Characterization and QC of biopharmaceuticals by MS-based 'multi-attribute method': advantages and challenges

“Multi-attribute method is the future ... it can replace some, but not all, current conventional assays for characterization of biopharmaceuticals.”

First draft submitted: 5 January 2017; Accepted for publication: 12 January 2017; Published online: 16 February 2017

Keywords: biopharmaceuticals • characterization • mass spectrometry • multi-attribute method • quality control • quality-by-design

Over the past 30 years, the market of biopharmaceuticals has grown rapidly due to the demand for new biotherapeutics. Because of the potential market value of tens to hundreds of billions of dollars, and higher approval rate (approximately threefold over small-molecule drugs), more companies are becoming actively involved in development of biopharmaceuticals and biosimilars. A majority of the biopharmaceuticals are protein therapeutics including monoclonal antibodies, vaccines, hormones and so on, which are commonly produced by recombinant DNA technology. Differing from small-molecule pharmaceuticals, protein biotherapeutics have unique characteristics such as:

- Larger molecular sizes;
- Molecular heterogeneity due to post-translational or post-production covalent modifications;
- Higher order structure changes upon modifications;
- Aggregations caused by misfolding.

All these structural characteristics contribute to the safety and efficacy of the protein biopharmaceuticals, and therefore it is critical

to characterize the biotherapeutics at various levels during drug discovery, development and quality control (QC) stages [1,2].

The extreme structural complexity makes the characterization a challenging task. In the past several decades, a vast array of bio-analytical methods have been developed to monitor various aspects of biopharmaceuticals. The conventional methods include immune assays, SDS gel-based approaches; chromatographic approaches, electrophoretic approaches, biophysical approaches and MS approaches. Most of these conventional methods usually only monitor one of the characteristics indirectly, and some methods cannot be readily applicable to characterization of biopharmaceutical products due to limitations of testing conditions. Using the conventional methods, it is costly and time consuming to perform a comprehensive characterization of biopharmaceuticals. Following the advances in ionization methods including ESI and MALDI, MS has become an indisputable technique of choice and has been widely used in the characterization of protein therapeutics because it provides high sensitivity, selectivity and specificity [2–4].

Recently, regulatory agencies have recommended a quality-by-design (QbD) approach for manufacturing therapeutics. The QbD guidelines require a deep understanding of

Yun Zhang

Analytical Development, Novavax, Inc. 20 Firstfield Road, Gaithersburg, MD 20878, USA

Jingzhong Guo

Author for correspondence: Analytical Development, Novavax, Inc. 20 Firstfield Road, Gaithersburg, MD 20878, USA
tguo@novavax.com

**FUTURE
SCIENCE**

part of

fsg

the critical quality attributes (CQAs) at the molecular level, and insuring the desired product quality is met at the end of production. Monitoring properties of the biotherapeutic molecule and formulation is important to identify CQAs for product safety and efficacy during biotherapeutic development. The current trend in biotherapeutic development among leading biopharmaceutical organizations is to apply MS to routine assays, in order to access more attributes in fewer analyses [1].

“...it may become a burden to interpret tons of data which provides little additional value for risk assessment, safety, among others.”

Using the QbD principles, Amgen (CA, USA) has developed an MS-based multi-attribute method (MAM) to simultaneously monitor an extensive array of product quality attributes (PQAs) of the protein biotherapeutics. The MAM is based on a peptide mapping method which uses Thermo Scientific Orbitrap™ (Thermo Scientific, MA, USA) MS for acquiring high resolution and highly accurate mass data, while using dedicated software Pinpoint™ (Thermo Scientific) for automated identification and relative quantitation of post-translational modifications (PTMs). The Pinpoint provides automated data processing, which targets MS1 precursor ions by retention time, accurate mass and isotopic distribution and outputs the area for the MS1 precursor. In addition, MS2 is also important to build the Pinpoint workbooks for in-depth characterization of biotherapeutic molecules. This method was developed to monitor and quantify multiple PTMs of biotherapeutic molecules [1]. The MAM represents an optimized solution to accelerate product release when providing greater knowledge of product attributes. The rationale for developing an MAM is to move analytics through QbD to more modern methodologies and get rid of the conventional methods such as gel, which are time consuming and only provide an indirect analysis of some of the PQAs. MAM is also a more complete analysis of the product quality profile during and after processing, compared with current methodologies. In addition, the MS-based MAM has the capability to simultaneously directly measure multiple CQAs and therefore can reduce the development cost by replacing several conventional assays used for process development, product release and in-process control. As an example, Amgen's single MAM could directly measure different forms of the protein therapeutic, for example, oxidation, deamidation and glycosylation. It could also be used online and provide the ability to monitor what is happening during product processing.

Amgen's MAM uses Thermo Scientific Orbitrap. The main advantages with Orbitrap include:

- High mass resolution (up to 250,000), high mass accuracy (~1 ppm) and high sensitivity, which ensure high specificity and high confidence for identification and relative quantitation;
- Simple to use and more robust due to the user-friendly design and minimal features such as one-button tuning and quick calibration;
- The technology allows 'plug-and-play' for peptide mapping analysis;
- Automated software are used to generate a comprehensive attribute target list, which allow automated quantitation;
- The method and instrumentation can be easily aligned for process development, process analytical technology control and product release;
- It allows the reduction of the number of assays used for process development, in-process control and product release and provides more detailed information.

A single MAM has the capability to simultaneously monitor over 20 different PQAs such as deamidation, glycation, methionine oxidation, signal peptide identification, glycosylation, tryptophan degradation, *N*-terminal pyroglutamate, galactosylation, fragmentation, host-cell protein profiling, mutations, C-terminal lysine, hydroxylysine, thioether, cysteine adducts, C-terminal amidation, fucosylation, residual protein A, *O*-linked glycans, identities and so on. Because the MAM provides better capability, higher stringency and specificity, it will surely improve productivity and reduce risk and cost for development of protein therapeutics.

