Bruker Daltonics

Application Note # ET-18

# Rapid Quality Control of Biopharmaceutical Products


## Abstract

Biopharmaceutical therapies, such as monoclonal antibodies, are set to replace traditional small molecule blockbuster drugs over the next few years. Biologics are usually very large, complex molecules. A biologic is manufactured in a living system usually by recombinant technology. These living systems can be very sensitive to minor changes in the manufacturing process. Small process differences may significantly alter the final biologic product, by introducing modifications, which may adversely affect the safety and efficacy of the drug. Strict quality control, of each biopharmaceutical batch is essential to ensure reproducibility between batches and to achieve regulatory approval.

QC of each biologic batch can rapidly be performed by non-technical experts using electrospray mass spectrometry (ESI-MS). The Bruker maXis™ ESI-UHR-TOF-MS, provides high-resolution, high-mass accuracy (confident low ppm) data at full sensitivity. The combination of high mass accuracy and resolution allows rapid detection of modifications, whilst high sensitivity assists detection of low level impurities within the biologic batch. In this application note we will describe an open access method for the rapid QC characterization of biotherapeutic drugs.


## Introduction

Biopharmaceuticals, in particular monoclonal antibodies, are set to revolutionize healthcare of the future. Antibodies are essential human proteins that are involved in the immune system's ability to recognize and eliminate pathogens. Due to their high affinity and high specificity antibodies have been shown to be successful in the treatment of cancers and autoimmune diseases. It is estimated that over the next few years around 30% of all new drug approvals will be antibodies or antibody fragments.

Biotherapeutic agents are now routinely produced by recombinant technology using animal and bacterial cell lines. These biological systems can be manipulated to produce proteins with very specific modifications, such as glycosylation, which are essential for therapeutic function. However, biological systems are very susceptible to subtle changes in the manufacturing process such as cell growth conditions which may result in incorrect modifications or alteration of glycosylation patterns which can significantly alter biological function. Strict quality control of each biopharmaceutical batch and comparison with reference standards is essential to ensure reproducibility between batches and to achieve regulatory approval.

| LC system | Agilent 1200 binary pump |
|---|---|
| LC column | Zorbax SBC8 Rapid Resolution Cartridge, 2.1 x 30 mm, 3.5 µm |
| Solvent A | 0.1% HCOOH in water |
| Solvent B | 0.1% HCOOH in ACN |
| Gradient | 0 min: 0%B, 3 min: 0 %B, 10 min: 100 %B, 13 min: 100 %B, 13.1 min: 0 %B |
| Column flow rate | 300 µl/min |
| Column temperature | 70°C |
| Mass spectrometer | Bruker maXis UHR-TOF MS, positive ion mode, internal calibration |

The biopharmaceutical industry requires efficient, accurate and comprehensive generation and interpretation of data from each protein batch to comply with quality control regulations. The combination of liquid chromatography with mass spectrometry provides an excellent technique for rapid characterization of proteins. Measuring the intact mass of a protein quickly confirms if the protein drug has been correctly manufactured and purified and provides information about heterogeneity and any potential contaminants. In this way batches can be easily filtered using pass / fail QC criteria either as acceptable for use or marked for re-processing due to the appearance of an impurity or heterogeneity which may cause adverse drug reactions.

Until recently, mass spectrometric experts were required to interpret LCMS datasets. However, recent developments in automated data acquisition and interpretation, using generic methods for analysis of different proteins, now facilitate rapid QC of biopharmaceuticals by non-expert operators using a simple workflow on a single platform.

The Bruker maXis ESI-UHR-TOF, provides high-resolution, high-mass accuracy (confident low ppm) data at full sensitivity. High resolution with high mass accuracy is a pre-requisite to characterize heterogeneous glycosylation patterns along with a range of other PTMs, whilst high sensitivity is essential to detect low levels of impurities.

In this application note we will describe an open access method for the rapid QC characterization of intact protein drugs and their comparison with reference standards to produce qualitative and quantitative information. We will also demonstrate the technical performance and suitability of the maXis for the analysis of biopharmaceutical proteins.

