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Be confident

Characterization from discovery to QC

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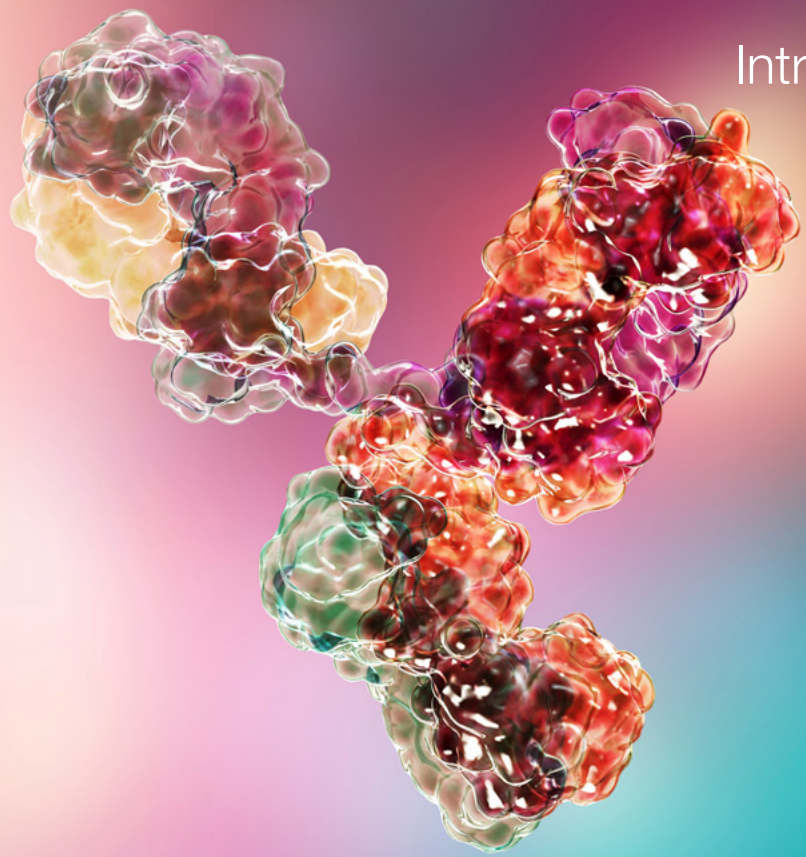
Introduction



Are you confident that your analytical solutions meet all your needs in the discovery and development of next-generation medicines?

You need a partner who has the expertise and innovative solutions for every step of your biopharmaceutical journey from discovery to quality control.

Thermo Fisher Scientific can provide solutions to your characterization challenges with exceptional separation, reproducibility, and high-quality data providing ever deeper insights in a compliance-ready data environment.

In the current climate biopharmaceutical companies are compelled to be more efficient and productive in delivering results without compromising quality. At the same time, technological advancements and innovations are available to improve operational efficiency and performance. Thermo Fisher Scientific has the cutting-edge expertise and technologies in the characterization and purification of biologics from discovery to quality control. Thermo Fisher Scientific is a leader in developing the latest methodologies and solutions to address your challenges in the biopharmaceutical industry.

This BioPharma Applications Compendium provides the latest methodologies and analyses, including peptide mapping, subunit, charge variant, host cell protein (HCP), glycan, antibody-drug conjugate (ADC), aggregate, oligonucleotide, and multi-attribute method (MAM) for the characterization, purification, and comparison of biologics. These techniques enhance sensitivity, reproducibility, productivity, and efficiency for rapid workflows in your laboratory and manufacturing environments.

