Analytical Technologies Europe: Symposium on the Practical Applications including CE, LC and MS in the Biopharmaceutical Industry (AT Europe 2018)

Symposium Co-chairs:

Christof Finkler, *F. Hoffmann - La Roche Ltd.*
Marta Germano, *Janssen Infectious Diseases and Vaccines*

6-9 March 2018
Crowne Plaza Barcelona – Fira Center
Barcelona, Spain

Organized by

CASSS

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CASSS AT Europe Student Travel Grants

CASSS is pleased to provide a limited number of student travel grants for PhD students and post-docs who present applicable posters at the Analytical Technologies Europe: Symposium on the Practical Applications including CE, LC and MS in the Biopharmaceutical Industry (AT Europe 2018). PhD students or post-doctoral fellows conducting research at academia throughout the world are eligible.

Why you should apply:
This Symposium is an active forum for discussion of recent developments and regulatory considerations of the practical application of analytical technologies amongst industry, academia and regulatory agencies. There will be a focus on capillary electrophoresis, mass spectrometry and chromatography for product characterization, process monitoring, formulation development and release testing in the biopharmaceutical industry. As a participant, you will have an excellent opportunity to meet, network and participate in exchanging knowledge for mutual education with your peers.

Requirements are:
- Present a poster on a MS, CE, LC topic relating to biopharmaceuticals
- Proof of studentship/post-doc status
- Recommendation from your supervisor

CASSS has awarded student travel grants to the following individuals:

Quantification of Endotoxins in Protein Samples
Martin Pattky, *University of Applied Sciences and Arts, Western Switzerland, Switzerland*

CE(SDS)-CZE-MS for the Analysis of Monoclonal Antibodies
Jennifer Römer, *Aalen University, Germany*

Evaluation of the Most Important Parameters of the Experimental Design In Ligand Binding Assays
Matthias Stein, *TU Braunschweig, Germany*

Social Program

Wednesday, 7 March
10:00 – 10:30
First Time Attendee Network Event

First time attendees of AT Europe are invited to attend special networking event in the Coral Ballroom where you will be able to meet with some of our CASSS members. Make sure you have your "New Member" ribbon on your badge to gain access to this event.

Wednesday, 7 March
18:10 – 19:30
Exhibitor Reception

Come network and visit exhibitors in the Rubí Ballroom.

Thursday, 8 March
19:30 – 23:30
Conference Event – Can Travi Nou

This event is open to all full conference attendees. One day only attendees will be charged extra to attend. Please see registration desk for pricing. Join us as we begin the evening with a tour of the city, highlighting the beautiful sights Barcelona has to offer, before arriving at Can Travi Nou for dinner. Situated in a beautiful and typical 18th century Catalan Masía, Can Travi Nou provides visitors traditional cuisine in an unusual urban location which enables them to eat in the city but surrounded by nature. While dining, we will be entertained by a group of Catalan Rumba dancers, a unique style that is derived from Flamenco and fused with Catalan and Andalusian chants.

Please meet CASSS staff in the lobby of the hotel at 19:15. Buses will be leaving for the tour at 19:30.

Acknowledgements

Symposium Co-Chairs:
Christof Finkler, *F. Hoffmann – La Roche Ltd.*
Marta Germano, *Janssen Infectious Diseases and Vaccines*

Scientific Program Committee:
François de l'Escaille, *Analis SA*
András Guttman, *University of Pannonia*
Carsten Jahn, *AbbVie Deutschland GmbH & Co KG*
Carl Jone, *UCB Pharma SA*
Katarzyna Kozakowska, *MedImmune Limited*
Anders Lund, *Sanofi*
Cari Sänger - van de Griend, *Kantisto BV*
Birgit Schmauser, *BfArM, Federal Institute for Drugs and Medical Devices*
Harold Taylor, *Merz Pharmaceuticals GmbH*
Jerome Thiebaud, *Sanofi Pasteur*
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AT Europe 2018
Scientific Program Summary

Tuesday, 6 March 2018

08:30 – 14:00 **Registration** (for course attendees ONLY) in the Lower Lobby Foyer

09:00 – 12:30

Short Course in the Cristal Ballroom
Fundamentals of Mass Spectrometry in the Analysis of Protein Therapeutics
Short Course Facilitator: Anders Lund, *Sanofi, Framingham, MA USA*

12:30 – 13:30 **Lunch** (for course attendees ONLY)

13:30 – 17:00

Short Course in the Cristal Ballroom
Applications of Mass Spectrometry to Characterize Protein Therapeutics
Short Course Facilitator: Anders Lund, *Sanofi, Framingham, MA USA*

User Group Meetings (Must Preregister Online)

14:00 – 18:00 **ProZyme, Inc.** in the Coral Ballroom

Wednesday, 7 March 2018

08:30 – 18:00 **Registration** in the Lower Lobby Foyer

09:00 – 09:15 **CASSS Welcome and Introductory Comments** in the Zafir Ballroom
Marta Germano, *Janssen Infectious Diseases and Vaccines, Leiden, Netherlands*

Keynote I in the Zafir Ballroom
Session Chair: Marta Germano, *Janssen Infectious Diseases and Vaccines, Leiden, Netherlands*

09:15 – 10:00 **Quality Aspects of Advanced Therapy Medicinal Products (ATMP): The Role of the Biologics Working Party (BWP)**
Sol Ruiz, *Spanish Medicines Agency (AEMPS), Madrid, Spain*

10:00 – 10:30 **Networking Break** – Visit the Exhibits and Posters in the Rubí Ballroom

10:00 – 10:30 **First Time Attendee Network Event –** Coral Ballroom

Analytical Technologies to Control Polysorbate and its Degradation Products
in the Zafir Ballroom
Session Chair: Anders Lund, *Sanofi, Framingham, MA USA*

10:30 – 10:55 **Quantification and Degradation Monitoring of PS80 in One Single Analysis using a QDa Mass Detector**
Pierre Guibal, *Sanofi, Vitry sur Seine, France*

10:55 – 11:20 **Complexity of the Analytical Characterization of Polysorbate – Case Studies for Degradation Profiling**
Klaus Wuchner, *Cilag AG, Schaffhausen, Switzerland*

11:20 – 11:45 **Effective Analytical Tools to Monitor Polysorbate Degradation on the Stability of Biotech Formulations**
Christian H. Bell, *F. Hoffmann – La Roche Ltd., Basel, Switzerland*

11:45 – 12:10 **Scientific and Regulatory Perspectives on Analytical Methods to Support Biotechnology Product Quality**
V. Ashutosh Rao, *CDER, FDA, Silver Spring, MD USA*

12:10 – 12:40 **Discussion**

12:40 – 13:40 **Buffet Lunch**

13:40 – 14:40 **Poster Session** in the Rubí Ballroom

Wednesday, 7 March 2018 continued

14:40 – 15:10

Technical Seminar

Accelerating Biotherapeutics Characterization with the new timsTOFpro and LC free MALDI Workflows
Romano Hebler and Detlev Suckau, *Bruker Daltonik, Bremen, Germany*

Sponsored by Bruker Daltonik **Zafir Ballroom**

Mass Spectrometry in the Zafir Ballroom
Session Chairs: Katarzyna Kozakowska, *MedImmune Limited, Cambridge, United Kingdom*
and Hansjörg Toll, *Sandoz Biopharmaceutical, Kundl, Austria*

15:10 – 15:35 **Using a Combined Mass Spectrometry Approach to Support Process Development of (ADC) Antibody Intermediates**
Nicholas Bond, *MedImmune Limited, Cambridge, United Kingdom*

15:35 – 16:00 **MAM Evaluation Update for Process Development Support**
Yann Fromentin, *Sanofi, Vitry sur Seine, France*

16:00 – 16:25 **Development of a Dual Data Independent Acquisition Approach Allowing both Global HCP Profiling and Absolute Quantification of Key HCP within a Single Analysis**
William Burkitt, *UCB Pharma, Slough, United Kingdom*

16:25 – 16:55 **Discussion**

16:55 – 17:05 **Mini Break**

17:05 – 18:05

Roundtable Discussions I in the Coral and Diamant Ballrooms
Session Chairs: Christof Finkler, *F. Hoffmann – La Roche Ltd., Basel, Switzerland*
and Marta Germano, *Janssen Infectious Diseases and Vaccines, Leiden, Netherlands*

18:05 – 19:05 **Exhibitor Reception** in the Rubí Ballroom

Thursday, 8 March 2018

08:30 – 17:30 **Registration** in the Lower Lobby Foyer

Keynote II in the Zafir Ballroom
Session Chair: András Guttman, *University of Pannonia, Debrecen, Hungary*

09:00 – 09:45 **Coupled Technologies for Glycan Analysis: From Sample Prep to Orthogonal Data Collection and Bioinformatics**
Pauline M. Rudd, *NIBRT, Co. Dublin, Ireland*

Keynote III in the Zafir Ballroom
Session Chair: András Guttman, *University of Pannonia, Debrecen, Hungary*

09:45 – 10:30 **Higher Throughput Glycosylation Analysis of Biopharmaceuticals**
Manfred Wuhrer, *Leiden University Medical Center, Leiden, Netherlands*

10:30 – 11:00 **Networking Break** – Visit the Exhibits and Posters in the Rubí Ballroom

CASSS Frantisek Svec Fellowships for Innovative Studies Award Winner in the Zafir Ballroom
Session Chair: Cari Sänger - van de Griend, *Kantisto B.V., Baarn, Netherlands*

11:00 – 11:25 **Towards the use of Reaction-modulators in an Integrated Multi-dimensional Liquid Chromatography System**
Bert Wouters, *Universiteit van Amsterdam, Amsterdam, Netherlands*

11:25 – 11:35 **Discussion**

11:35 – 12:05

Technical Seminar

CE in the Biopharmaceutical Industry, from Cell Line Screening to Characterisation and Quality Control
Jim Thorn and Stephen Lock, *SCIEX, Warrington, United Kingdom*

Sponsored by SCIEX **Zafir Ballroom**

12:05 – 13:05 **Buffet Lunch**

Capillary Electrophoresis in the Zafir Ballroom
Session Chairs: Carsten Jahn, *AbbVie Deutschland GmbH & Co KG, Ludwigshafen, Germany* and Jerome Thiebaud, *Sanofi Pasteur, Marcy l'Etoile, France*

13:05 – 13:30 **Capillary Electrophoresis in the Biotechnology Industry: Advances and Challenges through the Last Decade**
David Michels, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*

Thursday, 8 March 2018 continued

13:30 – 13:55 **ZipChip CE-MS, A New Analytical Tool for Monitoring Protein Manufacturing Process**
Li Zang, *Biogen, Cambridge, MA USA*

13:55 – 14:20 **On-line Mass Spectrometric Characterization of Impurities of Intact Monoclonal Antibodies after CE(SDS) and CZE Separation**
Christian Neusüß, *Aalen University, Aalen, Germany*

14:20 – 14:50 **Discussion**

14:50 – 15:20

Technical Seminar

Automated Data Processing and Analysis for Quality Monitoring of Biotherapeutics by Multi-attribute Method (MAM)
Marlis Zeiler, *Genedata GmbH, Munich, Germany*

Sponsored by Genedata **Zafir Ballroom**

15:20 – 16:20 **Poster Session** in the Rubí Ballroom

16:20 – 17:20

Roundtable Discussions II in the Coral and Diamant Ballrooms
Session Chairs: Christof Finkler, *F. Hoffmann – La Roche Ltd., Basel, Switzerland*
and Marta Germano, *Janssen Infectious Diseases and Vaccines, Leiden, Netherlands*

17:20 – 18:20

MAM Workshop in the Zafir Ballroom
Workshop Facilitator: Anders Lund, *Sanofi, Framingham, MA USA*
Jette Wypych, *Amgen Inc., Thousand Oaks, CA USA*

19:15 – 23:30 **Conference Event**

Friday, 9 March 2018

08:30 – 15:00 **Registration** in the Foyer

Keynote IV in the Zafir Ballroom
Session Chair: Christof Finkler, *F. Hoffmann - La Roche Ltd., Basel, Switzerland*

09:00 – 09:45 **Regulatory Considerations when Developing Relevant and Sustainable Analytical Technologies**
Patrick Swann, *Amgen, Inc., Cambridge, MA USA*

09:45 – 10:15 **Networking Break** – Visit the Exhibits and Posters in the Rubí Ballroom

Chromatography and Spectroscopy in the Zafir Ballroom
Session Chairs: Carl Jone, *UCB Pharma SA, Brussels, Belgium*
and Harold Taylor, *Merz Pharmaceuticals GmbH, Frankfurt Am Main, Germany*

10:15 – 10:40 **Large-scale Assessment of Extractables and Leachables in Single-use Bags for Biomanufacturing**
Jonathan Bones, *NIBRT, Co. Dublin, Ireland*

10:40 – 11:05 **Synergies between Spectroscopies: The SuperSpectra Approach for Biopharmaceutical Characterization**
George Tranter, *Chiralabs Ltd, Begbroke, United Kingdom*

11:05 – 11:30 **Characterization of Antibody Charge Variants by Ion-exchange Chromatography Coupled to Native Mass Spectrometry**
Rob Haselberg, *Vrije Universiteit Amsterdam, Amsterdam, Netherlands*

11:30 – 12:00 **Discussion**

12:00 – 12:30

Technical Seminar

Characterizing Biopharmaceuticals using UHPLC and High-Resolution MS
Jonathan Bones, *NIBRT, Co. Dublin, Ireland*

Sponsored by Thermo Fisher Scientific **Zafir Ballroom**

12:30 – 13:30 **Buffet Lunch**

Friday, 9 March 2018 continued

Qualification and Validation with a GMP Mindset in the Zafir Ballroom
Session Chair:
Birgit Schmauser, *BfArM, Federal Institute for Drugs and Medical Devices, Bonn, Germany*

13:30 – 13:55	**The Glycosimilarity Index and Its Potential Applications in Biopharmaceutical Development and Quality Control** Akos Szekrenyes, *mAbxience Research S.L., León, Spain*
13:55 – 14:20	**Analytical Technologies: From Company Laboratory to Pharmacopeial Method** Christopher Jones, *Retired from NIBSC, St Albans, United Kingdom*
14:20 – 14:45	**A Regulatory Perspective on Methods Used for Analytical Similarity Assessments** Kristen Nickens, *CDER, FDA, Silver Spring, MD USA*
14:45 – 15:15	**Discussion**
15:15 – 15:30	**Closing Comments** in the Zafir Ballroom Christof Finkler, *F. Hoffmann – La Roche Ltd., Basel, Switzerland*

NOTES:

Session Abstracts

Quality Aspects of Advanced Therapy Medicinal Products (ATMP): The Role of the Biologics Working Party (BWP)

Sol Ruiz

Spanish Medicines Agency (AEMPS), Madrid, Spain

During the last two decades an increasing number of biological medicinal products have been approved for many different clinical indications. More recently the development of gene- and cell-based products (referred to in European legislation as advanced therapy medicinal products or ATMP) has raised great expectations for alternative approaches for the prevention and treatment of human diseases. A very large number of medicinal products based on gene therapy and somatic cell therapy are already in clinical development for the treatment of inherited diseases, cancer, diabetes, and neurodegenerative disorders among many other therapeutic areas and 10 have got a marketing authorization in the European Union.

Despite the enormous experience gained in the evaluation of biologics, the peculiar characteristics of ATMP pose new challenges in various aspects such as production and control systems, non-clinical and clinical development, marketing, etc. Several aspects of the development and production of certain gene therapy medicinal products could be considered similar to vaccines and biotechnological products. However, a large number of cell therapies that are under clinical development are from autologous origin, that is, the patient's own cells (after being isolated, purified or modified in the laboratory) will be the medicinal product and, therefore, many of the approaches described in the pharmaceutical legislation need to be adapted to this new kind of product.

The Biotechnology Working Party (BWP) has been developing its work since the beginning of 1995, date of the creation of the European Medicines Agency (EMA), but it has precedent in the so-called Ad Hoc Working Group on Biotechnology and Pharmacy, constituted in 1986, to advise the CHMP (Committee for Medicinal Products for Human Use; then CPMP) on quality aspects of medicinal products produced by biotechnological processes. An important part of the current BWP activity is the evaluation of quality aspects of biological medicinal products, especially recombinant proteins but also including ATMP, vaccines, and plasma-derived medicinal products. The large experience gathered so far in the evaluation of these quality aspects has resulted in a solid and robust knowledge and harmonization in the EU and other regulatory areas through ICH cooperation efforts.

NOTES:

Quantification and Degradation Monitoring of PS80 in One Single Analysis using a QDa Mass Detector

Pierre Guibal

Sanofi, Vitry sur Seine, France

Polysorbates, such as PS80, are amphiphilic non-ionic surfactants commonly used in biopharmaceuticals formulation.

PS80 is supposed to be a polyoxyethylene sorbitan monooleate but due to its synthesis process, it's a heterogenic mixture covering a wide range of physico-chemical properties and including different fatty acids. PS80 is known to be prone to autoxidation and enzymatic hydrolysis of the esterified fatty acids. This complexity along with degradation possibility represents a tremendous challenge for PS80 quantification and degradation monitoring.

Different methods already exist for PS80 monitoring, but to our knowledge no method is able to quantify intact PS80 and monitor its potential degradation – oxidation or hydrolysis – in one single analysis. A liquid chromatography mass spectrometry method benefiting from previous work on the use of low m/z marker ions was developed. In previous work, this required a triple quadrupole mass spectrometer in order to control dissociation in the second quadrupole. As QDa is a single quadrupole mass detector, the distinctive feature of this method is to rely on the ion source versatility. By optimizing ionization parameters, we were able to enhance in source dissociation and subsequently follow low m/z marker ions signal in single ion recording for quantification purpose.