## Experimentals

Recombinant human IgG was expressed in Chinese Hamster Ovary (CHO) cells. Proteins were separated with a Zorbax SBC8, Rapid Resolution Cartridge (2.1 x 30 mm, 3.5 µm) within 15 minutes and directly analyzed by the maXis. IgG was reduced and alkylated to release the heavy (HC) and light chain (LC).

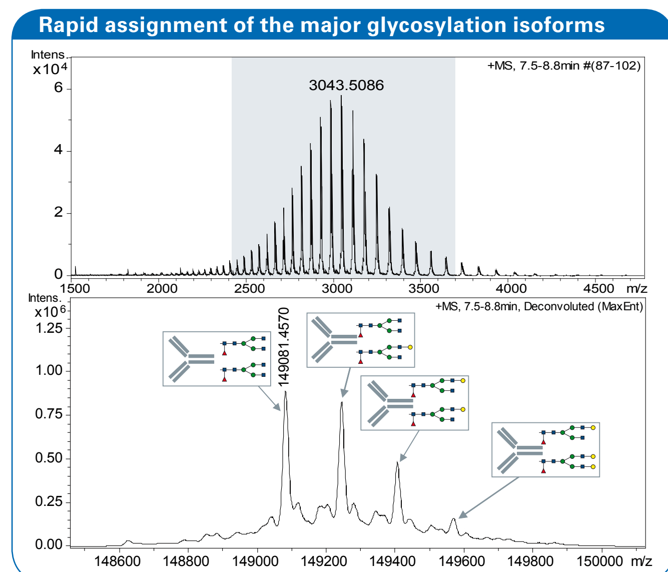



Figure 1: The mass spectrum before and after charge deconvolution applying the Maximum Entropy algorithm. The mass deviation between measured and theoretical mass is better than 2 ppm. The high resolving power of maXis™ rapidly identified heterogeneous glycosylation patterns, the major glycosylated species have been annotated.

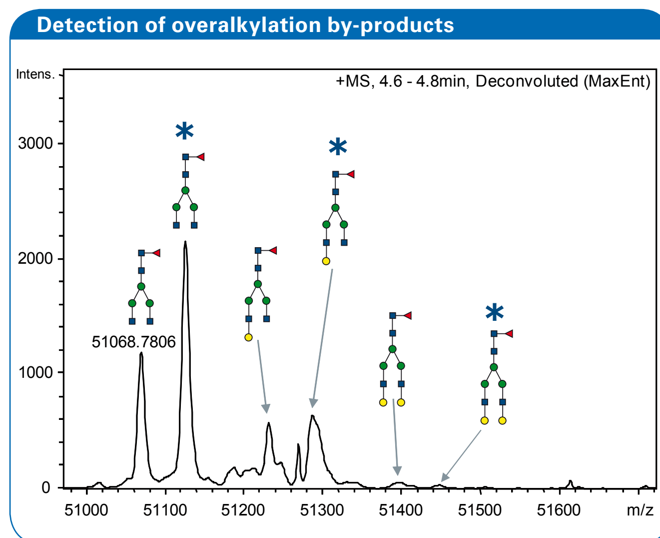



Figure 2: Maximum Entropy deconvoluted ESI-UHR-TOF spectrum of the released heavy chain of the human IgG1. Taking into account average atomic weights from organic sources according to Zhang et al., the calculated mass of the mass labeled glycosylation form is 51068.88Da, which deviates <2ppm from the measured mass. Annotated peaks labeled with * represent chemical artifacts due to overalkylation.

## Results

Figure 1 demonstrates the ability of the Bruker maXis ESI-UHR-TOF instrument to generate high-resolution, high accuracy mass data of intact proteins allowing rapid assignment of the major glycosylation isoforms of human IgG1. Maximum Entropy deconvolution of the data yields a mass for the major glycosylation form, which fits with the expected average mass range based on a calculation of atomic weights from organic sources according to Zhang et al. (deviation between measured mass and expected mass based on average atomic masses from organic sources: 2ppm). Further glycosylation isoforms were assigned based on characteristic mass distances of 162Da indicating a rising number of galactose units.