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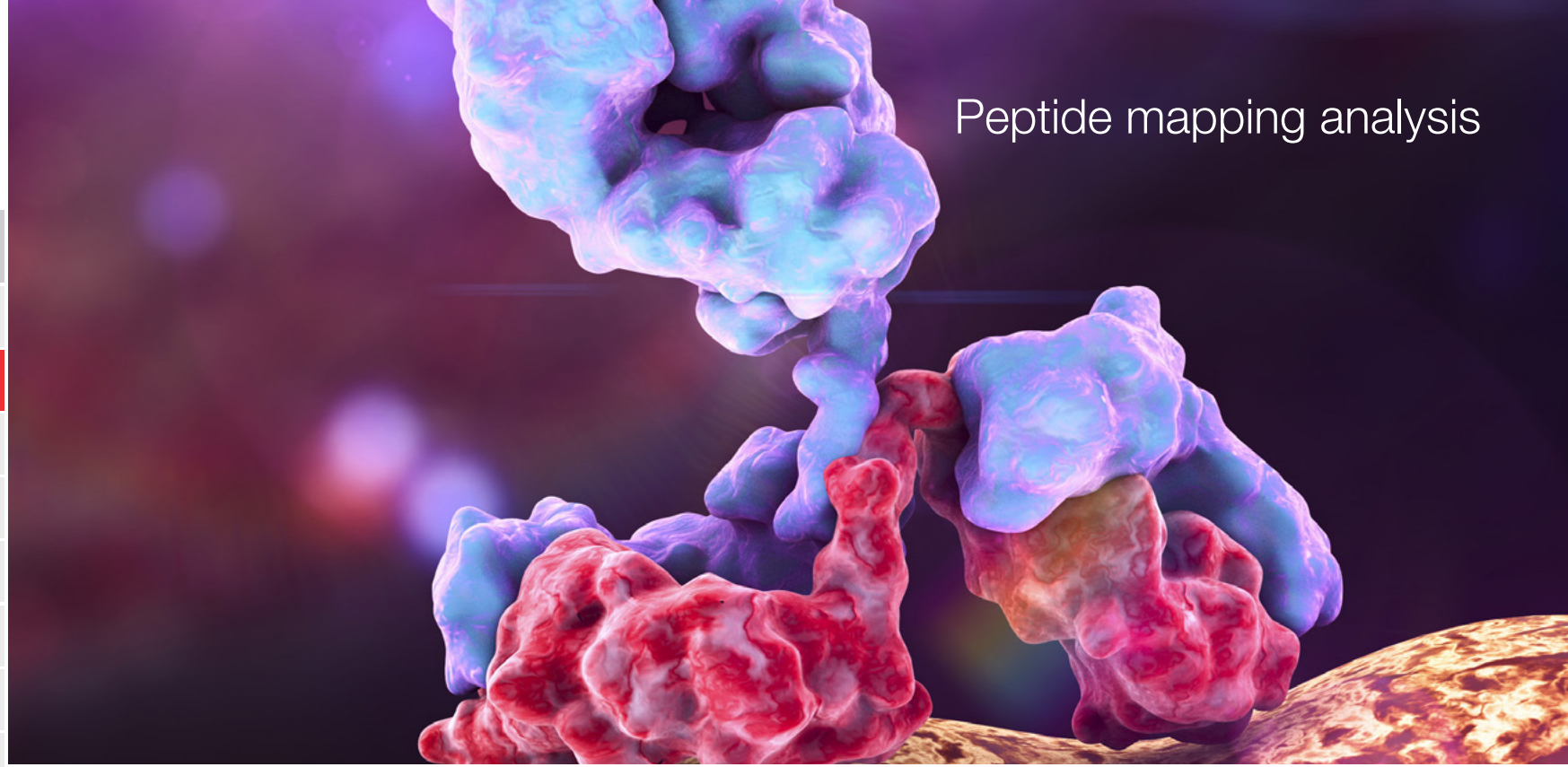

Peptide mapping analysis

Peptide mapping is a critical tool for biopharmaceuticals and has become a widely used method for comprehensive characterization of proteins, providing information on post-translational modifications (PTMs), sequence, degradation products and stability. Typical peptide mapping workflows involve enzymatic digestion followed by LC-UV, LC-MS, CE-MS or direction infusion MS analysis.

The use of liquid chromatography in combination with UV detection is common in stability studies, process control, quality control to extrapolate important attributes of the peptide sequence from the chromatogram. For more in-depth protein mapping characterization, high-resolution accurate mass (HRAM) mass spectrometry is needed to clearly resolve the large number of peptides and peptide variants in such workflows, as well to provide confident assignment of peptide identifications.

The high-resolution, accurate mass of the Orbitrap family of mass spectrometers, combined with various fragmentation capabilities and the selectivity of MS/MS, provides a superior range of MS options for in-depth peptide mapping characterization applications. High speed automated peptide mapping can be performed using Thermo Scientific™ BioPharma Finder™ software, providing in-depth profiling of biotherapeutics. Thermo Scientific sample preparation products, columns and chromatography systems offer a complete range of solutions for peptide mapping needs as demonstrated in the following application notes.

To learn more, visit Thermo Scientific Peptide Mapping Learning Center

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Peptide mapping analysis

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Confident Monoclonal Antibody Sequence Verification by Complementary LC-MS Techniques

Amy Farrell,[1] Sara Carillo,[1] Jonathan Bones,[1] Kai Scheffler,[2] and Ken Cook,[3]
[1]National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland; [2]Thermo Fisher Scientific, Germering, Germany; [3]Thermo Fisher Scientific, Hemel Hempstead, UK.