QDa is able to perform both positive and negative electrospray ionization and follow several SIR signals in one analytical run. Here one SIR was devoted to intact PS80 quantification – with external calibration – by following a specific low m/z marker ion of oleate ester structure. A marker of oxidized PS80 was recorded in another SIR. In negative ionization mode, SIR related to oleic acid resulting from a potential PS80 hydrolysis was recorded for quantification with external calibration.

The method was evaluated towards neat and degraded PS80 in aqueous solution and antibodies formulation.

This is the first method allowing quantification of intact PS80 covering all known degradation in one single analysis.

NOTES:

Complexity of the Analytical Characterization of Polysorbate – Case Studies for Degradation Profiling

Klaus Wuchner[1], Wendelin Koch[2], Eleonora Corradini[2], Andrea Hawe[2]

[1]Cilag AG, Schaffhausen, Switzerland, [2]Coriolis Pharma, Munich, Germany

Polysorbates (PS) are the most common surfactants in biopharmaceutical products and used to stabilize proteins against various (interfacial) stresses during the product life-cycle. Commercially available PS are complex mixtures with a large structural heterogeneity and variable composition. Additionally, PS may degrade in the therapeutic protein formulation by oxidation or hydrolysis (chemical or enzymatically driven) forming multiple degradants which even may precipitate out and form subvisible and visible particles.

This complexity poses a challenge for the analytical characterization of PS at different stages of product development. Multiple and complementary analytical methods are necessary to monitor the concentration, composition and degradation of PS and to understand their impact on product quality of the drug product. PS needs to be characterized as neat PS (raw material), during processing as diluted PS solutions and ultimately in the biopharmaceutical formulation throughout manufacturing, storage and use.

Different case studies will be presented demonstrating the application of an analytical toolbox used to assess the degradation profile during stress studies of different PS grades (including "Chinese all oleic acid ester grade" PS80), to identify potential degradation markers in bio-pharmaceutical products and to characterize the composition and nature of subvisible PS-related particles. The analytical methods include assays for PS content and for degradation profiling by liquid chromatography coupled with charged aerosol detector (LC-CAD) or with high-resolution mass spectrometry (LC-MS).

Effective Analytical Tools to Monitor Polysorbate Degradation on the Stability of Biotech Formulations

Christian H. Bell, Robert Kopf, Janina Pfaff, Kishore Ravuri

F. Hoffmann-La Roche Ltd., Basel, Switzerland

Polysorbate degradation has been in focus in the recent years due to its potential impact on drug product quality and formation of visible particles due to insoluble free fatty acid formation and precipitation. In this regard the analytical control strategy of the surfactant assumes importance. Factors such as right choice of the quantification assay, monitoring during development and assessment of likely impact of the degradation on drug product are crucial for the overall analytical control strategy. The talk will focus on case studies on analytical capability of various quantification methods of PS20 and will touch on aspects of surfactant control during development.

NOTES:

Scientific and Regulatory Perspectives on Analytical Methods to Support Biotechnology Product Quality

V. Ashutosh Rao

CDER, FDA, Silver Spring, MD USA

Elements of the analytical toolbox needed to successfully support optimal formulation, control strategies, and manufacturing process development are generally driven by the scientific understanding of the product and the process. Excipients are an integral component of biotechnology drug products. Consequently, optimal formulation and control of excipients are key aspects of an overall control strategy by drug manufacturers aimed at consistent product quality, safety, and efficacy. Scientific and regulatory perspectives on analytical methods for product characterization and development of control strategies will be discussed.

NOTES:

Using a Combined Mass Spectrometry Approach to Support Process Development of (ADC) Antibody Intermediates

Nicholas Bond

Medimmune Limited, Cambridge, United Kingdom

During the manufacture and storage of therapeutic proteins, modifications can occur that could influence the physicochemical and pharmacological properties of a given protein therapeutic. Over recent decades much has been learnt about the product/process interaction for monoclonal antibodies (mAb) and the circumstances under which these product variants are generated. Much less, however, is known for some of the novel therapeutic protein formats.

Popular amongst mAb-like formats are antibody drug conjugates (ADCs) which are designed to deliver a highly toxic payload to tumours by covalently attaching it to target-specific mAb. Since their emergence both the protein scaffold (antibody intermediate) and small molecule (payload) have been subject to significant innovation in an attempt refine the molecular design and achieve the maximal therapeutic index. Whilst improvements in payload have sought to increase potency and exhibit more favourable physicochemical properties, engineering approaches have been employed to generate antibody intermediates that confer site specific conjugation and reduce the heterogeneity of conjugated variants.

Case studies will be provided where several mass spectrometry approaches have been used in combination to support the pre-clinical development of an antibody intermediate intended for site-specific conjugation. Consideration will be given to how these approaches can provide insight into product variants and how to monitor these through process development, manufacturing and upon stability.

NOTES:

MAM Evaluation Update for Process Development Support

Yann Fromentin

Sanofi, Vitry sur Siene, France

Sanofi Biopharmaceutical development aims to minimize the Time to Clinic (TTC) for new biotech products, such as monoclonal or, bi/tri-specific antibodies, ADCs, or protein fragments… from early development to licensure. The analytical testing of such biotherapeutics remains an everyday challenge to provide support from research to late phase development and manufacturing. Multi attribute Monitoring (MAM) is a fast-growing field focused on tracking critical quality attributes (CQAs) all along the product lifecycle. In order to achieve the aggressive TTC goals, automated protein A purification, digestion and MAM analysis of the biotherapeutic product were implemented across Characterization and Bioprocess Analytics teams from three Sanofi sites (Framingham, Frankfurt, Vitry). A harmonized routine testing paradigm to support bioprocess development will be presented In addition our struggle to design proper testing paradigm across automation for sample purification digestion and LC-MAM will be presented.

NOTES:

Development of a Dual Data Independent Acquisition Approach Allowing both Global HCP Profiling and Absolute Quantification of Key HCP within a Single Analysis

William Burkitt, Ben Holmes, Johanna Paris, Nisha Patel, John O'Hara

UCB Pharma, Slough, United Kingdom

Peptide mapping mass spectrometry is one of the most informative techniques used for the characterisation of biopharmaceuticals. It is routinely applied to the analysis of samples for the purposes of comparability, understanding of the manufacturing process and degradation profiling. Peptide mapping mass spectrometry of biopharmaceuticals provides confirmation that the expected sequence is present and an assessment of the levels post-translational modifications and sequence variants, additionally it is possible to interrogate the data for the presence of non-hypothesis modifications.

Despite its routine use in the biopharmaceutical industry challenges remain in the methods for the interpretation and understanding of the data produced. These include the consistent determination of the levels of PTMs, comparison of sample sets obtained on separate occasions, determining which of the many new peaks present in each assay are of relevance.

Approaches taken at UCB to address these issues will be described. These include the use of isotopically labelled versions of the biopharmaceutical products and statistical methods to determine which modifications attention should be focused on. These will be exemplified using real world examples of problems encountered throughout the product life cycle. Futures requirement for data analysis will also be covered.

NOTES:

Coupled Technologies for Glycan Analysis: From Sample Prep to Orthogonal Data Collection and Bioinformatics

Pauline M. Rudd, Mark Hilliard, Roisin O'Flaherty, Radka Saldova

NIBRT, Co. Dublin, Ireland

Glycosylation is a key post-translation modification for most biological drugs many of which are designed to interact with the immune system and the inflammatory pathways. These drugs are used to treat major diseases such as cancer and autoimmunity as well as in organ transplantation and the use of stem cells. The glycans attached to the proteins are critical features that are controlled to ensure drug safety and efficacy. It is therefore necessary to install appropriate glycoanalytical technologies for the detailed glycan analysis in all labs involved in the production of such phamaceuticals. To this end, over the last decade the glycoscience field has made major improvements in separations technologies that focus on intact glycoproteins, glycopeptides and released glycans have been driven by the needs of the pharmaceutical industry to manufacture safe and efficacious biological drugs as well as new opportunities that are opening up in basic research. As well as providing detailed structural analysis and assisted data interpretation, accurate quantitation, robustness, speed and reporoducibility are also vital for the analysis of the glycans attached to biological drugs at all stages of production from understanding the roles for the glycans through clonal selection and production, downstream processing and QC. The relevance of glycosylation to understanding the systems biology of cells in culture is now widely recognised. Linking the glycome directly to genetics, epigenetics, transcription, metabolomics and proteomics is now feasible. The use of biological drugs in precision/personalised medicine, including mapping changing glycosylation of disease related glycoproteins, is important in clinical decision points, such as determining which patients will benefit for particular treatments. The technology platform includes coupled UPLC/MS/MS technologies underpinned in a total workflow by bioinformatics programmes integrating both LC and MS data and a plate-based sample preparation robot. Orthogonal technologies including exoglycosidase enzyme arrays and CE will be discussed.

NOTES:

Higher Throughput Glycosylation Analysis of Biopharmaceuticals

Manfred Wuhrer

Leiden University Medical Center, Leiden, Netherlands

Characterizing protein glycosylation is of great importance during the entire lifetime of a biopharmaceutical, ranging from early discovery to post-approval monitoring. Application fields of glycoanalytical methods include clone selection, monitoring of glycoengineering, optimization of culturing conditions, monitoring of batch effects, and research into glycosylation structure-function relationships, in order to infer relevant critical quality attributes.

Trends in the field of biopharmaceuticals including the rise of biosimilars and the move towards fusion proteins have resulted in a demand for glycoanalytical methods with higher throughput, resolving power and versatility allowing e.g. multi-attribute monitoring. Mass spectrometry (MS) has proven particularly valuable in addressing these needs. Examples will be given including MS methods to analyse the glycosylation of biopharmaceuticals from culture broths for batch comparison, the MS analysis of both N- and O-glycosylation at the levels of released glycans as well as glycopeptides at higher throughput, the integrated analysis of protein glycosylation and other post-translational modifications (PTMs) at the intact protein level. Importantly, these MS glycoanalytical methods are complemented by data processing tools that allow the robust extraction of glycosylation information from iterative MS analyses.

Developments in MS nourish the expectation that its role in the characterization of biopharmaceuticals will ever increase covering a wide range of attributes comprising glycosylation, other modifications including drug load for antibody-drug conjugates, aggregation, and host cell proteins.

NOTES:

Towards the use of Reaction-modulators in an Integrated Multi-dimensional Liquid Chromatography System

Bert Wouters[1], Bob Pirok[1], Niall P. Macdonald[2], Joan M. Cabot[2], Sinéad Currivan[3], Brett Paull[4], Michael C Breadmore[2], Peter J. Schoenmakers[1]

[1]Universiteit van Amsterdam, Amsterdam, Netherlands, [2]ARC Centre of Excellence for Electromaterials, Hobart, Australia, [3]Australian Centre for Research on Separation Sciences, Hobart, Australia

High-performance liquid chromatography is an analytical tool widely used for the separation and identification of analytes in a mixture. To reach higher separation efficiencies, two-dimensional liquid chromatography (2D-LC) can be used. For coupled-column 2D-LC, effluent fractions from the first-dimension column are transferred to a second-dimension column. The conventional approach is 'passive modulation', where empty loops are used for transfer. Alternatively, 'active modulation' modifies fractions during transfer (*e.g.*, dilution with weaker solvent, use of trap columns for desalting or reducing fraction volume). Similarly, we propose the use of immobilized-enzyme reactors (IMERs) as "reaction modulators" implemented in a 2D-LC system.

An IMER is a microfluidic reactor wherein enzymes are immobilized in a confined space, allowing high enzyme-to-substrate ratios and short diffusion distances. A cyclic-olefin-copolymer microfluidic reactor has been constructed, containing trypsin immobilised on a polymer monolithic material. Its potential has been demonstrated for the offline IMER-LC-MS analysis of complex protein mixtures in dried blood spots. However, when using polymer materials such as cyclic-olefin-copolymer, chemical resistance and UV transparency may be limiting, and alternative materials and prototyping methods are of interest. In recent years, 3D-printing has emerged as an alternative approach for prototyping. For instance, stereolithography (SLA) is an established 3D-printing technique for producing structures from a liquid photopolymer resin by means of scanning laser or light source. Recently, the exciting possibility to 3D print in transparent fused-silica glass has been introduced. Hereby, nanocomposite materials created by the suspension of silica nanoparticles in a monomeric matrix are used for stereolithography 3D printing, after which the objects are converted to glass via a heat treatment procedure. We are exploring digital light processing (DLP)-SLA based 3D printing fused-silica glass devices to create devices such as immobilized-enzyme reactors and micro-mixers for use as reaction-modulators in 2D-LC. Additionally, application of the IMER to polyesters is being investigated.

NOTES:

Capillary Electrophoresis in the Biotechnology Industry: Advances and Challenges through the Last Decade

David Michels

Genentech, a Member of the Roche Group, South San Francisco, CA USA

Since the introduction of first CE applications in the routine control system for biotherapeutics nearly two decades ago, capillary electrophoresis (CE) has been increasingly utilized as a key analytical tool to support various areas of product development in the biotechnology industry. The applications include formulation studies, process development, product characterization and validated lot release and stability testing of both commercial and clinical products. In this talk, an overview of the major applications and analytical strategies implemented at Genentech will be presented. The platform applications are divided into the following areas: 1) CE as a replacement for slab gel electrophoresis, particularly, capillary electrophoresis-sodium dodecylsulfate and capillary isoelectric focusing; 2) CE to monitor protein charge heterogeneity as an orthogonal technique to the traditional ion-exchange chromatographic methods, 3) and CE automation and miniaturization. In recent years, a strategy based on platform assay and high throughput formats have been implemented in order to meet the demands for the increased demand. In this talk, various practical applications of those methodologies will be discussed along with future directions and needs for CE in the biopharmaceutical industry.

NOTES:

ZipChip CE-MS, A New Analytical Tool for Monitoring Protein Manufacturing Process

Li Zang

Biogen, Cambridge, MA USA

ZipChip CE-MS offers a microfluidic solution to high-efficiency separation integrated with online mass spectrometry detection. The technology applies to large diversity of analytes, from intact proteins to small molecule metabolites and nutrients in culture media. In this talk, application of ZipChip CE-MS to direct analysis of protein product quality, nutrients and metabolites from cell culture media will be presented. The comprehensive understanding of cell culture obtained within a few minutes per analysis provides a potential approach to monitoring protein manufacturing process in a timely manner, which may enable establishment of feedback controls as part of a next-generation control strategy of protein manufacturing process.

NOTES:

On-line Mass Spectrometric Characterization of Impurities of Intact Monoclonal Antibodies after CE(SDS) and CZE Separation

Christian Neusüß[1], Kevin Jooß[1], Cristina Montealegre[1], Jennifer Römer[1], Johannes Schlecht[1], Steffen Kiessig[2], Bernd Moritz[2]

[1]Aalen University, Aalen, Germany, [2]F. Hoffmann-La Roche Ltd., Basel, Switzerland

Capillary Electrophoresis is a key technology for the separation of variants and impurities of therapeutic antibodies. However, identification by e.g. mass spectrometry is difficult since on one hand upscaling and fraction collection is difficult and on the other hand most CE-methods require the use of ESI-interfering constituents in the background electrolyte (BGE) which prevent an on-line coupling to mass spectrometry.

Recently we introduced the concept of two-dimensional CE applying a mechanical valve in order to couple the first (non-MS-compatible) dimension in a heart-cut approach to CZE-MS. The second CE-dimension separates the analytes of interest from all interfering constituents of the first dimension using a MS-compatible BGE [1]. In this way it is possible to couple any kind of capillary-based electromigration technique to mass spectrometry. We demonstrated this for CZE using phosphate buffer [1] or tricine buffer [2] and CIEF for protein separation [3], and, most recently, for the detailed mass characterization of charge variants of intact antibodies in routinely used CZE applying ε-aminocaproic acid as BGE [4].

In this presentation, recent results of the coupling of SDS-capillary sieving electrophoresis to CZE-MS is presented. In order to remove both SDS, non-volatile buffer ions and the pseudo-gel matrix a method based on the co-injection of complexing agents and organic solvents has been developed [5]. Incorporation of this method in the CE-CE-MS set-up allows for the first time a mass spectrometric characterization of CE(SDS)-separated impurities in biopharmaceutical antibody samples. Examples and limitations of the technology will be presented and discussed.

References

[1] Kohl, Montealegre, Neusüß *Electrophoresis,* 2016, 37(7-8), 954-958.

[2] Neuberger, Jooß, Ressel, Neusüß, *Anal. Bioanal. Chem.,* 2016, 408(30), 8701-8712.

[3] Hühner, Neusüß, *Anal. Bioanal. Chem.* 2016, 408(15), 4055–4061.

[4] Jooß, Hühner, Kiessig, Moritz, Neusüß *Anal. Bioanal. Chem.* 2017, 409, 6057–6067.

[5] Sánchez-Hernández, Montealegre, Kiessig, Moritz, Neusüß, Electrophoresis, 2017 38(7), 1044-1052.

NOTES:

Regulatory Considerations when Developing Relevant and Sustainable Analytical Technologies

Patrick Swann

Amgen, Inc., Cambridge, MA USA

During review of applications, regulatory authorities often focus on analytical technologies that measure product quality attributes that can be directly linked to safety and efficacy. For example, cell-based bioassays are thought to better reflect the product's mechanism of action relative to binding assays. Therefore, applicants are often requested to develop a cell-based bioassay for release for a well-characterized biopharmaceutical unless binding assay(s) can be justified as a suitable alternative.

With respect to biochemical methods for release of biopharmaceuticals, multiple companies are pursuing use of multi-attribute methods (MAM) including as a possible replacement for analytical technologies traditionally used to measure charge and/or size variants[1]. The specificity of MAM allows for direct measurement and control of molecular variants with known structure-activity relationships and, in addition, can provide improved understanding of the impact of process parameters on specific quality attributes. This keynote presentation will:

- Focus on Amgen's extensive efforts to date to develop MAM as a substitute for some traditional lot release tests
- Summarize current concerns from regulatory authorities and the path forward for resolution of those concerns, and
- Identify analytical lifecycle management considerations to improve sustainability of MAM methods as used for biopharmaceutical product lot release.