The released heavy chain of the intact human IgG was also analyzed by ESI-UHR-TOF (see Figure 2). The measured mass was found to be in excellent agreement with the expected mass, showing a 2ppm deviation from the calculated mass according to Zhang et al. In addition, the high-definition data provided by the maXis also allows unambiguous assignment of chemical artifacts which were generated during sample preparation. In the example shown here, spectral peaks that did not match the masses of expected protein sequence/glycosylation structures (denoted with * in Figure 2) could be assigned to non-desired by-products originating from the alkylation step, leading to an overalkylation, yielding additional peaks shifted by 57Da.

In Figure 3 it can be observed that there is an exact match between the calculated and experimental isotopic pattern of IgG light chain, illustrating the technical power of the maXis for the analysis of intact proteins. Of equal importance to the technical performance of the mass spectrometer is the ability to rapidly generate QC reports. Automated generic MS acquisition methods can be created to acquire MS data from the intact protein, these methods include the generation of QC sample reports. The sample reports contain details such as TIC, deconvoluted protein mass and qualitative and quantitative comparison with a reference standard to quickly identify any incorrect products or impurities within the new batch. The rapid QC workflow can be seen in Figure 4 with typical reports shown in Figure 5. Additionally, acceptance criteria may be set by the user to rapidly visualize samples which meet QC acceptance requirements and those which fail or require further more detailed investigation Figure 6. Such acceptance requirements may include; average mass deviation of sample peaks, number of observed or missing peaks in comparison to a reference sample, number of unexplained potential impurity peaks and intensity of sample compared to reference peaks.

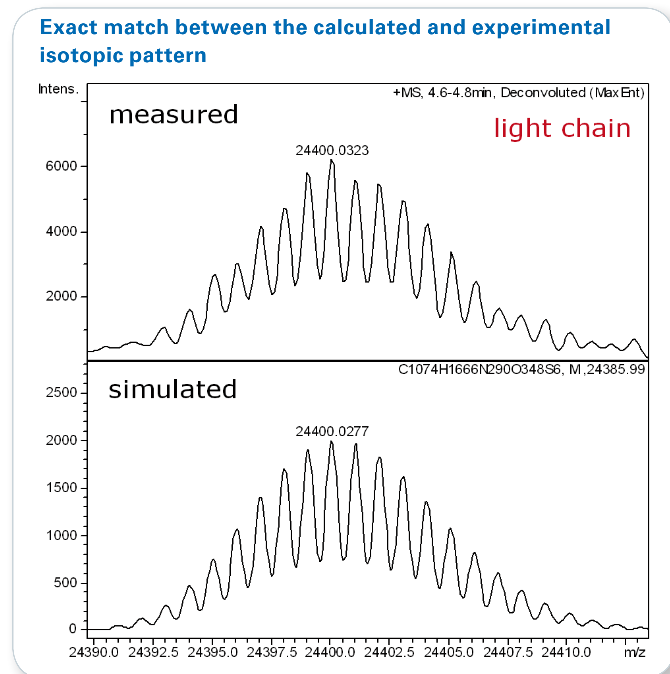



Figure 3: Comparison of the experimentally obtained isotopic pattern of the reduced and alkylated Light Chain of the IgG antibody compared with the expected theoretical isotopic pattern showing a mass deviation of < 0.2ppm.

