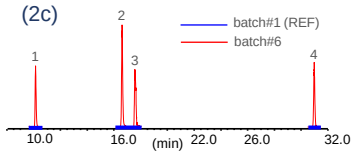
	30 min	60 min	120 min	4 h	16 h
Sequence Coverage	81.97	86.91	86.48	91.20	91.20
% Missed Cleavages	20.37	17.35	15.03	10.85	2.19
% Nonspecific	2.28	4.01	6.53	12.69	33.27

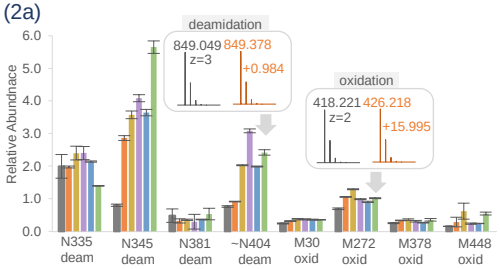
Table 2. List of CQAs monitored for Enbrel fusion protein

Category	Modification (Fc and TNFR domains)	
Charge variant	Deamidation C-term Lys	N335, N345, N381, N404/N409
Oxidation	Met oxidation	M30, M272, M378, M448
N-glycosylation		N317, N149, N171
O-glycosylation		T8, S186, S199, T200, T245

Table 3. Frame filters for NPD

PR Element	=0
PR Size	>1
Charge	between 2 and 5
Ratio	>=99999





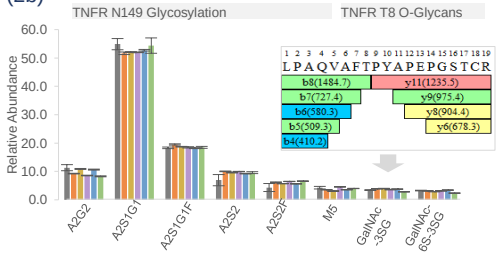


Figure 2. (a) and (b) Some CQAs levels for batch-to-batch comparison where some differences can be observed. (c) Frame plot showing new detected peaks for batch#6 using batch#1 as reference.

CONCLUSIONS

The results suggest that it is possible to seamlessly transfer a method to a QC environment that would give the benefit of HRMS data to the quick confirmation of complex biotherapeutic products such as fusion proteins.

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