[1]Rogers, R.S., Abernathy, M., Richardson, D.D. et al. AAPS J (2018) 20:
7. https://doi.org/10.1208/s12248-017-0168-3

NOTES:

Large-scale Assessment of Extractables and Leachables in Single-use Bags for Biomanufacturing

Jonathan Bones, Noemi Dorival-Garcia, Sara Carillo, Christine Ta

NIBRT, Co. Dublin, Ireland

Single-use technologies (SUTs) are widely used during biopharmaceutical manufacture as disposable bioreactors or media and buffer storage bags. Despite their advantages, significant concern exists regarding the risk of release of potentially toxic or inhibitory extractable and leachable (E&Ls) substances. E&Ls may detrimentally affect cell viability or productivity or may persist during purification and present a risk to the patient if remaining in the final drug product. A large-scale assessment was performed wherein 34 plastic films from single-use bags (SUBs) for cell cultivation were extracted with solvents selected based on the BPOG guidelines. SUBs were incubated at small-scale under accelerated-aging conditions that represented standard operational conditions of use. Leachables analysis was performed following dispersive liquid-liquid microextraction (DLLME) for analyte preconcentration and removal of matrix interference. Resulting extracts were characterized by GC-headspace for volatiles, high resolution GC-Orbitrap-MS/MS for semi-volatiles, high resolution LC-Orbitrap-MS/MS for non-volatiles and ICP-MS for trace elemental analysis. Multivariate statistical analysis of the analytical data revealed significant correlations between the type and concentration of compounds and bags features including brand, manufacturing date and polymer type.

The second stage of the experiment focused on the optimisation and application of accelerated solvent extraction (ASE) to perform worst case scenario extractions of the plastic films. Analysis of the ASE extractions revealed significantly more extractable compounds, many of which were not detected under the passive extraction conditions using during initial experiments to simulate working conditions of standard unit operations. Analysis of the ASE data set revealed the ability to track extractables that arise from particular additives based on transformation or degradation during exposure to environmental or operational conditions. Monitoring these transformation pathways facilitated an understanding of compounds that may become extractables or leachables even though the parent compound from which they are derived does not.

NOTES:

Synergies between Spectroscopies: The SuperSpectra Approach for Biopharmaceutical Characterisation

George Tranter

Chiralabs Ltd, Begbroke, United Kingdom

The numerous spectroscopic and physicochemical techniques each provide information on the nature and quality of a biopharmaceutical. However, by combining the data of multiple techniques together, as a "SuperSpectrum", it is possibly to take advantage of the synergies between techniques and provide greater insights into biopharmaceutical characterisation while improving efficiency. The presentation will cover the concepts of SuperSpectra and a broad overview of its use. Examples in optimising spectroscopic choices, fingerprinting biopharmaceuticals, early identification of production problems and prediction of product characteristics and impurity profiles will be discussed.

NOTES:

Characterization of Antibody Charge Variants by Ion-exchange Chromatography Coupled to Native Mass Spectrometry

Rob Haselberg

Vrije Universiteit Amsterdam, Amsterdam, Netherlands

Monoclonal antibodies (mAbs) are highly complex glycoproteins potentially exhibiting a wide range of micro heterogeneities due to the occurrence of post-translational modifications (PTMs). PTMs may significantly impact the pharmacological and biochemical properties of the protein. Therefore, extensive analysis important to ensure product quality and maintain consistency. Charge variants of mAbs can be efficiently separated by ion-exchange chromatography (IEX). Unfortunately, the salt gradients commonly used in IEX are not compatible with mass spectrometry (MS), hindering the selective characterization of mAb charge heterogeneity by on-line IEX-MS.

This study describes a new IEX method allowing the identification of mAb charge variants under native conditions. The combination of a cation-exchange stationary phase with a pH-gradient using volatile buffers at slightly alkaline conditions, enabled the separation of trastuzumab charge variants. Several column chemistries, buffer components, and pH gradients were evaluated to obtain optimal antibody charge variant resolution. The optimized MS-compatible method provided identical peak patterns as obtained with salt gradients. Moreover, efficient MS detection of mAb variants was possible while preserving the tertiary and quaternary structure of the proteins. The performance of the IEX-MS method for PTM characterization at the intact antibody level was investigated through the analysis of several different mAb products, such as Trastuzumab and Ustekinumab. IEX-MS allowed selective and clear distinction of glycoforms (both charged and neutral glycans), oxidation products, C-terminal lysine variants, and in some cases even deamidations. Further method evaluation showed that the IEX separation under MS-compatible conditions has satisfactory elution time and peak area repeatability and intermediate precision. A robustness study indicated that whereas elution times might shift, the separation efficiency remains constant.

NOTES:

The Glycosimilarity Index and Its Potential Applications in Biopharmaceutical Development and Quality Control

Akos Szekrenyes[1], Beata Borza[2], Laszlo Hajba[2], Andras Guttman[2,3]

[1]mAbxience Research S.L., León, Spain, [2]Horváth Csaba Laboratory of Bioseparation Sciences, Debrecen, Hungary, [3]SCIEX, Brea, CA USA

N-glycosylation is a very important post-translational modification that occurs on most therapeutic proteins. Its composition can directly affect the safety or efficacy profile of a given biologics. In IgG1 type monoclonal antibodies the attached carbohydrate chains determine major Fc related mechanisms, such as antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). While in other cases these carbohydrate structures can be directly linked to anti-inflammatory functions, immunogenicity or serum clearance. In addition, batch-to-batch alterations in glycosylation is an excellent indicator of process robustness. Glycosylation, therefore, is considered as one of the most important critical quality attributes of glycoprotein biotherapeutics, and consequently for their biosimilar counterparts. Thus, the carbohydrate moieties of such biopharmaceuticals should be closely scrutinized during all stages development and manufacturing.

However, it is still challenging to determine and describe the level of similarity between two N-glycosylation profiles, due the complexity of the obtained analytical profile as well as the nature and potential effect of the different structures on the safety and efficacy profiles. Assessing alikeness at the glycosylation level is one of the most crucial part of the analytical biosimilarity studies when the reference product is glycosylated. Here we introduce and describe a glycoanalytical profile-based similarity scoring approach, referred to as *The Glycosimilarity Index* that can be used to accurately calculate the level of similarity between the N-glycosylation profiles of any given reference and test items. We also review and discuss those applications of this similarity scoring approach as potentially becoming a matter of interest in various stages of drug development and Quality Control.

NOTES:

Analytical Technologies: From Company Laboratory to Pharmacopoeial Method

Christopher Jones

Retired from NIBSC, St Albans, United Kingdom

High technology analytical methods for biopharmaceutical or biological products developed in research or company quality control laboratories are typically optimised for specific instruments and products. Transitioning these methods for pharmacopoeial use, for a range of instruments and products, and using generic reference materials, creates a number of challenges. Different companies will not always have comparable instruments, will optimise experimental conditions for specific factors and will typically use product-specific reference materials. In the case of bacterial polysaccharides used in vaccine manufacture, materials from different manufacturers are sufficiently similar for a compendial assay to be proposed. In other cases, such as glycoprotein glycosylation, the best approach may be to specify a minimum performance for the analytical method, through the use of system suitability standards. The implications for the development of material-specific and system suitability reference standards will also be addressed.

NOTES:

A Regulatory Perspective on Methods Used for Analytical Similarity Assessments

Kristen Nickens

CDER, FDA, Silver Spring, MD USA

The FDA approval of biosimilar products includes a rigorous evaluation of an array of comparative data between the proposed biosimilar and the US-licensed reference product, which are generated from analytical, non-clinical and clinical studies. As the analytical similarity assessment is the foundation of the biosimilar development program, it is expected that a sponsor provides a complete physicochemical and functional comparison of the biosimilar product and of the US-licensed reference product using a comprehensive battery of tests and modern analytical technologies to allow for the discernment of potential differences between the proposed biosimilar and the reference product, and to understand the potential impact of those differences on product safety, purity and potency. This talk will discuss considerations that should be taken into the development of methods used for a robust analytical similarity assessment, such as the use of state of the art technologies, the assurance of the performance of methods through appropriate qualification/validation studies, and the demonstration of the ability of the methods to detect potential differences between the proposed biosimilar and the reference product.

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Technical Seminar

Wedenesday, 7 March 2018
14:40 – 15:10
Zafir Ballroom
Sponsored by Bruker Daltonik

Accelerating Biotherapeutics Characterization with the new timsTOFpro and LC free MALDI Workflows

Guillaume Tremintin[1], Romano Hebeler[2], Detlev Suckau[2]

[1]Bruker Daltonics, Inc., Billerica, MA USA, [2]Bruker Daltonik, Bremen, Germany

Attendees of this technology seminar will learn how Bruker solutions accelerate Biotherapeutics Characterization from upstream development to manufacturing QC.

One key technology is the new timsTOF Pro. This newly developed ultra-high resolution QTOF couples the potential of a dual Trapped Ion Mobility Spectrometry (TIMS) cell to increase duty cycle and MS/MS speed. Together with the Parallel Accumulation Sequential Fragmentation workflow (PASEF) it achieves an MS/MS spectra acquisition speed (> 100 Hz) without loss in sensitivity. The enhanced sample separation *in vacuo* improves sensitivity and quality of fragment ion spectra, which enables the identification of low concentration proteins such as HCPs in the presence of a high abundant peptides derived from the antibody. In addition, it reduces LC-MS/MS analysis cycles in measurements that thrive for high sequence coverage of individual biopharmaceuticals; 5 min gradients typically yield >95 % SQ of mAb digests on the timsTOF Pro.

Another MS technology broadly used in academia but not common in many BioPharma analysis labs is MALDI. Matrix- assisted Laser Desorption/Ionization MS is a surface technology and each sample can be analyzed in seconds to fractions of a second. This enables a sample throughput that is difficult to achieve with other techniques. Methods will be presented that highlight this potential, such as clone selection or rapid release identity testing.

Besides the established “bread and butter” LC and LC-ESI-methods, these LC-free MALDI workflows are now supported in a single piece of software – BioPharma Compass 3.0 – which can generate automatic result reports from hundreds of samples. The LC-ESI workflows are entirely controlled in BPC 3.0 from acquisition to reporting providing extensive support for 21 CFR part 11 compliance. The intact mass screening workflow of proteins, antibodies or antibody fragments for sequence errors and post-translational modification (PTM) profiles utilizes Bruker's industry-leading SNAP II algorithm. This yields in reliable monoisotopic mass determination on Bruker’s ultrahigh-resolution time-of-flight hardware with sub-ppm mass accuracy. Fast and specific Top-down and middle-down sequence analysis of large proteins can be done with both ESI-UHR-QTOF and MALDI-TOF/TOF. This workflow allows for sequence confirmation and to confirm protein N- or C-terminal modifications.

NOTES:

Thursday, 8 March 2018
11:35 – 12:05
Zafir Ballroom
Sponsored by SCIEX

CE in the Biopharmaceutical Industry, from Cell Line Screening to Characterisation and Quality Control

Jim Thorn, Stephen Lock

SCIEX, Warrington, United Kingdom

CE is a flexible analytical platform for the analysis of biopharmaceuticals. Recent innovations have focused on fast, simplified workflows to address challenges throughout development and production. Our first example will be a case study of the rapid and complete characterisation of the NIST reference material 8671 (NIST mAb). Next, we will present the identification of biopharmaceutical modifications such as deamidation and glycosylation by the connection of CE to mass spectrometry. Finally we will preview the acceleration of cell line development and process control through high throughput glycan screening.

Thursday, 8 March 2018
14:40 – 15:10
Zafir Ballroom
Sponsored by Genedata

Automated Data Processing and Analysis for Quality Monitoring of Biotherapeutics by Multi-attribute Method (MAM)

Marlis Zeiler[1], Albert van Wyk[2], Claudio Schmid[3], David Bush[4]

[1]Genedata GmbH, Munich, Germany, [2]Genedata Ltd, London, United Kingdom, [3]Genedata AG, Basel, Switzerland, [4]Genedata, Inc, Lexington, MA USA

Biopharmaceutical firms adopt complex and costly process monitoring strategies and quality systems to ensure final product quality. Critical quality attributes (CQAs) are currently monitored using an array of analytical techniques. Although routinely used as release tests, these techniques generally do not measure attributes at the molecular level. In this context, many industrial players are exploring the adoption of innovative analytical approaches employing mass spectrometry (MS) to enable direct measurement of CQAs at the molecular level. In addition, MS-based methodologies offer the benefit of measuring many different quality attributes on a given biotherapeutic with a single test. The multi-attribute method (MAM) can potentially reduce development and manufacturing costs and at the same time increase product quality.

We present an implementation of MAM using a single software platform for the data processing, analysis, and management of MS data. In this approach, dedicated workflows were tailored to measure the CQAs for a given biomolecule, while testing for impurities (new peak detection), as well as checking the instrument qualification (system suitability). Optimized data processing was applied to large data sets and execution times scaled linearly with the number of samples. Browsing and downstream data analyses, including statistical tests, visual verification of the results, and generation of customized reports, were performed. This approach can be fully automated and employed as part of a bioprocess control strategy. In this case, we show as an example the real-time monitoring of quality attributes of the materials produced in a bioreactor. A compliance module including GxP functionalities such as audit trails, electronic signatures and data security allows the deployment of this MAM implementation in regulated environments.

NOTES:

Friday, 9 March 2018
12:00 – 12:30
Zafir Ballroom
Sponsored by Thermo Fisher Scientific

Characterizing Biopharmaceuticals using UHPLC and High-Resolution MS

Jonathan Bones

NIBRT, Co. Dublin, Ireland

Biopharmaceuticals have revolutionised the treatment of many diseases and progress within the field continues at pace. These complicated recombinant proteins require a suite of high resolution analytical methods to enable their complete characterisation. A variety of strategies exist for the characterisation of biopharmaceuticals, including analysis as intact entities, partial digestion and sub unit analysis, peptide mapping for the identification of posttranslational modifications and quantitative structural analysis of glycans if present. In this seminar we will present an overview of the use of the Thermo Fisher Scientific solutions for the characterisation of biopharmaceuticals using UHPLC and high resolution Orbitrap mass spectrometry. A variety of workflows will be presented across all domains of analysis, demonstrating the speed, simplicity and performance of the MAbPac and Accucore column chemistries and SMART digestion kits when combined with the Vanquish UHPLC and Q-Exactive Plus hybrid quadrupole Orbitrap high resolution mass spectrometer with extended mass Biopharma Option.

NOTES:

MAM Workshop

Thursday, 8 March
17:20 – 18:20

Implementation of LC-MAM in a High Throughput Process Environment

Moderator:
Anders Lund, *Sanofi, Framingham, MA USA*
Jette Wypych, *Amgen Inc., Thousand Oaks, CA USA*

Panelists:
Nicholas Bond, *MedImmune Limited, Cambridge, United Kingdom*
William Burkitt, *UCB Pharma, Slough, United Kingdom*
Yann Fromentin, *Sanofi, Vitry sur Seine, France*

Every biopharmaceutical is tasked with driving product to market faster and more efficiently. With a complex paradigm surrounding each and every new program, from complex ADCs, to sequence liabilities (deamidation/isomerization), to heterogeneity (glycosylation) and sequence identification, the role of analytical biochemist is complex. Although the process in not new, the concept of tracking multiple attributes in a single assay (LCMS MAM) holds significant hope for the beleaguered analytical Mass Spectroscopist. This workshop will openly discuss the limitations around the current hardware and software used for the detection of attributes. The need around high and low resolution will be discussed, the strengths and weaknesses of each approach. Panelist will have experience with products from orbitrap (high resolution) and single quad (low resolution) detectors in the processing of samples.

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Roundtable Discussions

Roundtable Session I
Wednesday, 7 March 2018
17:05 – 18:05

The Roundtable Session will be a truly interactive workshop to connect and discuss real issues with your peers. These sessions were designed to be informal (but structured) discussions on topics which are of interest to participants. **Participation will be on a first come, first serve basis**. Each topic will include a facilitator, whose role is to help assist the discussion and ensure a lively exchange, and a scribe, whose role is to make general, anonymous notes about the discussion. Notes will be posted on the CASSS website two weeks after the conference.

Listed below is a quick view of the roundtable topics, facilitators and scribes. Abstracts can be found in the CASSS app or on the meeting website.

Table 1 **Mass Spec in QC**
Facilitator: Carsten Jahn, *AbbVie Deutschland GmbH & Co KG*
Scribe: Katarzyna Kozakowski, *MedImmune Limited*

Table 2 **Charge Variant Analysis (cIEF / IEC / CZE)**
Facilitator: Cari Sänger - van de Griend, *Kantisto BV*
Scribe: Jerome Thiebaud, *Sanofi Pasteur*

Table 3 **Setting Specifications with Limited Batches and Analytics to Understand Process and Assay Variability**
Facilitator: Harold Taylor, *Merz Pharmaceuticals GmbH*
Scribe: Birgit Schmauser, *BfArM, Federal Institute for Drugs and Medical Devices*

Table 4 **How to Translate Physicochemical Properties into Claims of Safety and Efficacy**
Facilitator: Marta Germano, *Janssen Infectious Diseases and Vaccines*
Scribe: Hansjörg Toll, *Sandoz Biopharmaceutical*

Table 5 **Analysis of Polysorbate and Its Degradation Products**
Facilitator: Friederike Junge, *AbbVie Deutschland GmbH & Co KG*
Scribe: Hilde van Hattum, *Janssen Infectious Diseases and Vaccines*

Table 6 **Phase Dependent Requirements for Method Validation Across the Globe**
Facilitator: Francisca Maria Alberti Aguilo, *Bayer*
Scribe: Martin Pattky, *HES-SO Valais*

Table 7 **Implementation of USP<129>**
Facilitator: Carl Jone, *UCB Pharma SA*
Scribe: Elisabeth Ruge, *F. Hoffmann – La Roche Ltd.*

Table 8 **Automation**
Facilitator: Mette Dahl Anderson, *Novo Nordisk A/S*
Scribe: Anders Lund, *Sanofi*

Table 9 **Software Considerations for Methods Development and Data Evaluation**
Facilitator: Simone Albrecht, *Pfizer Ireland Pharmaceuticals*
Scribe: András Guttman, *University of Pannonia*

Table 10 **Control Strategy for Extractables and Leachables**
Facilitator: Christian Bell, *F. Hoffmann – La Roche Ltd.*
Scribe: Rob Haselberg, *Vrije Universiteit Amsterdam*

Roundtable Session II
Thursday, 8 March 2018
16:10 – 17:100

The Roundtable Session will be a truly interactive workshop to connect and discuss real issues with your peers. These sessions were designed to be informal (but structured) discussions on topics which are of interest to participants. **Participation will be on a first come, first serve basis**. Each topic will include a facilitator, whose role is to help assist the discussion and ensure a lively exchange, and a scribe, whose role is to make general, anonymous notes about the discussion. Notes will be posted on the CASSS website two weeks after the conference.