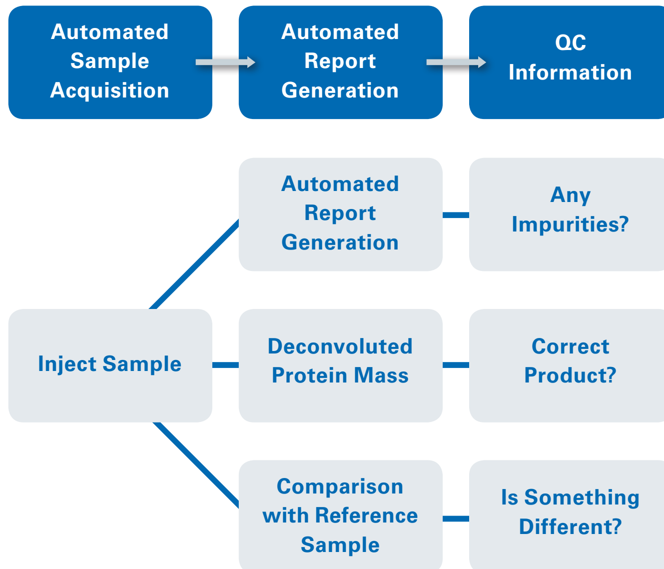
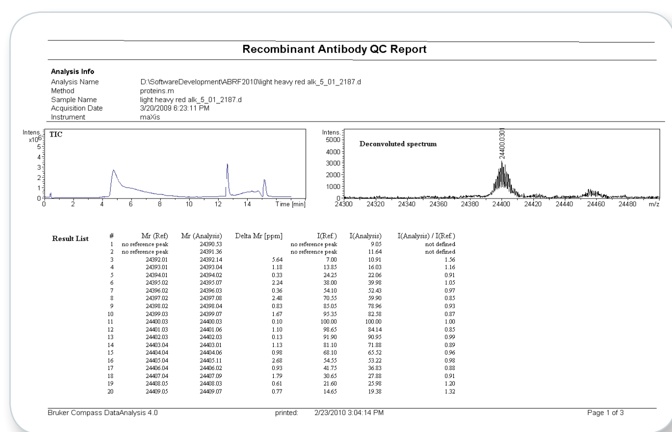



Figure 4: Quality Control Workflow. Intact protein data is automatically acquired, deconvoluted and compared with a reference standard. QC reports can be automatically generated containing the TIC for detection of impurities, the deconvoluted mass to ensure the correct product has been made and comparison with a reference standard to draw attention to any differences between sample and the reference standard.

## Conclusion

LC coupled to maXis ESI-UHR-TOF provides unbeatable technical performance for the analysis of biotherapeutic drugs with data produced at high-resolution, with no sensitivity compromise, combined with highly accurate mass data. We have described a rapid quality control workflow which offers fully automated MS acquisition and data processing from intact proteins, along with the generation of automated reports from each sample. In addition we present a high-throughput visualization tool for immediate identification of batch samples which either pass or fail QC acceptance criteria.

Fully automated high-throughput QC workflows provide several benefits to the scientist and their company. As the MS methods, processing and reporting are fully automated, routine QC evaluation can be carried out by non-qualified personnel, thus releasing technical specialists to concentrate on tasks which require higher levels of scientific input. The high-throughput automated QC workflow in combination with high technical performance also results in significant time and cost savings for the company. Products which pass QC criteria can be quickly released to the market, whereas faulty batches are instantly detected and retained for further analysis to check for incorrect products or impurities which may affect drug safety, stability or efficacy.



**Recombinant Antibody QC Report**

**Analysis Info**

| | |
|---|---|
| Analysis Name | D:\SoftwareDevelopment\ABRF2010\light heavy red alk_5_01_2187.d |
| Method | proteins.m |
| Sample Name | light heavy red alk_5_01_2187.d |
| Acquisition Date | 3/20/2009 6:23:11 PM |
| Instrument | maXis |