Overview

Due to the inherent complexity of monoclonal antibody (mAb) therapeutics, regulatory agencies require comprehensive characterization of mAb features to ensure product quality, safety, and efficacy. Primary sequence verification is an important characterization step for therapeutic proteins and is frequently performed using peptide mapping methods. The protein is treated with a protease (e.g., trypsin) to produce a series of peptides which are separated, detected, and analyzed by liquid chromatography – tandem mass spectrometry (LC-MS/MS), and then interpreted using bioinformatics software. In addition, peptide mapping enables the identification and relative quantitation of post-translational modifications (PTMs), such as deamidation, oxidation, and glycosylation. Confirmation of primary structure is also critical for the characterization of mAb biosimilars, as biosimilars must have an amino acid sequence that is identical to the reference drug product in order to achieve regulatory approval in Europe and the United States.

This application note demonstrates the benefits of primary sequence identification and shows the application of multiple, orthogonal techniques in characterization. Furthermore, it displays the importance of investigating primary protein sequences at different domains and generating and analyzing data produced by high-resolution accurate mass spectrometry techniques with a high level of certainty.

Key points

- Increased information using multiple, orthogonal characterization methods for therapeutic protein primary sequence verification
- Thermo Scientific™ MAbPac™ RP columns for intact and middle-up applications, offer good separation with minimal carryover
- The Thermo Scientific™ Q Exactive™ Plus and Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometers enable high-resolution accurate mass MS analysis of monoclonal antibodies on the intact, subunit, and peptide levels with high confidence.

Instrumentation

- Thermo Scientific™ Vanquish™ Flex Binary UHPLC system
- Q Exactive HF Hybrid Quadrupole-Orbitrap mass spectrometer
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer

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Peptide mapping analysis

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An Automated High-throughput Workflow for Peptide Mapping to Monitor Post-translational Modifications of Monoclonal Antibodies

Silvia Millán-Martín, Craig Jakes, Giorgio Oliviero, Sara Carillo, and Jonathan Bones, Characterisation and Comparability Laboratory, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

Post-translational modifications are the covalent and generally enzymatic modification of proteins during or after protein biosynthesis. PTMs affect structural and functional aspects of therapeutic proteins, and the effects of heterogeneity can be detrimental. Many approved or in-development therapeutic proteins bear at least one or more PTMs. Furthermore, variants of proteins produced for medicinal purposes can occur during production, purification, and storage, and can impact the activity and stability of the biotherapeutic. Peptide mapping is commonly used in the biopharmaceutical industry to establish product identity by confirming the primary structure of a product. It is used for the initial "proof of structure" characterization of the desired amino acid sequence and characterizes PTMs, supporting bioprocess development and clinical trials.

This application note reports on the benefits of peptide mapping techniques of the five top-selling monoclonal antibodies (Bevacizumab, Cetuximab, Rituximab, Trastuzumab, and Adalimumab) using the magnetic Thermo Scientific™ SMART Digest™ Trypsin Kit as the bulk resin option on the Thermo Scientific™ KingFisher™ Duo Prime purification system. The study focuses on the reproducibility protein sequence coverage and identification of PTMs.

Key points

- Simple and rapid protein digestion for a peptide mapping workflow of different monoclonal antibodies (chimeric, humanized, and fully human) in less than two hours
- Excellent recovery of samples with great reproducibility and efficiency with less hands-on time
- High confidence in results with excellent quality data sequence coverage and low observed levels of sample preparation-induced post-translational modifications

Instrumentation

- KingFisher Duo Prime purification system
- Vanquish Flex Binary UHPLC system
- SMART Digest Trypsin Kit, Magnetic Bulk Resin option
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- Thermo Scientific™ Nanodrop™ 2000 Spectrophotometer

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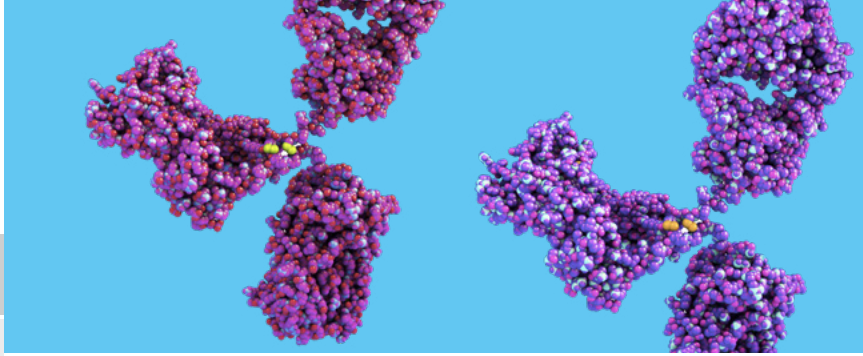