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Facilitator: Birgit Schmauser, *BfArM, Federal Institute for Drugs and Medical Devices*
Scribe: Katarzyna Kozakowski, *MedImmune Limited*

Table 3 **Setting Specifications with Limited Batches and Analytics to Understand Process and Assay Variability**
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Scribe: Marta Germano, *Janssen Infectious Diseases and Vaccines*

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Facilitator: Carl Jone, *UCB Pharma SA*
Scribe: Jennifer Römer, *Aalen University*

Table 5 **Analysis of Polysorbate and Its Degradation Products**
Facilitator: Anders Lund, *Sanofi*
Scribe: Pierre Guibal, *Sanofi*

Table 6 **Phase Dependent Requirements for Method Validation Across the Globe**
Facilitator: Elke Dietel, *F. Hoffmann – La Roche Ltd.*
Scribe: Elisabeth Ruge, *F. Hoffmann – La Roche Ltd.*

Table 7 **Implementation of USP<129>**
Facilitator: Christopher Jones, *Retired from NIBSC*
Scribe: Bert Wouters, *Vrije Universiteit Amsterdam*

Table 8 **Automation**
Facilitator: Harold Taylor, *Merz Pharmaceuticals GmbH*
Scribe: Francisca Maria Alberti Aguilo, *Bayer*

Table 9 **Software Considerations for Methods Development and Data Evaluation**
Facilitator: Christof Finkler, *F. Hoffmann – La Roche Ltd.*
Scribe: Mette Dahl Anderson, *Novo Nordisk A/S*

Table 10 **Control Strategy for Extractables and Leachables**
Facilitator: Jason Wood, *Bruker Daltonics, Inc.*
Scribe: Bernd Moritz, *F. Hoffmann – La Roche Ltd.*

Poster Abstracts

Analytical Technologies to Control Polysorbate and its Degradation Products

P-101-W

Impact of Mono- and Poly-ester Fractions on Polysorbate Quantitation using Mixed-mode HPLC-CAD/ELSD and the Fluorescence Micelle Assay

Janina Pfaff

F. Hoffmann-La Roche Ltd., Basel, Switzerland

Determination of excipient content in drug formulation is an important aspect of pharmaceutical formulation development and for analytical testing of the formulation. In this study, the influence of polysorbate subspecies, in particular mono- and poly-esters, for determining polysorbate (PS) content were investigated by comparing three of the most widely used PS quantitation approaches, the Fluorescence Micelle Assay (FMA) and Mixed-Mode High Performance Liquid Chromatography coupled with Charged Aerosol Detection (MM-CAD) or Evaporative Light Scattering Detection (MM-ELSD). FMA and MM-CAD were employed to investigate the quantitation behavior of PS20 and PS80 subspecies and corresponding degradation products in placebo formulations using forced degradation conditions at 40 °C for up to 12 weeks. While both methods allowed accurate and comparable quantification of neat PS at the beginning of stress studies, pronounced differences in content determination between the methods were observed at later time points, which were attributable to substantial differences in the contribution of individual mono and poly-esters to the overall quantitation results. It was particularly surprising to find that the main component of PS20, polyoxyethylene sorbitan monolaurate, did not show a signal at the studied concentration using FMA. Moreover, the degradation of polysorbate poly-esters, was reflected much stronger in FMA than MM-CAD results. Additional experiments employing chemical oxidation and base hydrolysis to degrade PS20, quantified by FMA and MM-ELSD, also show preferential reduction in certain subspecies depending on the degradation pathway involved. For PS20 degraded by chemical oxidation, quantitation results were lower for FMA than MM-ELSD, while the opposite trend was observed with base hydrolysis.

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Capillary Electrophoresis

P-102-T

Use of Electrophoretic Methods in Therapeutic Fc Fusion Protein Characterization

Francesco Cristofaro[1], Roberta Russo[1], Chiara Arzilli[1], Natascia Sciamanna[2]

[1]Merck Serono S.p.A., Rome, Italy, [2]Merck Serono S.p.A., Guidonia, Italy

The use of Fc fusion proteins to improve pharmacological properties of small bioactive peptides has increased significantly in the last decades and, during recent years, some of these experimental drugs have reached the market.

Various analytical methods are used to monitor and characterize several product-related and host cell-related impurities in these samples. Product-related impurities include disulfide-related scrambled forms (misfoldings), clips, oxidation products, unfolded single chain species, and both covalent and noncovalent aggregates. To ensure the safety, quality, integrity, and efficacy of a therapeutic protein, it is critical to characterize and monitor the product-related variants throughout the discovery and quality control processes and Capillary Electrophoresis (CE) methods are routinely used to monitor such species.

Generally, species identification observed in CE-SDS is challenging to carry out due to the difficulty of collecting significative analytical amounts of fractionations separated in the capillary.

In the present study, we discuss the high potential of combing SDS-PAGE and CE–SDS methods with mass spectrometry to elucidate the nature of fragments, to fully characterize single species and disulfide-related forms (scrambled or correctly folded) in a complex fusion protein. In addition, for the first time, a fractionator system was newly introduced to collect the different Fc fusion protein species for subsequent mass spectrometry structural analysis and for further electrophoresis characterization.

The presented study is a clear example about how several analytical techniques are required for a deep characterization of recombinant protein for human therapeutics to shed light on their molecular structure and activity.

NOTES:

P-103-W

GlycanAssure™: Simple and Sensitive Fully Integrated N-Glycan Analysis Solutions for High Throughput and QC Release Applications

Bérengère Francois

TF, St Genis les Ollières, France

We have developed a magnetic bead-based sample prep that improves the N-glycan analysis process for both Ultra High-Performance Liquid Chromatography (UHPLC) and Capillary Electrophoresis (CE) analytical platforms. We have simplified sample preparation by reducing the hands-on time, eliminating lengthy centrifugation and vacuum drying steps, and avoiding the use of toxic chemicals. The magnetic bead-based procedure provides a streamlined workflow for glycoprotein denaturation and deglycosylation, APTS labeling of released glycans, and excess free dye clean up. This workflow is automated on a cartridge-based platform making the overall sample prep a hands-free, walkaway solution. Samples from both manual and automated workflow can be analyzed by high throughput 3500 multi-capillary CE or Vanquish UHPLC. This provides an end-to-end single sample prep solution that can be used from high throughput clone selection applications to low throughput drug product QC release applications. N-glycan data generated from multi-capillary CE and UHPLC instruments is presented.

NOTES:

P-104-T

Quantitative N-glycosylation Comparison of the Innovator and Biosimilar Versions of Etanercept

Beata Borza[1], Marton Szigeti[2], Akos Szekrenyes[1], Laszlo Hajba[3], Andras Guttman[4]

[1]Horvath Csaba Laboratory of Bioseperation Sciences, Debrecen, Hungary, [2]University of Debrecen, Debrecen, Hungary, [3]Universiy of Pannonia, Veszprem, Hungary, [4]SCIEX, Brea, CA USA

The carbohydrate decoration on the polypeptide chains in most glycoprotein based biotherapeutics and their biosimilars plays essential roles in such major mechanisms of actions as antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, anti-inflammatory functions and serum clearance. In addition, alteration in glycosylation may influence the safety and efficacy of the product. Glycosylation, therefore, is considered as one of the important critical quality attributes of glycoprotein biotherapeutics, and consequently for their biosimilar counterparts. Thus, the carbohydrate moieties of such biopharmaceuticals (both innovator and biosimilar products) should be closely scrutinized during all stages of the manufacturing process. In this paper we introduce a rapid, capillary gel electrophoresis-based process to quantitatively assess the glycosylation aspect of biosimilarity (referred to as glycosimilarity) between the innovator and a biosimilar version of etanercept (Enbrel® and Benepali®, respectively), based on their N-linked carbohydrate profiles. Differences in sialylated, core fucosylated, galactosylated and high mannose glycans were all quantified. Since the mechanism of action of etanercept is TNFα binding, only mannosylation was deemed as critical quality attribute for glycosimilarity assessment due to its influence on serum half-life.

NOTES:

P-105-W

Using Electrokinetic Injection to Increase Throughput and Improve Sensitivity in the Detection of Proteins by CE-MS

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Capillary electrophoresis (CE) is an orthogonal technique to LC separating compounds based on their charge. The properties of CE enable the reduction and often elimination of carryover and wall absorption which effects peak resolution and sensitivity of LC. CESI [the integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into a single process in a single device] is now enabling the easy connection of CE to mass spectrometers. In this work we will describe how a CESI-MS method has been developed to detect intact proteins between 15 -25,000 amu.

Protein standards were prepared in a variety of different solvents and injected by either pressure or electro-kinetically onto a neutrally coated capillary under various conditions. The CE separation used MS amenable background electrolyte (BGE) consisting of a mixture of Acetic acid, Acetonitrile and Water. Proteins were detected by a MS system in MRM mode (using a capillary 30 μm ID, 91 cm long) which was fitted with a NanoSpray® III source which was used in either full scan or single ion monitoring mode with an ionspray voltage of 1600 - 1800 V which was optimized to the BGE being used.

The CESI-MS method was developed with the capability of detecting intact protein standards at <10 ng/mL. This study showed that electro kinetic injection was >40 fold more sensitive that an isotachophoresis (ITP) injection technique and a also more sensitive than a traditional LCMS approach and followed a similar trend as shown previously for neuropeptides. The response obtained was linear over the 2-3 orders tests with very low levels of carryover observed. Sensitivity depended on how well the protein ionized and one of the major factors shown to affect sensitivity on the electro kinetic injection was the levels of acetonitrile in the sample.

NOTES:

P-106-T

CESI-MS - A Sensitive and Versatile Approach for Metabolomics

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The analysis of the metabolites can present an analytical challenge, particularly those involved in the central carbon metabolism and neurotransmission. Often, structural isomers can't be differentiated by MS or MS/MS alone, making a separation crucial. Unfortunately, the LC separations prove difficult for some metabolites; some hydrophilic analytes are poorly retained by RP-LC, while column-to-column reproducibility can sometimes be more challenging with HILIC separations. In contrast, capillary electrophoresis (CE) is well suited towards metabolomics analyses. CESI-MS integrates CE and electrospray ionization (ESI) into a single device and this combines the benefits of a high-resolution separation with the increased MS sensitivities that result from the ultra-low flow rates. In this case, we demonstrate CESI-MS for the analysis of these challenging metabolites.

A single protocol was established for the separation and detection of both cationic and anionic metabolites. Electrophoretic separations were performed using 30 kV to generate a field strength of 333 V/cm and normal and reversed CE polarities were employed for cationic and anionic metabolites respectively. MS compatible background electrolytes (BGEs) facilitated highly efficient separations, achieved in less than 30 mins. Enhanced sensitivity was achieved using transient isotachophoresis with CESI directly coupled to either a SCIEX TripleTOF® 6600 or QTRAP®6500+ system.

Cationic metabolites were characterized using normal polarity with positive ESI while the anionic metabolites were analyzed from the same sample by simply switching the polarity of the CE and MS. The CESI-MS technology proved powerful for the targeted analysis of the central carbon metabolism, particularly the small organic acids of the tricarboxylic acid (TCA) cycle and the isobaric phosphorylated sugars of the pentose and glycolysis pathways. In addition, CESI-MS was used to detect metabolites involved in neurotransmission such as those in the tryptophan pathway. Despite an injection volume of less than 50 nL, low nanoMolar concentrations were easily detected.

NOTES:

P-107-W

How Does CE-MS Compare with LC-MS in PTM Analysis?

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Post translational modifications (PTMs) are important indicators of change in cells. Two of the top four most abundant PTMs are deamidation and phosphorylation and the location of the phosphorylation site is an important factor in understanding the effect of this modification on the activity of proteins. Traditional LC-MS methods struggle with the identification of positional isomers of phosphorylated peptides as they are identical in mass and have very similar fragmentation patterns. Phosphorylated peptides are often polar, and elute early in reverse phase chromatography (especially when more than one phosphorylation site is present on the peptide) which can make them difficult to detect by LC-MS. LC-MS methods also struggle to identify and quantify Aspartate and iso-Aspartate isomers (associated with deamidation) as they have the same mass and similar fragmentation patterns which often result in false positive identifications.

As CE separates analytes based on their charge and size (a different mechanism to LC) it can often overcome some of the separation challenges seen by LC. In this poster we will show how CESI-MS has been used to tackle these challenges and how it compares to LC-MS and find out how it has been applied to real biological samples in proteomics research.

NOTES:

P-108-T

Assessment of Antibody-derived Therapeutics at the Intact and Middle-up Level by CESI-MS

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Antibody-based pharmaceuticals often contain complex structural heterogeneity which requires enhanced analytical methods for reliable characterization of variants and degradation products. In this poster we will describe how CE-MS (CESI-MS which integrates CE and electrospray ionization (ESI) into a single device) in combination with high-resolution MS detection has been used for profiling antibody therapeutics. By using a neutral capillary coating (to provide near-zero electroosmotic flow) and an acidic background electrolyte intact model proteins were detected with overall migration-time RSDs below 2.2% (using 3 different capillaries). Various therapeutic proteins, including mono- and bivalent nanobodies, and three monoclonal antibodies (mAbs) were tested.

Intact nanobodies were resolved from their degradation products, which could be assigned to deamidated, cleaved, and truncated forms at the C-terminal tag with excellent resolution of isomeric deamidated products obtained.

The mAbs were analysed intact and after digestion by the IdeS endoproteinase (middle-up approach). CE-MS of intact mAbs resolved clipped species (e.g. light chain and light chain-heavy chain fragments) from the native protein as well as glycoforms containing sialic acids from their non-sialylated counterparts. For IdeS-digested mAbs, $F(ab)_2$ and Fc/2 where efficiently resolved. While migration of Fc/2 fragments were fairly similar for the three mAbs, the migration of the $F(ab)_2$ strongly depended on the mAb. All Fc/2 charged variants, which included glycoforms containing sialic acids and other PTMs such as loss of C-terminal lysine or deamidation of Asn, were nicely separated in less than 20 min. This allowed a detailed and reliable assessment of the Fc/2 heterogeneity (18-33 proteoforms) for the mAbs studied.

NOTES:

P-109-W

Enhancement of Capillary Zone Electrophoresis for Charge Heterogeneity Testing of Biopharmaceuticals

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Capillary zone electrophoresis is a powerful technique for charge heterogeneity testing of biopharmaceuticals [1]. In an extensive intercompany study, it was previously shown that capillary zone electrophoresis is very robust and can be easily implemented in labs that did not perform it before [2].

The distribution of different charge species strongly depends on the individual characteristics of the examined protein and may result in suboptimal resolution. Possibilities for further method optimization were investigated in several DOE studies [3]. This resulted in the determination of several factors that are most important for improving CZE separation. Despite of product specific DOE optimization, the obtained methods can be translated to other antibodies or product formats as well which demonstrates generic applicability. However, adaptation to individual molecular properties is sometimes still required. The set-screws that were identified in this study are well suited for this specific optimization.

Another application of CZE is flow-through partial-fill affinity capillary electrophoresis (FTPFACE). It was applied for a mixture of two similar therapeutic mAbs that are typical for novel co-formulation approaches. The goal was to separate the two mAbs and enable a mAb specific charge heterogeneity profiling without interference by the respective co-formulated mAb [4]. It was shown that mAbs can get complexed by a mAb specific antigen that shifts its peaks. Since the co-formulated mAb is not binding to this antigen, its charge profile is isolated and can be monitored independently.

[1] Y. He et al., J. Sep. Sci. 2011, 34, 1-8

[2] B. Moritz et al., J. Chrom. B, 2015, 983-984, 101-110

[3] B. Moritz, V. Locatelli et al., Electrophoresis, 2017, 24, 3136-3146

[4] A. Hutanu et al., manuscript in preparation

NOTES:

P-110-T

CE(SDS)-CZE-MS for the Analysis of Monoclonal Antibodies

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During production and storage of monoclonal antibodies, impurities can be observed, and structural modifications may occur. Due to their possible influence on the therapeutic activity and function, a detailed characterization of these modifications and impurities are of outstanding importance [1]. For the purity assessment and quality control, CE(SDS) is a widely used analytical method in pharmaceutical industries. Mass spectrometry (MS) is a powerful tool for the identification of these impurities. A two-dimensional CE-system with a mechanical valve as interface and an online SDS removal strategy was developed in our research group.

With the CE(SDS)-CZE-MS system, the MS identification of impurities in stressed intact antibodies is possible. Additionally, a reduced antibody with two different LC structures was analyzed. With the commercial available SDS-buffer, it was not possible to separate these two LCs. After optimization of the SDS separation buffer, a baseline separation was achieved and the MS identification with the 2D-CE-system of the LCs was done.