In combination with compatible software support, other mass spectrometers can also be used in the MAM workflow. Currently, Thermo Scientific provides an MAM workflow using BioPharma Finder™ (2.0) and Chromeleon™ (7.2) with its Orbitrap MS series. Waters (MA, USA) promotes its Acquity QDa™ with the Enpower 3™ software for the MAM workflow [5]. Recently, Bomans *et al.* from the Roche Diagnostics (Basel, Switzerland) have successfully applied Water's Synapt G2 mass spectrometer (MA, USA) with MassMap (MassMap GmbH & Co. KG, Wolfratshausen, Germany) and GRAMS AI software (Thermo Scientific) in an MAM workflow [6]. Following this new trend, Genedata (Basel, Switzerland) – a leading provider

of advanced software solutions for drug discovery and development, recently released off a new version (10.5) of its market-leading MS software platform Genedata Expressionist®, in particular support of the application of MAMs for the characterization of biotherapeutics [7].

Due to its promising potential, MAM has become a hot topic at several recent conferences. Since 2014, Amgen has been promoting MAM to streamline biopharmaceutical development and QC testing. Amgen has presented the MAM at the CASSS CMC Strategy and Mass Spec Forums since 2014 [8,9]. It will be a hot topic at the 8th annual Biotherapeutic Analytical Summit in March 2017 in Bethesda, MD, USA [10]. In a discussion held at the 2016 ASMS conference by the biotherapeutics interest group, people discussed the potential of MAM to replace other conventional methods and suggested the MAM represents an optimized analytical solution, but some people disagreed with this [11]. In our opinion, MAM will improve productivity and certainty for sure, but there are still some challenges that must be overcome before an MS-based MAM can be implemented in a GMP environment.

Instrument and method variability are two major challenges, which cause issues in accurately and consistently quantitating low-level signals or detecting trace levels of new impurity peaks. In addition, data processing automation and reliability, limited options of GMP-compliant data acquisition and processing software packages are also considered as major challenges. Moreover, the correlation between the MAM and other conventional analytical methods needs to be studied for each specific biopharmaceutical. As MS is a sensitive universal detector, the MS-based MAM provided complex profiles with great detailed information. Knowing more about the product is better, but it may become a burden to interpret tons of data

which provides little additional value for risk assessment, safety, among others. Furthermore, the reliability for quantitation of randomly distributed low-level modifications such as glycation is also a challenge; however, this challenge can be solved by directly monitoring using conventional analytical methods such as ion exchange chromatography [9]. In order to address some of the above challenges, it is necessary to utilize appropriate system suitability controls, develop robust sample preparation protocols, apply optimized LC-MS conditions and implement GMP-compliant software packages in the MAM workflow.

In conclusion, the MS-based MAM represents an optimized solution to accelerate product release while providing better quantitative information for PQAs than that obtained by the conventional analytical methods currently used for characterization and QC of biopharmaceuticals. It can align with QbD principles by monitoring CQAs through all stages of the manufacturing process. MAM has the capability of automated quantification via combining high-resolution MS and dedicated software, which can be used for QC application. MAM is the future of characterization of biopharmaceuticals. It can replace some, but not all, current conventional assays for characterization of biopharmaceuticals.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

- 1 Rogers RS, Nightlinger NS, Livingston B, Campbell P, Bailey R, Balland A. Development of a quantitative mass spectrometry multi-attribute method for characterization, quality control testing and disposition of biologics. *Mabs* 7(5), 881–890 (2015).
- 2 Fekete S, Guilleme D, Snadra P, Sandra K. Chromatographic, electrophoretic, and mass spectrometric methods for the analytical characterization of protein biopharmaceuticals. *Anal. Chem.* 88, 480–507 (2016).
- 3 Kaltashov IA, Bobst CE, Abzalimov RR, Wang G, Baykal B, Wang S. Advances and challenges in analytical characterization of biotechnology products: mass spectrometry-based approaches to study properties and behavior of protein therapeutics. *Biotechnol. Adv.* 30, 210–222 (2012).
- 4 Chen G, Warrack BM, Goodenough AK, Wei H, Wang-Iverson DB, Tymiak AA. Characterization of protein therapeutics by mass spectrometry: recent developments and future directions. *Drug Discov. Today* 16(1/2), 59–64 (2010).
- 5 McCarthy S. Multiple attribute monitoring of biopharmaceuticals using mass detection. Waters Webcasts. www.waters.com/
- 6 Bomans K, Habegger M, Bonnington L *et al.* Multi-attribute monitoring of antibody modifications by semi-automated liquid chromatography mass spectrometry peptide mapping. *Am. Pharm. Rev.* 19(7), 16–21 (2016).
- 7 Genedata expressionist extends support of MAM for biotherapeutics characterization. www.genedata.com/news-events/press-releases/detail/news/
- 8 Mire-Sluis A. Advancing the promise of analytics of the future using multi attribute methodology. <http://c.ymcdn.com/>
- 9 Wang Y. Simultaneous monitoring of multi-attributes for product attribute control (PAC) by mass spec based method. <http://c.ymcdn.com/>

- 10 Joint session: multi-attribute method for product and process characterization.
www.biotherapeuticsanalyticalsummit.com/
- 11 Houde D, Ruth A. Characterization of protein therapeutics by mass spectrometry, Biotherapeutics Interest Group Workshop,

64th ASMS Conference and Applied Topics. San Antonio, TX, USA, 6–9 June 2016.
www.asms.org/