Comparability Study for the Determination of Post-translational Modifications of Infliximab Innovator and Biosimilars by Automated High-throughput Peptide Mapping Analysis

Silvia Millán-Martín, Izabela Zaborowska, Craig Jakes, Sara Carillo, Jonathan Bones, Characterisation and Comparability Laboratory, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

In the development and production of biosimilars, their quality, efficacy, and safety profiles must show similarity to the reference product (innovator). The similarity between a proposed biosimilar product and the reference product can be affected by many factors. Monoclonal antibodies exist as a mixture of heterogeneous variants due to post-translational modifications that arise during cell culture, purification, and storage. Some of these modifications can alter biological activity, drug metabolism and pharmacokinetics (DMPK), and immunogenicity and thus may pose risks to patients. The biopharmaceutical industry needs fast and robust analytical platforms to meet regulatory requirements in the Biologics License Applications (BLA) process.

Extensive analytical testing platforms are needed for in-depth characterization and to ensure product stability, proper in-process controls, safety, and efficacy. This application note presents the benefits of the Magnetic SMART Digest method to perform a comparability study of PTMs for infliximab innovator with its biosimilars. It focuses on the determination of protein sequence coverage and identification of PTMs, including deamidation, oxidation, lysine clipping, glycation, and glycosylation. This efficient approach involves using the KingFisher Duo Prime purification system, high-resolution accurate-mass (HRAM) capabilities of the Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer, and chromatographic separation with the Thermo Scientific™ Acclaim™ VANQUISH™ C18 UHPLC column on a Vanquish Flex Binary UHPLC system.

Download Application Note 21849

Key points

- Rapid, automated digestion leading to highly reproducible results for innovator and biosimilar comparability studies with less hands-on time
- Simple reproducible protein digestion with minimal user intervention for peptide mapping analysis
- High confidence in workflow results with excellent quality data; excellent coverage and low levels of post-translational modifications (except for M34+oxidation site where noticeable levels were observed)

Instrumentation

- KingFisher Duo Prime purification system
- Vanquish Flex Binary UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- Nanodrop 2000 Spectrophotometer

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Peptide mapping analysis

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Comparability Study for the Determination of Post-translational Modifications of Biotherapeutic Drug Products and Biosimilars by Automated Peptide Mapping Analysis

Silvia Millán-Martín, Izabela Zaborowska, Craig Jakes, Sara Carillo, Jonathan Bones, Characterisation and Comparability Laboratory, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

In accordance with regulatory requirements, a biosimilar is a biotherapeutic that must have the similar quality, efficacy, and safety as the reference product (innovator). In the characterization of a biosimilar, methods are required to demonstrate the presence or absence of differences resulting from the manufacturing process. This is done by investigating the physicochemical and biological properties of the biosimilar molecule compared to the corresponding reference product (innovator).

In this application note, peptide mapping is used to evaluate the similarity of PTMs in rituximab and trastuzumab and their respective biosimilars. To automate and speed up the method, the Magnetic SMART Digest Kit on a KingFisher Duo Prime purification system was used. The efficiency and reproducibility of the platform was evaluated with a specific focus on the determination of protein sequence coverage and identification of PTMs including deamidation, oxidation, lysine clipping, glycation, and glycosylation.

Download Application Note 21850

Key points

- Rapid, automated sample preparation within 1 hour leading to highly reproducible results for innovator and biosimilar comparability studies, with less hands-on time
- Simple protein digestion with minimal user intervention for peptide mapping analysis
- High confidence in results with excellent data quality; approximately 100% sequence coverage and low levels of sample preparation induced post-translational modifications

Instrumentation

- KingFisher Duo Prime purification system
- Vanquish Flex Binary UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- Nanodrop 2000 Spectrophotometer

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Automated Chymotrypsin Peptide Mapping of Proteins by LC-MS

Amy Farrell,[1] Jonathan Bones,[1] Ken Cook,[2] [1]Characterisation and Comparability Laboratory, National Institute for Bioprocessing Research Training (NIBRT), Dublin, Ireland and [2]Thermo Fisher Scientific, Hemel Hempstead, UK.

Overview

Peptide mapping is used to measure several critical quality attributes (CQAs) required for the characterization of any biotherapeutic proteins. Trypsin is the most commonly used proteolytic digestion enzyme due to its high specificity for cleavage at arginine and lysine residues. However, there are proteins that do not digest well with trypsin because of too many or too few trypsin cleavage sites in the protein sequence. There are also cases where the cleavage sites can be too close together, creating very small peptides that are not retained on reverse-phase columns making them difficult to detect.