The importance of the 2D-CE system for the identification of CE(SDS) impurities and the analysis of antibodies with more complex structures will be shown. Additional, further improvements of the 2D-CE-system regarding the efficiency of SDS removal, robustness and sensitivity will be discussed.

1. Moritz B, Schnaible V, Kiessig S, Heyne A, Wild M, Finkler C, et al. Evaluation of capillary zone electrophoresis for charge heterogeneity testing of monoclonal antibodies. J Chromatogr B. 2015;983–984:101–10

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NOTES:

Liquid Chromatography

P-111-W

High Resolution Chromatography – Mass Spectrometry with a Novel Phenyl RPLC Column for Heightened Characterization of Hydrophobic Monoclonal Antibodies and Antibody Drug ConjugatesHigh Resolution Chromatography – Mass Spectrometry with a Novel Phenyl RPLC

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Monoclonal antibodies (mAbs) exhibit two functionally significant subunits: two equivalent antigen binding fragments (Fab domains) and one crystallizable fragment (Fc domain). Structural characterization of these subunits by mass spectrometry typically involves proteolysis by the Immunoglobulin Degrading Enzyme of S. pyogenes (IdeS). IdeS has high fidelity and specificity for a conserved Gly-Gly sequence motif in the lower hinge region of mAbs, and when used with disulfide bond reduction, IdeS produces three different ~25 kDa mAb fragments: the light chain along with two heavy chain Fd' and single chain Fc (scFc) subunits.

Characterization of these three mAb constituents can be readily achieved by means of reversed-phase liquid chromatography (RPLC) coupled to ultrahigh-resolution mass spectrometry (MS). Many times, however, it is challenging to develop an optimal separation for all three subunit components due to strong and complicated adsorption mechanisms that can exist between protein analytes and the stationary phase of a C4 RPLC column. Unfortunately, these chromatographic challenges are only exacerbated upon analysis of the corresponding antibody drug conjugate (ADC). To yield quantitative recovery and optimal resolution, the RP separation requires disconcertingly high column temperatures and/or relatively high concentrations of strong ion pairing agents, which can sometimes cause on-column hydrolysis or compromise the quality of protein mass spectra.

Using a newly-developed RPLC column comprised of a novel phenyl bonded phase and a highly efficient superficially porous particle, it has been possible to mitigate numerous shortcomings of an RPLC-based product characterization strategy. With application to mAb and ADC subunit profiling, this column has afforded optimal resolution and analyte recovery at 20°C lower temperatures and significantly lower concentrations of the ion pairing additive. In turn, the development of a new platform method for mAb and ADC subunit profiling is now envisaged, wherein it will be possible to obtain higher

NOTES:

P-113-T

Assessing Performance and Method Transfer of Monoclonal Antibody and Peptide Bioseparation Methods Using a Novel Biocompatible UHPLC System

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Many of the top-selling pharmaceuticals currently on the market and in the pipeline are biologics. Because biologics are more complex than small molecules, analytical methods for analysis and regulatory requirements tend to be less straightforward. To date, many of the methods used in development and quality control laboratories are HPLC-based. While this may be sufficient in some cases, there are noted advantages of updating legacy systems and methods with more modern instrumentation. Regulators also recognize and support the notion for improving process performance through lifecycle management in an effort to enhance product quality and patient safety.

The product lifecycle includes development and manufacturing activities as well as technology transfers. When adopting new technology, it is of critical importance that instrumentation be robust and easily deployed. In this work, a new-to-market biocompatible UHPLC platform will be used to demonstrate the benefits of laboratory modernization in support of lifecycle management. Ion exchange, size exclusion, and peptide mapping are among the methods used to demonstrate equivalency across HPLC, UHPLC, and UPLC platforms. By updating from an HPLC platform, better resolution, shorter run time, and greater peak capacity can be achieved. As analytical assays are transferred to various in-house laboratories and contract organizations, it is imperative that results are consistent among sites. Method transfer across multiple instrument platforms is also demonstrated and assessed using retention time and peak area percent. By modernizing laboratory instrumentation, legacy methods can be successfully reproduced or updated to take advantage of new column technologies and lower dispersive systems for various improvements in performance.

NOTES:

P-114-W

Quantification of Endotoxins in Protein Samples

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Endotoxins (ET) are a pyrogenic contaminant in non-sterile biological production streams and must be removed for products with intended human use. If introduced into the blood stream, they can cause a septic shock even at low amounts of only a few ng. According to current FDA guidelines, products for parenteral administration must not contain more than 5 endotoxin units (EU) per dose and kg body weight (10EU ~ 1ng ET).

ETs contain a lipid part and a sugar part, which may contain a variable number of repeating glycan units. ET average MWs range from 2kDa - 42kDa for different ETs or ET preparations. Due to their amphiphilic, extremely heterogenic molecular structure, their tendency to form various aggregates up to > 6000kDa and lack of chromophores, they are difficult to analyze with conventional analytical methods. Today, ET analysis is mainly performed using the Limulus Amebocyte Lysate (LAL) assay and the rabbit pyrogen test. These biological tests show very high variability. In some cases, ETs were spiked to monoclonal antibody formulations and could be detected with the rabbit test, but not by LAL testing (LER phenomenon).

In this study, we analyzed spiked and unspiked protein solutions with respect to their ET content. Protein solutions were filtered with ET specific filter sheets developed by FILTROX AG (St. Gallen, CH). The filters quantitatively retained up to 90 % of the spiked ETs. ET concentration was measured by a chemical ET assay (patent pending). In principle, conservative ET building blocks, present to a defined amount in all ETs, are detected sensitively with an LOQ of 50EU/ml (ca. 5ng/ml ET). The assay allows the quantification of ETs even in concentrated protein solutions. Results from the chemical assay will be compared to the results of the LAL test.

NOTES:

P-115-W

A Rapid and Universal Monoclonal Antibody Charge Variant Characterization Platform using pH-Gradient Ion Exchange

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Monoclonal antibodies (mAbs) are prone to modifications such as sialylation, deamidation or C-terminal lysine truncation. Traditionally, salt gradient cation exchange chromatography has successfully been used for the assessment of the mAb charge variant profile. However, significant efforts are often required to tailor salt gradient methods to individual mAbs and generally long run times are needed to achieve the desired resolution. In the fast-paced drug development environment, standardized, rapid and robust platform methods are desirable, accommodating the majority of mAbs analyzed. Here, we present the charge variant profile of top-selling mAbs, analyzed by strong cation exchange with a linear pH gradient method utilizing next generation UHPLC technology. The pH gradient method serves as a platform method for the mAb charge variant analysis, covering a pH range from 5.6 – 10.2, allowing to determine the pI value of the charge variants when combined with an on-line pH monitor. Bevacizumab, Cetuximab, Infliximab, and Trastuzumab were analyzed on a small particle MAbPac SCX-10 column using a full pH gradient of 10 min. Separations of multiple charge variants was achieved for all mAbs analyzed. Additional resolution improvements and a significant shortening of the analysis time was achieved by optimizing the utilized pH range for each mAb in conjunction with the application of an elevated flow rate to further decrease the applied gradient slope. This easy and fast method optimization approach allowed the registration of the charge variant profile for each mAb within 5 min while retaining the high-resolution separation normally only associated with longer gradient runs.

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P-116-W

A Novel Phenyl-Based RPLC Stationary Phase for High Throughput, High Resolution Characterization of Protein Therapeutics

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Protein therapeutics can be effectively characterized using the capabilities of liquid chromatography (LC). Because of its high resolving power and amenability to mass spectrometric (MS) detection, reversed phase liquid chromatography (RPLC) has become one of the most heavily relied upon techniques. However, RPLC stationary phases notoriously suffer from performance limitations, including their strong dependence on ion pairing and elevated separation temperatures that can cause on-column degradation.

To address these limitations, a novel column technology has been designed. This column technology is based on an optimized 2.7 µm superficially porous particle that by van Deemter analyses has proven to be effective in minimizing intra-particle diffusion, thereby affording a kinetic efficiency advantage. For the porous layer of this stationary phase, an optimal pore diameter has also been carefully selected. Comprehensive analysis of intact and IdeS-digested monoclonal antibodies (mAbs) has shown that the average pore diameter needs to be at least 400 Å, particularly when ion pairing is minimized for MS compatibility, where proteins are more likely to adopt extended structures. Moreover, the capability of this column technology is augmented by a novel surface chemistry that is synthesized using a multistep silanization process to yield a phenyl-based bonded phase which is both high in coverage (up to 6 µmol phenyl moiety/m^2) and comprised of rigidly constrained carbons. This novel bonded phase is believed to limit silanol interactions by extensively masking the silica base particle, to facilitate more discrete desorption at lower temperatures by minimizing the conformational heterogeneity of protein adsorption, and to improve resolving power by being highly retentive. Using either HPLC or UHPLC instrumentation, it will be shown that this technology has made it possible to better characterize mAb and ADC therapeutics by delivering unprecedented resolution as well as higher fidelity, higher quality data.

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P-117-T

A New Multi-attribute Method - Ion Exchange Chromatography of Biopharmaceutical Proteins Coupled Directly to High Resolution Mass Spectrometry

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Thorough characterisation of Bio-therapeutic proteins is essential at all stages of development through to manufacture and final product quality control. Each protein will have several different variant forms due to multiple post translational modifications that can occur during production, purification and storage. These modifications can alter the charge distribution on the surface of the protein and are characterised by charge variant analysis using ion exchange chromatography. All modification requires characterisation and control to ensure product quality and reproducibility as they could have an impact on efficacy or safety. Identification of structural variants is a critical challenge and Mass Spectrometry [MS] is used as a tool in the identification of the protein variants. However, the technique of ion exchange requires high salt eluents in the chromatography which is incompatible with MS so the structural variants exposed by these techniques must be collected separately off-line, then desalted before further characterisation by MS. Here we describe novel direct on-line coupling of ion exchange to MS in the characterisation of Mab variants. The technique has fast run times and greatly reduces analysis time and sample handling by avoiding fraction collection and separate desalting injections by reverse phase LCMS. The chromatographic resolution of MAb charged variants using pH gradient elution with a novel volatile buffer preparation compares favourably with traditional salt elution. The proteins enter the Orbitrap MS system in the native state with a reduced charge distribution and an elevated mass to charge ratio. Variants found with this direct on-line coupling in addition to the charge variant profile include fragments, glycosylation and lysine truncation. The Mass accuracy is also much improved due to the separation of near isobaric variants.

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Mass Spectrometry

P-118-W

Antibody Analysis with Native Mass Spectrometry and Parsimonious Charge Deconvolution

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Intact mass analysis under native conditions offers a number of advantages over denaturing conditions. Native MS preserves structure (e.g., folded and partially unfolded protein will charge differently) and non-covalent binding (e.g., dimerization, ADCs with cysteine conjugation), and also gives greater separation between charge states in the m/z spectrum so that charge states of heavily modified proteins are less likely to overlap. Native MS, however, poses some challenges to charge deconvolution algorithms: higher masses, lower and fewer charge states, wider and less predictable peak shapes, and co-occurrence of problematic mass pairs such as monomers and dimers. The most commonly used charge deconvolution algorithm, MaxEnt, can incorrectly split a wide peak into two narrow peaks, or produce "harmonic" artifacts such as masses at one-half or twice the true mass that can be easily confused with minor species. In this poster, we present a novel "parsimonious" charge deconvolution algorithm that more faithfully represents the m/z data. The new algorithm is especially well-suited to high-resolution native mass spectrometry of intact glycoproteins and protein complexes. We will show intact mass analysis under native conditions of four therapeutic antibodies: cetuximab, daclizumab, infliximab, and obinutuzumab. The four antibodies present interesting challenges, including N-terminal extensions and clipping, abundant C-terminal lysine, Fab glycosylation, and engineered Fc glycosylation. Native MS reveals these complexities in qualitative and quantitative detail, and bottom-up analysis confirms the results from the intact mass analysis.

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P-119-T

Charge Variant Orbitrap Mass Spectrometry Analysis Dramatically Improves Effective Resolution and Dynamic Range of Intact Mass Measurements

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Therapeutic proteins are microheterogeneous mixtures in which many unique, albeit isobaric molecular compositions, may be present simultaneously. Conventional denaturing intact protein MS utilizes aqueous/organic mixtures at low pH for sensitive ESI; however, complex protein spectra may be complicated by significant spectral interferences of overlapping successive protein charge states. Native MS utilizes aqueous buffers within physiological pH ranges and reduces charge state values to simplify spectra; however, there are currently few options for combining separations with native MS analysis. Ion exchange chromatography (IEC) is a powerful technique used to separate proteins on the basis of small changes in isoelectric point in the outer surface of proteins. IEC is commonly used to separate charge variants of biotherapeutic proteins. Conventional IEC employs buffer salts which suppress protein ionization. In this report we demonstrate a charge variant intact mass analysis (CV-MS) workflow in which IEC mobile phases can be substituted for volatile salt buffers which allow direct MS compatibility. We utilize pH gradient elution as an alternative method for IEC, allowing separations to be performed at low salt concentrations. We perform a multi-dimensional evaluation of the CV-MS data (mass resolution, chromatographic resolution, dynamic range) to understand the advantages of employing our workflow versus the conventional limitations of intact mass analysis performed using reverse phase or direct infusion coupled to MS.

For LC-MS analysis, mAb sample was directly injected into the LC flow path without any pre-treatment. For mass spectrometry we utilized a commercially-available Thermo Scientific™ Q Exactive™ HF-X Orbitrap™ mass spectrometer operated in High Mass Range mode for native LC-MS intact protein analysis. Data were analyzed using the ReSpect algorithm in Thermo Scientific™ BioPharma Finder™ 3.0 software.

NOTES:

P-120-W

Detection and Characterization of N- and O-Glycosylation: Streamlined Methods for Intact Mass Analysis and Bottom-Up Proteomics

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Glycoprotein characterization presents unique analytical challenges. Although *N*-glycosylation sites can be predicted, site occupancy can be hard to quantitate. Additionally, *O*-glycan modifications have to be empirically identified since they are difficult to model from the primary protein sequence. Mass spectrometry (MS) is becoming the analytical standard for biotherapeutics, as user-friendly instrumentation allows precise identification and quantitation of protein features at relatively low costs. These methods apply to peptides or proteins, before or after deglycosylation under mild, MS-compatible conditions. We report here the use of streamlined methods using a combination of enzymes that readily reveal whether *N*- and/or *O*-glycans are present, allowing the estimation of site occupancy. Well-characterized monoclonal antibodies and fusion proteins were used as models for validation.

Antibodies (mouse monoclonal IgG2, rituximab) or fusion proteins (etanercept and abatacept) were completely deglycosylated using Rapid PNGase F and/or the Protein Deglycosylation Mix II in 10 min or 1h respectively. The intact mass of samples treated with Rapid PNGase F compared to samples treated with the Protein Deglycosylation Mix II, readily shows whether a protein of interest contains one or more *O*-glycan groups. This treatment increased peptide coverage for abatacept and etanercept: peptides containing *N*- or *O*-glycans were only detected after treatment. The comparison between control and deglycosylated samples allowed the rapid identification of the *N*- glycan and *O*-glycan sites.

In complex serum samples treatment with the Protein Deglycosylation Mix II increased peptide coverage. For example, the sequence coverage of serotransferrin, a well-known diagnostic reporter for a number of rare genetic defects, increased from 34 to 47% after deglycosylation. Site occupancy was also calculated using a combination of endoglycosidases under optimized conditions. The GlcNAc scar left on the asparagine residue was used as a marker for *N*-glycosylation to identify rates of occupancy.

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P-121-T

How Accurate is N-glycan Quantitation in Monoclonal Antibodies? A Comparison Across Different Domains of Analysis

Sara Carillo[1], Jonathan Bones[1], Amy Farrell[1], Raquel Raquel Pérez-Robles[2], Natalia Navas[2]

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There is a growing interest in the analysis of recombinant monoclonal antibodies (mAbs) and the study of their interaction mechanisms. N-glycans present on the Fc region of the monoclonal antibody have an important role not only in monoclonal antibody stability but also the mechanism of action of the drug. It has been showed that afucosylated glycans can enhance the antibody-dependent cellular cytotoxicity (ADCC) as the binding affinity of the Fc region of mAbs to the Fcγ receptor increases.[1] As well, higher levels of galactosylation can impact mAb effector function[2], while a different type of activity is attributed to high mannose glycans[3]. It is clear that glycan analysis is of crucial importance in biotherapeutic analysis for quality control and product development (biosimilars) to ensure product safety and maximize desired effects.

N-glycans relative abundances can be obtained with different analytical techniques ranging from peptide mapping to intact mass analysis to the isolation and analysis of N-glycans. Each of these techniques has strengths and weaknesses and has as a final step LC-MS analysis. To achieve confident results, the analysis needs high mass accuracy and resolution, which are easily achieved using the Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer.

In this study, a comparative analysis on the N-glycans profiles of 3 of the top 5 biotherapeutics (rituximab, trastuzumab and bevacizumab) across different domains of analysis is reported. N-glycans profiles will be evaluated and quantified at:

- intact mass level, discussing differences between denatured and native condition of analysis
- heavy/light chain level
- IdeS digested subunits level
- peptide mapping
- N-glycans level, discussing the influence of the fluorescent label during the analysis.

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2. Raju, TS & Jordan, RE *mAbs* 4, 385, (2012).

3. Bowden, TA et al. *Journal of the American Chemical Society* 134, 17554, (2012).

NOTES:

P-122-W

Absolute Quantification of Proteins: Forget Amino-Acid Analysis and Move to ICP-MS!

Arnaud Delobel, Juliusz Bianga, Philippe De Raeve

Quality Assistance sa, Donstiennes, Belgium

Despite all recent advances in analytical technologies dedicated to biotherapeutics, accurate protein quantification remains a challenge for the biopharmaceutical industry. UV spectrophotometry is commonly used for batch testing, but it requires the knowledge of the extinction coefficient of the protein, whose experimental determination requires the accurate concentration of a reference standard obtained by an absolute quantification method.