**Result List**

| # | Mr (Ref) | Mr (Analysis) | Delta Mr [ppm] | I(Ref.) | I(Analysis) | I(Analysis) / I(Ref.) |
|---|---|---|---|---|---|---|
| 1 | no reference peak | 24390.53 | | no reference peak | 9.05 | not defined |
| 2 | no reference peak | 24391.36 | | no reference peak | 11.64 | not defined |
| 3 | 24392.01 | 24392.14 | 5.64 | 7.00 | 10.91 | 1.56 |
| 4 | 24393.01 | 24393.04 | 1.18 | 13.85 | 16.03 | 1.16 |
| 5 | 24394.01 | 24394.02 | 0.33 | 24.25 | 22.06 | 0.91 |
| 6 | 24395.02 | 24395.07 | 2.24 | 38.00 | 39.98 | 1.05 |
| 7 | 24396.02 | 24396.03 | 0.36 | 54.10 | 52.43 | 0.97 |
| 8 | 24397.02 | 24397.08 | 2.48 | 70.55 | 59.90 | 0.85 |
| 9 | 24398.02 | 24398.04 | 0.83 | 85.05 | 78.96 | 0.93 |
| 10 | 24399.03 | 24399.07 | 1.67 | 95.35 | 82.58 | 0.87 |
| 11 | 24400.03 | 24400.03 | 0.10 | 100.00 | 100.00 | 1.00 |
| 12 | 24401.03 | 24401.06 | 1.10 | 98.65 | 84.14 | 0.85 |
| 13 | 24402.03 | 24402.03 | 0.13 | 91.90 | 90.95 | 0.99 |
| 14 | 24403.04 | 24403.01 | 1.13 | 81.10 | 71.88 | 0.89 |
| 15 | 24404.04 | 24404.06 | 0.98 | 68.10 | 65.52 | 0.96 |
| 16 | 24405.04 | 24405.11 | 2.68 | 54.55 | 53.22 | 0.98 |
| 17 | 24406.04 | 24406.02 | 0.93 | 41.75 | 36.83 | 0.88 |
| 18 | 24407.04 | 24407.09 | 1.79 | 30.65 | 27.88 | 0.91 |
| 19 | 24408.05 | 24408.03 | 0.61 | 21.60 | 25.98 | 1.20 |
| 20 | 24409.05 | 24409.07 | 0.77 | 14.65 | 19.38 | 1.32 |

Bruker Compass DataAnalysis 4.0 printed: 2/23/2010 3:04:14 PM Page 1 of 3

Figure 5: Automated report containing essential QC information such as TIC, deconvoluted protein mass and qualitative and quantitative comparison with a reference standard. This information rapidly highlights incorrect products or impurities within a batch.

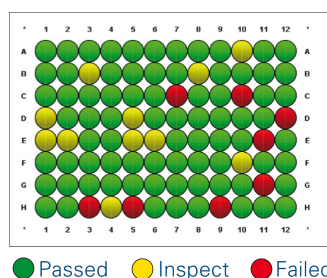



Figure 6: QC analysis of 96 samples, where QC acceptance criteria has been set by the user. A visual overview of samples is automatically created displaying those samples which meet QC acceptance criteria and those which fail.

## References


[1] Z. Zhang, H. Pan, X. Chen, Mass Spectrometry Reviews, 2009, 28, 147-176

[2] K. Sparbier, A. Asperger, A. Resemann, I. Kessler, S. Koch, T. Wenzel, G. Stein, L. Vorwerg, D. Suckau, M.Kostrzewa, Journal of Biomolecular Techniques, 18, 2007(4), 252–258

[3] M. Wuhrer, C.H. Hokke, A.M. Deelder, Rapid Commun. Mass Spectrom., 18, 2004, 1741-1748

[4] A. Ceroni, K. Maass, H. Geyer, R. Geyer, A. Dell, S. M. Haslam, J. Proteome Res., 7, 2008(4), 1650–1659

[5] A. Asperger, D. Wunderlich, M. Macht, P.-O. Schmit, In-depth characterization of the N-glycosylation of recombinant monoclonal antibodies by means of MALDI and ESI mass spectrometric analysis, poster contribution (no. 721) presented at IMSC 2009, Bremen, Germany


## Authors


Laura Main, Wolfgang Jabs, Arndt Asperger, Dirk Wunderlich, Marcus Macht, Pierre-Olivier Schmit; Bruker Daltonik, Bremen, Germany



| Keywords | Instrumentation & Software |
|---|---|
| N-glycosylation | maXis |
| Antibodies | |
| Recombinant proteins | |
| Protein sequencing | |


For research use only. Not for use in diagnostic procedures.


Bruker Daltonics is continually improving its products and reserves the right to change specifications without notice. © Bruker Daltonics 03-2010, ET-18, #269416



www.bdal.com

**Bruker Daltonik GmbH**

Bremen · Germany
Phone +49 (0)421-2205-0
Fax +49 (0)421-2205-103
sales@bdal.de

**Bruker Daltonics Inc.**

Billerica, MA · USA
Phone +1 (978) 663-3660
Fax +1 (978) 667-5993
ms-sales@bdal.com