A quantitative MS-based multi-attribute method (MAM) approach for new biological entities and biosimilar candidates presented by a full method qualification for an IgG1 market product

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Summary

- Development of an MS-based multi-attribute method (MAM) for monitoring the product quality attributes (PQA) on a biotherapeutic protein IgG1 market product
- Characterization and quantification of PQAs: amino acid sequence, terminal processing, truncation, deamidation, oxidation and glycosylation
- Introduction of a concept for full method qualification of a MAM approach using reversed-phase chromatography in combination with high resolution mass spectrometry
- Evaluation of the qualification parameters: analyte autosampler stability, repeatability, intermediate precision, linearity and specificity of the method using appropriate stress samples
- MAM approach offers the technical potential for validation and usage as GMP release testing

Analytical Method Qualification

The qualification of analytical methods serves to prove that the method is suitable for the intended application with method results which are meaningful and reliable. Various qualification parameters are addressed. In accordance to quality assurance qualified equipment and Good Documentation Practice should be mandatory. A qualification study identifies the method performance and can still be a part of the method development process with the option to optimize the method, if required. In contrast to validation, results have to meet strictly predetermined acceptance criteria, the qualification can serve to define acceptance criteria.

Autosampler Analyte Stability

<table>
<thead>
<tr>
<th>Deamidation</th>
<th>Oxidation</th>
<th>Glycation</th>
<th>N-Glycosylation profile</th>
<th>Disulfide linkages, thioether, trisulfide, free thioles</th>
</tr>
</thead>
</table>

Deamidation level [%]

| 0.00 | 6.00 | 16.0 |

Oxidation level [%]

| 0.00 | 6.00 | 16.0 |

Glycation level [%]

| 0.00 | 6.00 | 16.0 |

N-Glycosylation profile

| Monodeglycosylated, Di- and Oligo-glycosylated compounds |

Methods and Instrumentation

Sample
- Monoclonal antibody, IgG1
- Quality sample, Innovator Lot
- Specificity sample, stress induced innovator Lot

Reduction and Alkylation
- Dithiothreitol
- Iodoacetamide

Digestion
- Trypsin (Promega)
- Trypsin-Chymotrypsin
- Chymotrypsin (Roche)

Chromatography
- UltiMate™ 3000 RSLC (Thermo Fisher Scientific)
- Accucore RP, 2.1×100 mm, 2 μm particle size

Spectrometry
- Q Exactive™ Orbitrap MS (Thermo Fisher Scientific)
- R: 140,000, scan range: 300−1400 m/z
- MS/MS: TOP 4 HCD, NCE: 25%

Data Analysis
- Byonic® and Byologic® software packages (Protein Metrics Inc.)

Methods

1. Preparation of the sample for analysis
2. Installation of the instrument
3. Method development
4. Method validation
5. Method transfer
6. Method implementation

Fig. 1: XIC of deamidated and unmodified PENNY-peptide for quantitative evaluation of modification level. The co-elution and resulting overlapped integrated mass window of deamidated peptide with first 14C isotope of unmodified peptide is overcome with Byologic deconvolution algorithm.

Fig. 2: Monitored Product Quality Attributes (PQAs) by MAM

- Amino acid sequence and sequence variants
- Terminal truncations, signal peptide residue
- N-Terminal pyro-Glu
- C-Terminal Lys and Amidation
- Deamidation
- Oxidation
- Glycation
- N-Glycosylation profile
- Disulfide linkages, thioether, trisulfide, free thioles

Fig. 3: Bar chart showing the comparison of deamidation and oxidation levels among different samples.

Fig. 4: Deamidation PENNY-peptide

Fig. 5: Total modification level

Fig. 6: N-glycan structures attached to the IgG1 Fc N-glycosylation site peptide were quantified via XICs over the tested range.

Fig. 7: An unweighted linear regression was performed to test the hypothesis that the slope of the trendline is zero. The site-specific N-glycan analysis was determined as linear for the tested range 6.25% to 150% for all N-glycan species (all p-values ≤0.05), even for low abundant N-glycans.

Methods

Comparative analysis of impurity samples (stressed sample, controls (other IgG5))

Assessment

- Coefficient of variation (CV) determination

Specificity

C-Terminal Variants

Sample for specificity testing to evaluate the analysis of C-terminal cleavage was generated by Carbosyphate B (CPB) treatment.

The specificity of the method is confirmed as differences in the Lys-clipping level were detected between untreated IgG1 and specificity sample.

Outlook: MAM and GMP

LC-MS is an extremely powerful, but several considerations and necessary precautions should be addressed to use MS in fully compliant routine analyses.

- LC-MS is a highly sophisticated technique with many different instrument types
- Method validation is feasible, if robustness of the complex MAM method is addressed properly by the creation of robust protocols for sample preparation and the analytical workflow
- Implementation of system suitability tests with appropriate acceptance criteria
- GMP-compliant software for data acquisition, automated data analysis validated by manufacturer and in user environment

Fig. 8: Precision of N-Glycosylation analysis at LOQ

Speciation of specificity sample

C-Terminal HC | Unfractured gly | Specificity sample CPB

C-Term HC | 2.72 | —
SLSLEPO C-Term HC | 97.23 | 99.95
SLSLEPO + C-Term HC | 0.05 | 0.05