Most protein quantification techniques (separation techniques such as LC or CE, colorimetric assays, immunoassays…) cannot be considered as absolute as they also require a reference standard.

Amino acids analysis after complete hydrolysis of a protein is probably the most commonly used method for the absolute quantification of a single protein or peptide. However, hydrolysis and derivatization are time-consuming procedures and, very often, result in low precision and accuracy.

In order to address the need for a fast-analytical method capable to accurately quantify a protein without any specific reference substance, an isotope dilution ICP-MS method was developed and validated, based on sulfur determination, allowing very accurate determination of a single protein in solution after microwave digestion.

The method was validated (ref. ICH Q2A) using a NIST certified BSA solution: precision is < 1 % RSD and accuracy shows less than 2 % bias over the range of concentrations tested.

Ref : Bianga *et al.*, *Spectroscopy*, 31 (2016), 25-30

NOTES:

P-123-T

Orthogonal Liquid Chromatography-Mass Spectrometry Methods for the Comprehensive Characterization of Therapeutic Glycoproteins, from Released Glycans to Intact Protein Level

Arnaud Delobel, Eric Largy, Fabrice Cantais

Quality Assistance sa, Donstiennes, Belgium

A large number of pharmaceuticals are glycosylated proteins, including monoclonal antibodies (mAbs) and other recombinant proteins (fusion proteins, cytokines, growth factors). An adequate glycosylation is critical for therapeutic glycoproteins in terms of safety, bioactivity, solubility, stability, and pharmacokinetics and dynamics. Consequently, the glycosylation profile of therapeutic glycoproteins must be thoroughly analysed. However, these proteins are typically produced in different expression systems, whose glycosylation machineries function through sequential and competitive steps, hence creating micro- (glycans nature for a given site) and macro heterogeneities (number and location of sites) of glycosylation. This creates a challenging analytical puzzle that requires a number of orthogonal analytical techniques, at different levels of analysis (released glycans, peptides, intact and subunits), to be solved.

We will present the use of mass spectrometry to characterise both the N- and O-glycosylation of Etanercept, a tumor necrosis factor-a (TNFa) antagonist, commercialised as Enbrel®. It is produced by recombinant DNA technology as a fully human, dimeric fusion protein, each monomer consisting of a TNFa-receptor linked to an Fc/2 of IgG1 (minus its CH1 domain). Etanercept glycosylation accounts for roughly a third of the protein's apparent molecular weight (150 kDa).

The methods used for this complete characterisation were:

- N-glycans profiling by UPLC/FLR/MS using RapiFluor-MS labelling
- O-glycans profiling by LC/MS using a porous graphitic carbon column
- Sialylation profiling by mixed-mode chromatography with fluorescence detection
- Sialylation quantification by RP-UPLC/FLR
- Site-specific N-glycans profiling by HILIC-ESI-QTOF/MS
- Determination of O-glycosylation sites by RP-UPLC-ESI/MS with ETD fragmentation
- Characterisation at the subunit level using Widepore HILIC ESI-QTOF/MS

The combination of all these methods allowed a full characterisation of Etanercept N- and O-glycosylation.

Ref : Largy *et al. Journal of Chromatography A*, 1498 (2017) 128–146

NOTES:

P-124-W

Epitope Mapping of an Interleukin Receptor for Three Therapeutic Antibodies by Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS)

Arnaud Delobel, Eric Largy

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Monoclonal antibodies (mAbs) constitute a major and fast-growing biotherapeutic class, thanks notably to their outstanding selectivity for specific targets. Their physico-chemical characterisation is complex due to their size, numerous variants caused by PTMs, or their tendency to aggregate. Besides, there is a need for better understanding how these aspects may alter their structure and function. This prompts the study of the precise nature of the interaction between mAbs and their antigens at the molecular level.

Several methods can be used to map interactions between proteins. X-Ray crystallography is probably the gold standard in such endeavours but suffers from a few drawbacks. It is a lengthy process that relies on the amenability of the proteins to crystallise, and "only" yields a snapshot of one energetically accessible conformation. Solution-based techniques are best suited to study dynamic systems as they consider all attainable conformations. NMR experiments provide similar resolutions but are often too difficult to carry out in this context. Other methods such as circular dichroism or mutagenesis usually lack in resolution or do not provide direct evidences, respectively.

We take advantage of the HDX/MS approach, which is much faster and easier to carry out than the aforementioned methods. Although it does not provide atomic resolution, it allows the determination of epitopes at the scale of a few amino acids whatever the complexity of the sample, requires less sample amounts, tolerates impurities, formulants and operates in solution in near-physiological conditions. Furthermore, it provides insight into structure and interaction dynamics on a large time scale.

We describe the epitope mapping of three distinct mAb candidates targeting an interleukin receptor. The goal is to establish the binding mode of these mAbs, and explain possible differences observed in *in vitro* binding and *in vivo* function.

NOTES:

P-125-T

LC-MS Method to Evaluate Incidence Rate of Protein Sequence Variants in Biopharmaceuticals

Paul Getty

Lonza Ltd., Slough, United Kingdom

Protein sequence variants are unintended amino acid sequence changes resulting from genomic nucleotide change or translational misincorporation. Understanding the propensity for different protein expression systems to generate sequence variants enables an effective risk mitigation strategy for cell line and process development. We have therefore determined the overall rate of unintended amino acid variant incorporation within the Xceed Expression System™. Mechanisms of misincorporation and correlation with cell line stability at generation numbers representative of large-scale biomanufacturing were investigated. Finally, variability of detection limit for sequence variants at different locations within an antibody product was investigated.

Antibodies were expressed using the Xceed Expression System™ in AMBr miniature bioreactors using a platform cell culture process. Culture supernatant was purified by Protein A affinity. Samples were denatured, reduced and digested using proteolytic enzymes. The resulting peptides were separated by reverse phase chromatography at nanoflow scale and identified using an Orbitrap Fusion Q-OT-LIT mass spectrometer and a data-dependant decision tree workflow with HCD and EThcD fragmentation. Data analysis was performed using Progenesis QI for Proteomics and PEAKS Studio 7.0.

Three engineered amino acid changes were expressed based on the trastuzumab sequence, which was spiked into trastuzumab at defined relative concentrations. It was determined from the development projects that many variants could be confidently detected at levels of less than 0.01%. The spike recovery approach tested the ability of the method to identify variants in possible “blind spots” where the amino acid sequence challenged this method. This was found to substantially increase the limit of detection to 1%.

Five cell line constructions for four different products were tested to determine an interim variant rate. This resulted in a misincorporation rate of 6%, representing the percentage of cell lines that showed a variant at above 0.2% at either early or late generation for the Xceed Expression System™.

NOTES:

P-126-W

Evaluating the Analytical Power of the TripleTOF 6600 MS Platform to Assess Monoclonal Antibodies Quality Attributes

Ricardo Gomes

iBET, Lisboa, Portugal

Monoclonal antibodies (mAb) are a major class of biopharmaceuticals used for the treatment of oncologic conditions, chronic autoimmune, respiratory, metabolic and central nervous system disorders, among other. The biopharmaceutical industry accounts nowadays for 20% of the pharma market with R&D investment of 58.8 billion dollars in 2015 for US alone. As patents expire, biosimilar and biobetter development is also on the rise using a Quality by Design (QbD) approach. The development of new mABs as well as of biosimilars, and optimization of the production processes require a comprehensive characterization at the molecular level of antibodies heterogeneity. This includes depicting post-translational modifications (PTMs), including glycosylation, identifying product impurities, detecting process related contaminants such as host-cell protein and evaluating sequence variations. In an industry where time is crucial, analytical characterization must be fast and produce an array of robust and relevant information used to guide the development stage.

Considering all these facts, in our work we demonstrate the analytical power of TripleTOF 6600 MS platform for comprehensive analysis at different molecular levels of an in-house produced mAb. Four characterization levels were considered:

1) protein analysis with mass measurement of intact and deglycosylated mAb for the detection of high and low-abundance molecular isoforms (Ab heterogeneity);
2) protein analysis with mass measurement of reduced and reduced+deglycosylated for the assessment of mAb heavy and light chains;
3) peptide analysis of tryptic digested mAb to characterized peptide and glycopeptides (detection of PTMs, N-terminal modifications…);
4) N-glycan structures analysis (from PNGase F digestion).

The main aim of this work was to develop a robust MS-based methodology where sample preparation procedures are kept at a minimum, providing an extensive collection of valuable data in a short period of time.

NOTES:

P-127-T

Enhanced Peptide Mapping with a PASEF Enabled Ultra High Resolution QTOF

Romano Hebeler[1], Detlev Suckau[1], Guillaume Tremintin[2], Stuart Pengelley[1]

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Peptide mapping by LCMS is always a compromise in between acquiring high quality and dynamic range MS1 data for quantitation, high quality MS2 data for unambiguous assignment of heterogeneities and high sensitivity to minimize the risk of low abundance forms going undetected.

A newly developed ultra-high resolution QTOF – the timsTOF Pro - harnesses the potential of a dual Trapped Ion Mobility Spectrometry (TIMS) cell to simultaneously increase ions utilization and MS2 speed. Together with the Parallel Accumulation Sequential Fragmentation workflow (PASEF) this enables high sequencing speed of over 100 Hz without losing sensitivity by synchronizing the quadrupole isolation mass window with the elution time of the specific peptide packages from the TIMS funnel.

These hardware enhancements can benefit the detection of isobaric peptides as separation in the mobility cell prior CID fragmentation reduces the occurrence of chimeric spectra. The enhanced sensitivity also enhances the general quality of MS2 spectra simplifying the data review tasks and the identification of low concentration proteins such as HCPs.

Using the NISTmab reference material as the basis for this case study, the capabilities of this novel instrument to support the development of monoclonal antibodies were evaluated in the context of high speed peptide maps as well as longer methods for the sensitive detection of low abundance heterogeneities.

NOTES:

P-128-W

Automated Data Processing and Analysis for Quality Monitoring of Biotherapeutics by Multi-attribute Method (MAM)

Marlis Zeiler[1], Claudio Schmid[2], David Bush[3], Albert van Wyk[4]

[1]Genedata GmbH, Munich, Germany, [2]Genedata AG, Basel, Switzerland, [3]Genedata, Inc, Lexington, MA USA, [4]Genedata Limited, Cambridge, United Kingdom

Biopharmaceutical firms adopt complex and costly process monitoring strategies and quality systems to ensure final product quality. Critical quality attributes (CQAs) are currently monitored using an array of analytical techniques. Although routinely used as release tests, these techniques generally do not measure attributes at the molecular level. In this context, many industrial players are exploring the adoption of innovative analytical approaches employing mass spectrometry (MS) to enable direct measurement of CQAs at the molecular level. In addition, MS-based methodologies offer the benefit of measuring many different quality attributes on a given biotherapeutic with a single test. The multi-attribute method (MAM) can potentially reduce development and manufacturing costs and at the same time increase product quality.

We present an implementation of MAM using a single software platform for the data processing, analysis, and management of MS data. In this approach, dedicated workflows were tailored to measure the CQAs for a given biomolecule, while testing for impurities (new peak detection), as well as checking the instrument qualification (system suitability). Optimized data processing was applied to large data sets and execution times scaled linearly with the number of samples. Browsing and downstream data analyses, including statistical tests, visual verification of the results, and generation of customized reports, were performed. This approach can be fully automated and employed as part of a bioprocess control strategy. In this case, we show as an example the real-time monitoring of quality attributes of the materials produced in a bioreactor. A compliance module including GxP functionalities such as audit trails, electronic signatures and data security allows the deployment of this MAM implementation in regulated environments.

NOTES:

P-129-T

Automated Workflow for Host Cell Protein Monitoring by Mass Spectrometry: From Raw Data to Final Report

Albert van Wyk[1], Marlis Zeiler[2], Claudio Schmid[3], Joe Shambaugh[4]

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Allowing unbiased identification and high throughput quantification of multiple low-abundant proteins, MS enables a thorough assessment of HCP contamination in biotherapeutics. We present an automated approach that, starting from the raw data, allows the identification, quantification, and routine monitoring of HCPs by MS.

HCP contaminations can span a wide range of concentrations, with low-abundant species present at the ppm level. To address this challenge, we applied optimized algorithms for mass recalibration, noise removal, and retention time alignment to obtain optimal peak detection even for low-abundant species. After that, two different strategies for the identification of HCPs were followed.

In the first approach, monitoring of low-abundant HCPs was accomplished using a two-stage identification procedure. The whole collection of signals belonging to the protein biotherapeutics (e.g. peptides, modifications, etc.) was identified before submitting the data to conventional peptide spectrum match searches. This allowed monitoring and quantification of the expected peptides reducing the chance of low-abundant features from the biotherapeutics to be falsely identified as HCP signals (false positives).

Submitting mass spectra from low-abundant signals to PSM algorithms also poses the risk of missing the identification of HCPs (false negatives). In the second approach, we developed a strategy to mitigate this risk. Potential HCPs were identified by PSM search of samples with enriched HCP content, and the information pertaining to the respective peptides was then used by the software as identification criteria for any other samples. In particular, retention time and m/z coordinates of known impurities were stored in a dedicated knowledge base and were used for matching of the respective signals in single-stage MS data. These libraries can be used in combination to annotate signals in downstream samples and allow for proper identification even in cases where no fragmentation data is available.

NOTES:

P-130-W

Enterprise Mass Spectrometry Software Solution Enabling Characterization of Biotherapeutics from Discovery and Development to Production and Quality Control

Claudio Schmid[1], Marlis Zeiler[2], Albert van Wyk[3], Joe Shambaugh[4]

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Mass spectrometry has rapidly become a key technology for the complete characterization of biotherapeutic candidates, a task that is required throughout the entire discovery, development, and manufacturing cycle. Often data analysis has become the bottleneck limiting sample throughput and quality.

In this poster we present Genedata Expressionist®, a software solution that revolutionizes mass spectrometry data analysis and data management processes company-wide, from raw data to electronically signed reports. Offering complete automation across instruments and labs, the software streamlines complex processes such as peptide mapping, intact protein mass, released glycan, and host cell protein analysis. Genedata Expressionist provides a flexible workflow system enabling method development and implementation of standard operating procedures, setting a new benchmark in standardization and reproducibility.

NOTES:

P-131-T

High-sensitivity HDX-MS to Identify Chemical Modification-induced Conformational Changes of Biopharmaceuticals

Felix Kuhne

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Higher order structure information of biopharmaceuticals gains in relevance. Questions pertaining to the assessment of chemical modifications as quality attributes and their impact on structure and function are more diverse for upcoming molecule formats and formulations. We established a high sensitivity method for hydrogen/deuterium exchange mass spectrometry (HDX-MS) to determine structural changes as induced by chemical modifications. Significant deuterium uptake differences for low level oxidations and glycosylation variants could be verified down to the lowest increments tested. This method could hence be applied to structurally differentiate other relevant chemical modifications and systematically investigate their influence on structure and function.

NOTES:

P-132-W

Mass Spectrometry as a Powerful Tool Box for Host Cell Protein Analysis

Barbara Kurth[1], Heiner Falkenberg[2], Anke Schnabel[2], Gerhard Körting[2], Anja Dommermuth[2], Thomas Flad[2], Roland Moussa[2]

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Developing and producing recombinant biopharmaceuticals in mammalian cells requires unambiguous monitoring of HCPs impurities. Immunoassays are still the method of choice for release testing, for which it is recommended to demonstrate the suitability of antisera in a QM-regulated environment to meet regulatory demands. However, peptide analysis by MS has been proven to be a powerful tool box by providing complementary data for HCP characterization.

We show that MS supports the data interpretation of methods for antisera characterization, like high resolution 2D gel electrophoresis combined with immunoblotting to demonstrate antisera coverage, and anti-CHO affinity chromatography to specify the antigen coverage.

Furthermore, peptide analysis by MS offers orthogonal solutions for detection and monitoring of residual HCPs. The advantage compared to immunological methods is the unbiased discovery of HCP impurities to reveal the HCP identities.

By using label-free MS quantification the removal of HCPs during downstream processing was monitored and demonstrated the suitability of the technique for quantitative analysis of trace impurities and thus elucidating effectiveness of individual downstream processing steps.

Additionally, isotopic labeled peptides were applied to quantify single host cell proteins within the processed drug substance. The use of internal standard combined with mass spectrometric LC-SRM (selected reaction monitoring) is a reliable method for absolute quantification. The robust nature of this approach offers the technical potential for validation and usage as GMP release testing for biopharmaceuticals to address the purity. However, multiple challenges need to be solved, to use MS within a release testing.

NOTES:

P-133-T

Comparability Study of Infliximab Innovator and Biosimilars by Automated High Throughput Peptide Mapping Analysis and Intact Mass Analysis

Silvia Millan, Izabela Zaborowska, Craig Jakes, Sara Carillo, Jonathan Bones

NIBRT, Co. Dublin, Ireland

MAbs are the fastest growing class of biotherapeutics due to their high specificity, long serum half-life and ability to treat a wide range of ailments. As the patents expire, the development of biosimilars with similar quality, safety and efficacy profiles is expanding globally. Regulatory bodies worldwide have prepared guidelines to regulate their development receiving authorization based on an abbreviated regulatory application containing comparative quality, nonclinical and clinical data that demonstrate similarity to a licensed biological product. The demonstration of biosimilarity represents a significant challenge and is required to show the presence or absence of differences resulting from the manufacturing process by investigating the physicochemical and biological properties of biosimilars compared to the corresponding reference product (innovator). The analysis of entire mAb can provide information about the protein as a whole (mass, protein sequence, structural integrity, and PTMs with relatively large mass shift), but cannot determine the precise location of a PTM. Therefore, peptide mapping is a "gold standard" tool used to measure critical quality attributes (CQA's) by making the identification of particular modifications at specific domains easier. This study evaluates PTMs during enzymatic digestion of Infliximab drug product and biosimilars using a Magnetic Bulk resin option of SMART Digest™ kit on a KingFisher™ Duo Prime Purification System. The efficiency and reproducibility of the platform was evaluated with a specific focus on the determination of protein sequence coverage and identification of PTMs (deamidation, oxidation, lysine clipping, glycation and glycosylation). Supplementary high throughput intact antibody characterization is also described. Assessment of the primary and quaternary structure was achieved successfully by coupling the Vanquish Flex UHPLC system and high-resolution accurate-mass (HRAM) capabilities of the Q Exactive™ hybrid quadrupole Orbitrap mass spectrometer, providing excellent mass accuracy and highly sensitive results. BioPharma Finder™ Software was used to interrogate provided data sets.

NOTES:

P-134-W

A Software for Biopharmaceutical Routine Analysis from Discovery, Development to QC Work Under Regulated Conditions

Stuart Pengelley[1], Eckhard Belau[1], Anja Resemann[1], Heiko Neuweger[1], Guillaume Tremintin[2], Detlev Suckau[1]

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Support by chromatographic and mass spectrometric analytical techniques is an integral part of biopharmaceutical development and manufacturing. Throughout this range of applications involved, from originator characterization to clone selection, structure verification to stress response studies or release identity testing there is a requirement for fast analysis return times and minimized user intervention to generate analytical reports.

We developed a dedicated software package with client-server architecture supporting a workflow wizard-driven user-interface concept and optional support of work under regulated conditions (21 CFR part 11).

With the introduction of the workflow-orchestrated transfer of raw measurement data acquired on ESI instruments, BioPharma Compass 3.0 enhances the data integrity capabilities of the solution. Right after the acquisition, LC-MS datasets are transferred from the acquisition PC in an audit trail controlled workflow process. This prevents any uncontrolled manual user interference and streamlines data processing and storage. Raw data are thus never changed and stay available for further reprocessing.

NIST mAb reference material was used to verify several workflows including intact mAb or IdeS plus reduction-generated Fc/2, Fd and LC analysis for structure verification and glycan profiling. In addition, bottom-up workflows and top-down/middle down analyses were supported to target PTMs as well as artefactual modifications and to confirm the overall sequence.

Large numbers of samples/sequences were analyzed in batches with reports utilizing a traffic light reporting system facilitating user acceptance decisions on individual samples, thus minimizing analysis times and costs.

NOTES:

P-135-T

Monitoring Critical Quality Attributes Supporting Development of Biopharmaceuticals

Kerstin Pohl[1], Sibylle Heidelberger[2], Ferran Sanchez[3], Harini Kaluarachchi, Annu Uppal[4]

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A rapidly emerging trend is the application of mass spectrometry as a key application for identification and tracking of biopharmaceutical critical quality attributes. A particular challenge in implementing these approaches has been the complexity of mass spectrometry and limited informatics solutions to address these needs. Complicating this further is the need for implementable solutions which can be used effectively by non-expert users.

Several monoclonal antibody biotherapeutics were used for this study. Molecules were subjected to a standard digestion protocol. In some cases, antibody samples were subjected to stress conditions including oxidation agents, heat, and pH to investigate the impact of conditions on protein post translational modification and to verify the ability to the analytical approach to detect changes. Additionally, known components were added to some samples in order to mimic new components in these samples. In all cases, samples were separated using a reversed phase UHPLC separation and detected with a QToF instrument. Data was processed using dedicated biopharmaceutical software to detect and track sample components.

Both native and stressed monoclonal antibody samples were analyzed using reversed phase LS/MS/MS. Peptides were separated and detected to allow for accurate identification and tracking of species across samples. For the samples the extent of oxidation, deamidation was determined using dedicated software tools. Components were tracked across sample sets and plotted to easily track how critical quality attributes changed over time as a function of stress conditions. In addition, the presence and relative abundance of key glycoforms was also determined. Finally, samples were spiked with varying levels of known peptide to interrogate the ability of this method to detect new components in an untargeted manner. In all samples new components were detected at levels of 0.1% relative abundance or below.

NOTES:

P-136-W

Antibody Drug Conjugate Analysis using Automated Affinity Purification and Sensitive Intact Protein Based LC/Q-TOF Analysis

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Quantitation of proteins in biological systems is traditionally performed either by ligand binding assays (LBA) or multiple reaction monitoring (MRM). LBAs can be highly sensitive but do not provide information about the physical state of the biomolecules (i.e. mass) and can be affected by non-specific binding. MRM assays are sensitive, but surrogate peptides represent only a portion of the total protein. Both LBA and MRM assays may miss unexpected changes to biomolecules that can alter the efficacy and immunogenicity. We developed a workflow using automated affinity purification of antibody-drug conjugates (ADCs) from serum with streptavidin cartridges, followed by UHPLC separation coupled to a newly developed 6545XT AdvanceBio LC/Q-TOF providing a reproducible, sensitive and accurate quantitation method for bioanalytical analysis of intact proteins

NOTES:

P-137-T

In Depth Analysis of HCP Impurities in the NIST mAb Standard by 2D-LC-MS/MS

Miriam Reuleaux, Udo Roth, Annette Pieper

Sanofi, Frankfurt, Germany

Detecting and identifying residual host cell proteins (HCPs) in monoclonal antibody formulations is a key element for the process development of biotherapeutic proteins. In recent years, mass spectrometry is increasingly applied as an orthogonal method to the routinely aplied ELISA assays and provides valuable information on HCP content and identity. However, one of the major challenges with LC-MS based approaches is the huge dynamic range between the drug candidate protein and the residual HCPs during downstream processing.

This poster shows the benefits of two dimensional microflow chromatography (2D-LC) in combination with a hybrid orbitrap / linear ion trap mass spectrometer for maximizing the HCP coverage. With this approach, a total of 86 host cell proteins were identified in the NIST mAb, of which 36 have not been published before.

NOTES:

P-138-W

Robust and Sensitive Workflow for Qualitative and Quantitative Analysis of Intact Monoclonal Antibodies Using a MicroLC and TripleTOF Mass Spectrometry

Ferran Sanchez[1], Jason Causon[2]

[1]SCIEX, Alcobendas, Spain, [2]SCIEX, Warrington, United Kingdom

Protein biotherapeutics such as immunoglobulin G (IgG)-derived monoclonal antibodies (mAbs) are an attractive targeted therapy to treat an array of diseases like cancer, autoimmune disorders and infectious diseases. Understanding the primary structure, heterogeneity, and post-translational modifications of these biologics are essential to understanding function, developing novel therapeutics and ensuring product quality. LC-MS analysis has become an essential tool for the identification, characterization and quantification of intact mAbs and similar high-molecular-weight proteins. Here, we describe a robust and sensitive workflow using an M3 MicroLC-TripleTOF® 6600 mass spectrometer for qualitative and quantitative analysis of mAbs. Analytical LC-MS methods often deliver insufficient sensitivity. Microflow LC-MS, despite its inherent sensitivity advantage, has not been used extensively because the necessary off-line desalting and sample clean up can result in sample loss and long sample preparation cycles. The typical on-column desalting with a divert valve as used in analytical flow LC cannot be easily implemented in microflow LC, due to chromatographic dispersion at lower flow rates. Our method takes advantage of a different on-column desalting approach to decrease sample preparation time and increase throughput and sensitivity. Three different IgGs were used; results for all three mAbs showed quantitation with a high level of accuracy and a good coefficient of variation (CV) across 3 orders of linear dynamic range (0.1-100 ng). Successful characterization of the major mAbs glycoforms at levels down to 2.5 ng, on-column was achieved. This microflow LC-MS method allowed quantitation and characterization of intact IgG's with 5x more sensitivity compared to traditional flow LC-MS.

NOTES:

P-139-T

Comprehensive Analysis of Therapeutic Biologics by MS Spectrometer

Hsien-Yu Tsai, I-Ling Kou

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Biological drugs unlike small-molecule drugs are much more complicated and required comprehensive analytic methods to support due to the uncertainty of the manufacturing parameters in a living cell. The variations in biodrugs e.q. therapeutic antibodies are including potency, glycosylation, aggregation, charge variants and post-translational modification. Hence, characterization of biopharmaceutical product is essential as regulatory agency requested and also stated in the guidelines of International Conference on Harmonisation. Our analytic group has valuable experiences in successful analysis of regular antibody (trastuzumab), antibody-drug conjugates (trastuzumab emtansine), therapeutic protein (etanercept) and bispecific antibody in house. In this study, we present the characterization results of N and O glycosylation of etanercept by Waters Synapt G2-Si MS and the sequence of denosumab. Our data reveal there are 3 O-linked glycan and 17 N-linked glycan species on etanercept. The majority of N-linked glycan is G0F and G2FS1 glycoforms and the O-linked glycan is core 2 structures with mainly 2-3 linked sialic acids. The sequence of denosumab is DE novo sequenced and assembled by enzymatic digested peptide mapping. The sequencing results of denosumab are similarly to the CDR region searching form drugbank but with igg2 Fc format. In conclusion, the characterization of etanercept glycosylation and denosumab sequence from our results has an important role in the development of biosimilars and for the comparability test.

NOTES:

P-140-W

Determination of Higher Order Structure Comparability for A Highly Glycosylated Protein by Advanced HDX-MS Approaches

Shiaw-Lin Wu[1], Peter Li[1], Chen Li[1], Lionel Sison[2], Scott Li[2], Bernice Yeung[2]

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Effective correlations between a biologic drug's CQAs and higher-order structure are increasingly important in biopharmaceutical development. In these studies, we first designed HDX-MS methods for the detailed analysis of multiple stressed and un-stressed samples of Idursulfase, a highly-glycosylated therapeutic protein; and secondly, we developed and applied several HDX-MS data formatting and statistical analysis approaches to enable quantitative determinations of comparability among the different samples. Specifically, a high-resolution HDX-MS method was developed to achieve >95% sequence coverage of the protein in its native form for all samples, including 7 of 8 glycosylation sites containing a mixture of complex-type sialylated glycans, mannose-6-phosphate glycans, hybrid gylcans, and high-mannose glycans. Further, two different data analysis approaches were developed for assessing sample comparability. First, a "Butterfly Comparison" for comparative qualitative analysis of full sample sequences, as well as a quantitative "Similarity Scoring" approach to enable statistical comparisons of 6 key regions of the samples particularly related to glycosylation and the known active site of Idursulfase. In the Butterfly Comparison, the majority of the protein backbones were qualitatively similar, except for certain glycosylation regions in stressed samples. In the Similarity Scoring comparison, un-stressed DS and DP samples consistently met similarity score cut-offs and could be scored as "Highly Similar"; heat-stressed or de-phosphorylated samples fluctuated across defined cut-offs and were scored as "Similar"; and de-sialylated or de-sialylated plus de-phosphorylated samples consistently failed to meet the similarity scores, and were categorized as "Not Similar". By comparing key regions of each sample against a reference lot, statistical similarity could be achieved within a 95% confidence interval in multiple repeat measurements. From these results, desialylation appears to have the most destabilizing effects on the higher order structure of Idursulfase.

NOTES:

P-141-T

Advances in IgG2 Disulfide Isoform Characterization using MALDI and In-source Decay Fragmentation

Anja Resemann[1], Lily Liu-Shin[3], Fang Wang[3], Adam Fung[3], Guillaume Tremintin[2], Detlev Suckau[1], Gayathri Ratnaswamy

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IgG2 antibodies are produced as a mixture of three disulfide isoforms, namely A, A/B, and B. The distribution of disulfide isoforms is a critical product quality attribute as each disulfide configuration impacts higher order structure, thermal stability, and binding efficiency. RP-HPLC is the current standard to resolve and quantify the distribution of IgG2 disulfide isoforms, however, this assay involves optimization efforts for each IgG2 as the species are not baseline resolves. We have developed a highly automated and semi-quantitative approach for characterizing complex mixtures of isoforms via LC-MALDI-TOF/TOF of non-reduced digests. This poster presents the identification and validation of disulfide-bonded peptides that are unique to each disulfide isoform. The m/z of each peptide can be added to a database for future automated software quantitation of heterogeneous disulfide isoform mixtures.

NOTES:

P-142-W

Fast Analysis of Antibody Glycosylation in Cell Culture Samples

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The glycosylation of IgGs is a critical quality attribute (CQA) and thus needs to be analyzed during the cell line and bioprocess development. The current analytical methods demand rather high amounts of purified protein and use sophisticated protocols and equipment.

In this study we present data from several therapeutic IgGs as well as research material with different glycan profiles that were generated with bead-based microplate assays. This new technology uses capture beads in combination with fluorescence labeled plant lectins to detect N-Glycans (fucose, galactose, mannose and sialic acid) in a high throughput 384-well plate format. The sample preparation protocol is simple and does not require purified protein. One lectin can be assayed per well offering the possibility to test several lectins and multiple samples on each microplate.

The results demonstrate that PAIA assays are capable of quickly detecting differences in glycan patterns of different antibodies.

The different IgGs exhibit lectin binding profiles that correspond to the differences in glycosylation. High abundance of glycans leads to high binding rates of the lectin for the respective glycan. It was also demonstrated that Fc glycans are only accessible when denaturation of the IgG has been performed. Without denaturation only the Fab glycans are detectable, e.g. in Erbitux.

In addition, it was shown that the lectin binding rates for glycan variants of the same IgG correlate very well with the results obtained from 2-AB-UPLC.

We believe that bead-based assays with lectins have a great potential for monitoring product quality early in the development process.

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P-143-T

Fluorescence-based Bioprocess Monitoring: A Case-study of Viral Vector Production in Insect Cells

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Insect cells, particularly in association with the baculovirus expression system (IC-BEVS), are emerging as an important alternative platform for biotherapeutics manufacturing, with multiple products, mostly vaccines and gene therapy vectors, already approved for human and veterinary use and many others in clinical trials. In this work, we use the IC-BEVS platform to produce recombinant adeno-associated virus (AAV), one of the vectors of choice for gene therapy. Here a critical process parameter is the time of harvest (TOH), especially given the lytic infection that increases proteolytic activity and leads to degradation of product quality.

We have shown previously that fluorescence spectroscopy is a simple approach that can be successfully used in real-time monitoring of cell and recombinant antibody concentration in CHO cell cultures, either using 2D fluorescence maps or synchronous fluorescence scans. With insect cell culture, the medium has a stronger background fluorescence signal and the monitoring platform becomes more sensitive to changes in data processing. Here we present a benchmark of different data pre-treatment combinations, including data normalization and smoothing, as well as different predictive modelling algorithms, from partial least squares (PLS) regression to support vector machines (SVM).

Our results show that fluorescence can be used to monitor AAV production in IC-BEVS and that with a relatively easy optimization step we were able to improve prediction of cell concentration and AAV titer. Applying the same rational to relevant product quality attributes like *in vivo*therapeutic efficacy or to process parameters with impact downstream (e.g. intra or extracellular product localization) this platform can be expanded and applied to determine, in real-time, the best time of harvest for different products in the IC-BEVS platform.

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P-144-W

Rapid N-Glycan Sample Preparation Workflows for Liquid Chromatography and Capillary Electrophoresis Platforms

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The structure of N-linked glycans can play a critical role in the pharmacology of therapeutic proteins, potentially affecting immunogenicity, pharmacokinetics and pharmacodynamics. This makes the characterization of N-glycans an essential part of the biotherapeutic development process. N-Glycans do not contain a chromophore or fluorophore suitable for online detection with standard liquid chromatography (LC) or capillary electrophoresis (CE) separation techniques. Typically, enzymatically-released glycans must be derivatized with a tag to allow for fluorescence detection prior to analysis, a process that often requires numerous hours or days to complete. ProZyme Gly-X chemistry releases N-glycans with PNGase F in 5 minutes and released N-glycans are labeled with a choice of fluorescent dye: InstantPC, 2-AB, InstantAB, or InstantQ. The entire sample preparation protocol can be completed in less than 1.5 hours for instant dyes or 2.5 hours for reductive amination dye (2-AB), and the protocol can be automated on common laboratory liquid handlers. Three N-glycan dye options are discussed.

InstantPC is an N-glycan dye that allows for separation with hydrophilic interaction liquid chromatography (HILIC) with fluorescence (FLR) detection for relative quantitation of glycan species. The increased fluorescence signal of InstantPC in conjunction with its favorable properties for mass spectrometry (MS) allows for the detection of low abundance glycans.

The fluorophore 2-AB (2-aminobenzamide) has been used to generate labeled N-glycan data for over 20 years and is well established in many laboratories. Traditionally, labeling with 2-AB requires sample drying prior to the labeling reaction, a step that is eliminated with Gly-X 2-AB Express on-matrix labeling.

Finally, InstantQ is a charged N-glycan dye that facilitates separation of labeled N-glycans on the ProZyme Gly-Q CE system, using a run time of 2 minutes per sample with LED-induced fluorescence detection (LEDIF). The Gly-Q system enables relative N-glycan quantification for up to 96 samples within a single workday.

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True Automation or High Throughput

P-145-T

Library Based Automated Glycan Identification by Mass Spectrometry in Combination with Fluorescence Quantification

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The glycomes of mammalian and even insect or plant proteins are quite complex. However, LC-MS based methods are powerful tools to cope with the demands of the characterization of complex glycan mixtures. The effort to process the generated analytical raw data is tremendous, therefore manual evaluation of FLD-MS/MS data of complex glycan mixtures is ineffective with respect to time, precision and reproducibility.

In the present study we used proteins comprising different numbers of glycosylation sites (2 - 8): Trastuzumab, NIST mAb reference standard, FSH, EGFR. Products result from different rodent and human cell lines. N-glycans were released enzymatically from the proteins by N-glycanase F and subsequently labeled with fluorescent dyes. Data were recorded by using an HILIC-UPLC-FLD-ESI-QTOF MS/MS system (hydrophilic interaction ultra-performance chromatography with fluorescence detection coupled to electrospray ionization quadrupole time-of-flight tandem mass spectrometry). Recorded data were automatically processed (GlycoFiler, Glycotope GmbH) including deconvolution of MS/MS spectra and matching to a glycan library for structure assignment, integration of fluorescence peaks for quantification of glycan structures, calculation of glycan parameters of biological interest, comparison to standards, reporting to summarize all data in a certificate of analysis.

The absolute number of N-glycan structures automatically detected and quantified ranged from 46 (Trastuzumab) to 368 (EGFR) depending on the number of N-glycosylation sites rather than the expression cell line. The number and quality of structures automatically assigned for the NIST mAb reference standard is fully comparable to other methods. Increasing complexity of the N-glycosylation pattern does not compromise speed of the automated data processing ranging from 2 – 4 minutes. Importantly no compromise with respect to quality of the analyses was observed in comparison to manual data evaluation: The non-assigned peak area ranged from 1-2%, false positives were below 2%. Replicate analyses resulted in standard deviations of < 2%.

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P-146-W

Development of Oligosaccharide Labelling with Fluorescent Markers Applying Heterogeneous Catalysis in Flow

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Glycoproteins are important in biological processes. They facilitate receptor-ligand and cell-cell interactions, modulate immunogenicity, stabilize the quaternary and tertiary structures of proteins, just to mention a few. Their glycan moiety is generated via post-translational modification. The glycosylation pattern ought to be analyzed in great detail for both the biomedical and biopharmaceutical fields through quantitative analysis of the oligosaccharide chains, i.e., the glycan parts of the glycoproteins. Carbohydrates lack of UV- or fluorescently active groups, therefore, are often labelled with fluorescent markers, usually by reductive amination. The resulting labelled glycans can be separated and analyzed then by e.g. capillary electrophoresis with laser induced fluorescent detection (CE-LIF). Although reductive amination is achievable with cheap hydrogenating agents (picoline borane, sodium cyanoborohydride, etc.), the process is usually time consuming and may generate hazardous side products. Heterogeneous catalytic processes in flow can speed up such transformations without the generation of such side products.

As a model, we optimized the reductive amination reaction of a disaccharide with a UV-active marker applying heterogeneous catalysis in a commercial flow reactor. We have screened many heterogeneous catalysts and optimized the reaction conditions (temperature, inlet pressure and residence time) and we succeeded to reduce the reaction time to minutes. In continuation, we have tested the optimal oligosaccharide-labelling conditions with fluorophore markers and applied capillary electrophoresis to separate the labelled sugars.

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P-147-T

Fully automated GlycanAssure AutoXpress N-Glycan sample preparation for N-glycan Analysis on UPLC and CE

Bérengère Francois

TF, St Genis les Ollières, France

The glycans or polysaccharides attached to proteins after protein post-translation modification play critical roles in eukaryotic cell protein functions, such as protein assembling and folding stability, signal transduction, ligand binding, protein interaction, etc. In the therapeutic immunoglobulin, the N-glycosylation on amide nitrogen of asparagine is a critical quality attribute in the pharmacology, affecting immunogenicity, pharmacokinetics and pharmacodynamics. A great challenge for glycan analysis is glycan sample preparation since many sample preparation procedures are not effective, laborious and time-consuming.

We have developed a fully automated workflow for N-glycan sample preparation using Thermo Fisher GlycanAssure reagents. The magnetic beads-based, automated procedure provides streamlined process for glycoprotein denaturation, deglycosylation, APTS labeling of the released glycans, and removal of the excess free dyes. The hand-on time for processing 13 samples is as short as ~5 minutes because the operators only need to add their glycoprotein to micro-tubes before they start the automated run. The setup of the instrument is simple and easy as all of the reagents are prefilled in the prefilled cartridges, which are stable at -20ºC for at least one year. The total sample preparation time on the instrument is 1 hour and 45 min and the APTS labeled glycans are ready to be analyzed on UPLC and CE instruments. The fully automated platform is demonstrated to result in high quality of glycan profiles by UPLC and CE. The glycan profiles of NIST mAb, human serum IgG, RNase B and Fetuin are comparable to the GlycanAssure manual kits, as well as comparable to other glycan sample preparation kits on the market.

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P-148-W

Rapid MALDI-MS Assays for mAb Development, Production Control and Release

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During biopharmaceutical development (e.g., clone selection) and production (e.g., rapid release identity testing) there is a requirement for fast analysis return times to accelerate decision making and reduce costs. We utilized rapid protein digest methods and integrated MALDI-TOF sample analysis with a software workflow to compare measurements against a reference attribute profile. This comparison was used in clone selection workflows to screen glycan profiles in intact Fc-domains, and to provide antibody identities rapidly based on differentiating abundant peptides in peptide mass fingerprints. Protocols were developed to achieve analysis return times from intact antibody sample to automatic identity confirmation based on trypsin/Lys-C digests within 15min and for Fc-glycoprofiling within 30min. Spectra acquisition and processing were completed in less than 10 sec/sample.

Six commercially available mAbs were used in this study.

For clone selection they were IdeS digested, diluted into matrix for Fc-glycan profiling using MALDI-MS. Major glycans were assayed by direct profiling of the intact Fc-domain. Multiple attributes such as the match of the glycan profile with a reference profile or the test for G0F as being the base peak glycan were reported in BioPharma Compass 3.0 (BPC, Bruker) providing multiple data points for decision making of which clones to select for further rounds of screening.

For rapid release identity testing MALDI spectra were analyzed in BPC. The quality of the MALDI peptide mass fingerprints achieved from all tested antibody digests was high (average: 70% sequence coverage for LCs and 40% for HCs) and enabled assaying mAb identities largely based on the differentiating peptides, i.e., peptides derived from the variable N-termini of ~120 residues of the antibodies.

These workflows will be compatible with high throughput modes of operation with proper lab automation. With user defined acceptance criteria, software workflows were developed that provide the required information rapidly and automatically.

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P-149-T

High-precision, Automated Peptide Mapping of Proteins and mAbs

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The biopharmaceutical industry continues to develop protein-based biotherapeutics in increasing numbers. Due to their complexity and biotechnological production, there are many attributes that need to be analyzed to guarantee their safety and efficacy.

Peptide mapping is used to measure several critical quality attributes (CQA) required for the characterization of any biotherapeutic protein. The analysis is used to confirm that the correct sequence has been expressed for the protein and to check for post-translational and chemical modifications.

Mass spectrometry (MS) is coupled to liquid chromatography (LC) for peak identification and confirmation of the sequence. However, many quality control (QC) methods use detection by ultraviolet (UV) absorption only after the peaks identities have been confirmed.

Trypsin is the enzyme most commonly used for proteolytic digestion due to its high specificity. Although a widely accepted technique, in-solution trypsin digestion protocols required for sample preparation are labor intensive and prone to manual errors. These errors affect the quality of the analytical data compromising the ability to reproducibly characterize a protein to the required standard. In the most critical cases where workflows only employ UV detection without confirmation by MS, robust and stable sample preparation and separation methods are critical. The digestion must be reproducible, and chromatography must be extremely stable to allow unambiguous peptide identification based on chromatographic retention time.

In this poster, we detail the automated peptide mapping of cytochrome c, recombinant somatotropin, and infliximab drug product. These proteins were chosen to investigate the applicability and reproducibility of the automated digestion protocol and subsequent analysis.

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P-150-W

From Sample to Report: Automation of Intact Protein Characterization by Mass Spectrometry in Early Research

Hans Voelger

Roche Diagnostics GmbH, Penzberg, Germany

When biopharmaceutical projects enter development for clinical trials, the amino acid sequence of the therapeutic protein is fixed and remains unchanged throughout clinical testing. In addition to that, the molecule has already been extensively characterized during discovery and pre-clinical early research. Hence, typical side products or relevant modifications of the drug candidate are known. These two factors facilitate or enable setting up an automated workflow for mass spectrometry analysis of larger numbers of samples, i.e. during process development.

In contrast to this, the inherent variation of the protein samples in early research often prevents automated data evaluation of these samples and thereby critically limits throughput. This can lead to significant bottlenecks when highly automated processes for analysis of bispecific proteins in multiple formats, for example CrossMabs, or asymmetric formats should be set up.

In this talk, pRED LMR's approach to overcome this problem by linking our in-house database for protein registration with our specifically tailored in-house application for data evaluation of intact protein mass spectrometry data will be described. This automated solution ideally supports pRED LMR's 'screening in final format' efforts.

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P-151-T

Consideration of a High-Throughput (HTP) N-glycan Analysis Method for Process Characterization Studies

Jutta Vogelmann, Noreen Rippolz

Sanofi, Frankfurt am Main, Germany

N-glycosylation of biologics is a pivotal quality attribute influencing the efficacy of the molecule. Glycosylation patterns influence not only solubility of a molecule it could have also an impact on protein folding, effector functions and pharmacokinetics/clearance behavior. Further a few glycan species might be immunogenic and are therefore considered as a critical quality attribute.

Process stability is also reflected in a reproducible N-glycosylation pattern. This explains why N-glycan analytics is also an essential part of process characterization (PC) studies and an emerging number of samples have to be analyzed. The use of release methods for PC studies poses a challenge regarding time, cost and resource management.

Having established a HTP-N-glycan-analysis method showing excellent bridging data between the HTP-/release -method brings up the question about its possible use for PC studies. Considering the use of a HTP-N-glycan-analysis method changes requirements of the HTP-method. The method needs to be fast, robust, reproducible, cost efficient, sensitive and precise. The generated data needs to be highly comparable to data generated by the release method.

This poster presents an overall view on different aspects that have to be considered when a HTP-method is applied for process characterization studies.

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Other Processes and Technologies

P-152-W

Evaporative Fluorophore Labeling, An Effective Novel Tool in Glycosylation Analysis

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As analytical glycomics became to prominence, newer and more efficient sample preparation methods are being developed. Albeit, numerous reductive amination-based carbohydrate labeling protocols have been reported in the literature, the preferred way to conduct the reaction is in closed vials. Here we report on a novel evaporative labeling protocol with the great advantage of continuously concentrating the reagents during the tagging reaction, therefore accommodating to reach the optimal reagent concentrations for a wide range of glycan structures in a complex mixture. The mild conditions of the evaporative labeling process minimized sialylation loss, otherwise representing a major issue in reductive amination-based carbohydrate tagging. The approach was applied in conjunction with an improved PNGase F digestion mediated N-glycan release process, where the effect of enzyme/protein ratio was optimized. In addition, balanced dispersion of dry samples was accommodated by supplementing the low volume labeling mixtures (several microliters) with extra solvent (e.g., THF). Evaporative labeling also makes glycan labeling automation-friendly, suitable for standard open 96 well plate format operation.

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P-153-T

Novel Enzymes for O-glycan Analysis

Malin Mejare, Fredrik Leo, Rolf Lood, Maria Nordgren, Helén Nyhlén, Jonathan Sjögren, Stephan Björk, Fredrik Olsson

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The degree and composition of glycosylation of proteins are critical for a wide range of biological processes. Alterations of the glycan structures may impact the function and effect of the glycoprotein and thus, close monitoring of the glycan profile is required during development and manufacturing of biopharmaceuticals. The analyses of O-glycans have suffered from lack of specific enzymes and there is a great need for novel tools.

An O-protease (O-glycan specific endoprotease) was discovered in *Akkermancia muciniphila.* This enzyme recognizes the galactose residue of core 1 and core 2 O-glycans and digests the peptide backbone N-terminally of the Ser and Thr residues, carrying the glycan, without any additional amino acid sequence specificity or limitation. The inherent site heterogeneity of O-glycosylation together with the O-protease specificity for O-glycan structures generate overlapping peptides that can be used to map O-glycosylation sites. The activity of the enzyme is increased after asialylation, and for some analytical techniques, removing the remaining core structure is advantageous. The sialic acids are effectively removed using a novel sialidase mix from *Akkermancia muciniphila* acting on α2-3, α2-6 and α2-8 linked sialic acids. Furthermore, the use of a new enzyme, an endo O-glycosidase from *Streptococcus oralis* with the ability to hydrolyze asialylated core 1 and core 3 O-glycans of glycoproteins at native conditions, facilitates O-glycan analysis.

Workflows and analytical strategies in LC/MS for site-specific O-glycan characterization of proteins with these new enzymatic tools were developed and demonstrated by analyzing etanercept, an Fc-fusion protein with a highly O-glycosylated hinge region.

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P-154-W

Line of Sight to the Biopharmaceutical Control Strategy: A Plan for Commercial Success

Tim Schofield

GlaxoSmithKline, Rockville, MD USA

Line of sight to the biopharmaceutical control strategy should begin with the development of the quality target product profile (qTPP) and should anticipate commercial manufacturing conditions and control. Process studies and small-scale manufacturing experience yield meaningful insights into the appropriate control of the process and the product. Key to successful development leading to commercial success is a scientifically sound basis for specifications. With this understanding process development works closely with analytical development to deliver to a common target for quality. Process controls and formulation components are put in place to manage to these targets. When process and formulation controls are insufficient to ensure conformance to quality targets, analytical controls are used to manage supply of product to the market. The analytical controls should include opportunities to assess shifts and drifts, and to gain further insight into the process. A strong continued process verification (CPV) plan can provide further insight into the process and be a basis for continual improvement.

The biopharmaceutical control strategy should also include tools which manage quality in the course of anticipated process and analytical changes. Thus, plans should be devised to ensure continued quality after introduction of new raw materials, over equipment changes and through typical method changes such as standard qualifications and transfers. Those plans might be filed as part of the license application and managed through a robust quality system. Finally control of the product extends beyond release to the end of shelf life. Intermediates and final product might be released on the basis of stability and method information, and these aspects of product control should be verified throughout the product lifecycle.

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P-155-T

Evaluation of the Most Important Parameters of The Experimental Design In Ligand Binding Assays

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During all phases of drug development ligand binding assays play a crucial role. Therefore, high quality methods for determining binding properties are necessary. The basic principle of the most ligand binding assays is mostly the same. The change of a defined chemical response is measured in a range from ligand free analyte to the concentration of fully saturated analyte. In the simulated examples this chemical response is the change of the electrophoretic mobility in CE. The dissociation constant is calculated by nonlinear fitting of the chemical response and the corresponding ligand concentrations to the binding equation. In dependence of changing the most important four experimental parameters the uncertainty of the dissociation constant's determination was evaluated statistically by Design of Experiments (DOE). The DOE revealed combinations of measurement parameters in the experimental space where the overall error increases dramatically. The minimal values of each parameter should be higher than 0.05 for the maximum response range (MRR; maximal observed change in the chemical response) and 0.25 for both the data point range (DPR) and the data point position (DPP). More than one parameter near the lower threshold in the same measurement leads to an exorbitant amplification of the error. The number of different data points does not influence the uncertainty of the KD determination very strongly when there are at least a number of five measuring points (NoDP). The precision of the ligand binding assay can be maximized when the measurement conditions are in accordance with the following aspects. The MRR should be greater than 0.50. The DPR should cover at least 40 % (DPR > 0.40) of the binding hyperbola and the optimal data point positions should cover the middle to the upper parts of the binding-curve (DPP 0.50 – 0.80).

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P-156-W

Overcoming the Challenges of Biosimilar's Development with the Latest in High Field NMR with 1D PROFILE and 2D HSQC Analysis

Jason Wood

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Biologics drug development has reached a mature state and continues to be a multi-billion dollar a year market for many drug companies. However, many of these biologics are beginning to lose patent protection globally and, as such, new biosimilars of these drugs are starting to enter the market.

In this poster we discuss the latest advancements in high field NMR applications to the analytical challenges facing innovators and biosimilars characterization labs. Innovations in hardware, software and application specific workflows will be highlighted including several biotech-relevant case studies. In particular, we will be highlighting: 1) the latest in high-field NMR workflows, 2) simplified higher order structure determination (1D Profile), and 3) rapid, NMR-based determination, of ideal growth conditions during manufacturing (2D HSQC).

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Late Breaking

LB-01-T

Quality Methods by Design

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During the past years we have developed a way of working within Analytical Development that aims for delivering the right method at the right time. A process was implemented focusing on customer alignment, collaboration, risk management, multivariate design of experiments and method life cycle management. This resulted in high quality analytical tests to support process development and ensure quality and safety of our products.

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LB-02-W

High Throughput Multi-attribute Monitoring of Antibody Drug Conjugate Monoclonal Antibody Intermediates

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Antibody-drug conjugates (ADCs) are a novel class of therapeutic protein comprised of a target specific antibody covalently linked to a highly-potency cytotoxic drug payload (1). During manufacture, various product variants may form which include, but are not limited to; thiol modifications at introduced cysteines (intended for site-specific, cysteine mediated conjugation), modifications to residues in the complementary-determining region (CDR), reduction of intrachain disulphide bonds, and variation in glycoform profile of the Fc domain.

Modifications to quality attributes (QA) can contribute to structural heterogeneity that may influence bioactivity, stability, or pharmacokinetics and pharmacodynamics(2). Regulatory agencies strongly advocate a Quality by Design (QbD) approach, of which identifying, monitoring, and controlling these quality attributes, are crucial components. Traditionally, each quality attribute would require its own analytical method, consuming valuable time and resource.

Here we describe a high-throughput multi-attribute monitoring (MAM) method which uses targeted mass spectrometry to monitor and quantitate a number of quality attributes in a single 12-minute method. We share development of an MAM assay for site-specific ADC antibody intermediates, comparison with traditional and orthogonal methodology, and its application to support product and process understanding.

This method provides an elegant solution to streamline the monitoring of quality attributes that can be employed at any stage of process development. The high-throughput and multiplexed nature of the MAM assay reduces the testing burden of monitoring multiple quality attributes, enabling enhanced product and process characterization with a limited time and resource.

Refs:
1)Dimasi, et al; molecular pharmaceutics, 2017
2) Xu, et al; mAbs, 2017

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