Unlike trypsin, the chymotrypsin digestion pattern alters as the digestion time increases due to a slower rate of activity at its alternative digestion sites. Chymotrypsin will cleave primarily at the hydrophobic aromatic amino acid residues of tryptophan, tyrosine, and phenylalanine. With increased digestion time, chymotrypsin also cleaves at other sites such as leucine, histidine, and methionine, but with a lower level of specific activity. With a carefully controlled digestion time, chymotrypsin can provide an alternative digestion selectivity to tryptic digestion for proteins difficult to digest with trypsin. This application note demonstrates an easy-to-use, robust, high-precision, automated approach to a chymotrypsin peptide mapping characterization workflow.

Download Application Note 21834

Key points

- An easily automated proteolytic digestion protocol using chymotrypsin
- Orthogonal digestion to trypsin
- High reproducibility from a robust protocol and automation

Instrumentation

- KingFisher Duo Prime purification system
- Thermo Scientific™ Hypersep™ 96 well positive pressure system
- Thermo Scientific™ Vanquish™ Horizon UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer

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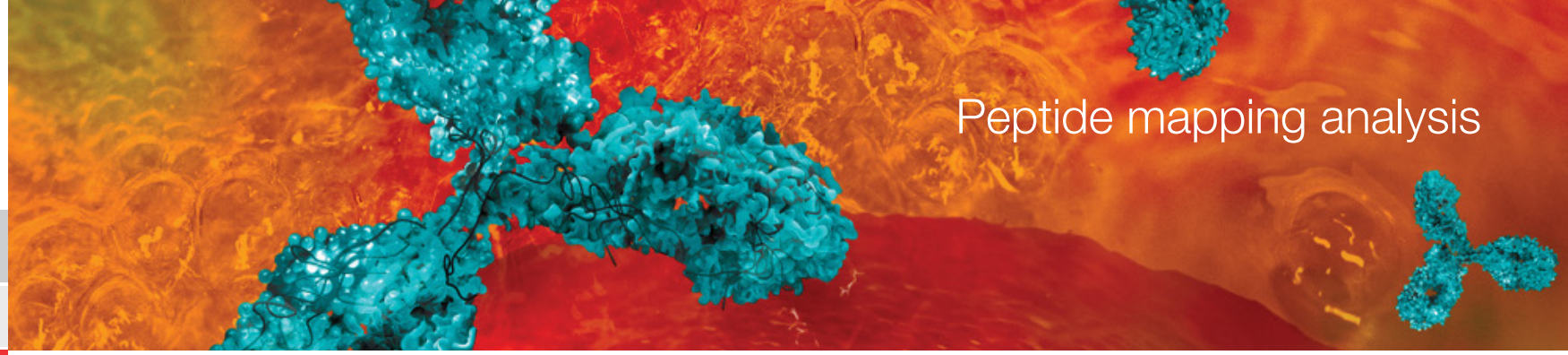

Peptide mapping analysis

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Comparison of Alternative Approaches to Trypsin Protein Digestion for Reproducible and Efficient Peptide Mapping Analysis of Monoclonal Antibodies

Silvia Millán-Martín, Craig Jakes, Noemi Dorival-García, Nicola McGillicuddy, Sara Carillo, Amy Farrell, Jonathan Bones, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

Peptide mapping is a “gold standard” tool in biotherapeutic characterization, and it is used to measure CQAs of mAbs to ensure product quality, efficacy, and safety. Peptide mapping consists of an enzymatic treatment of a protein, resulting in peptide fragments that are then separated, detected, and interpreted. Various approaches to peptide mapping have been developed utilizing different protein denaturation procedures and digestion times. However, there is a lack of reproducibility and confidence in the results with commonly used digestion protocols for peptide mapping. This leads to incurring excessive costs and reduced productivity due to laborious sample handling procedures and long digestion times. Furthermore, modifications such as deamidation and oxidation may be induced by sample preparation conditions, thereby distorting results. Hence, a simple, robust, and reproducible method is needed for peptide mapping.

In this study, a traditional overnight trypsin digestion method and a recently applied rapid digestion protocol were compared to two SMART Digest kit options (the standard kit and the magnetic bulk resin option) These kits contain thermally stable, immobilized trypsin that enables high temperature protein denaturation without a requirement for addition of denaturants. The study evaluated the reproducibility, protein sequence coverage, and identification of PTMs, including deamidation and oxidation.

Download Application Note 21782

Key points

- SMART Digest is a simple and rapid approach to peptide mapping analysis with protein digestion that takes only 45 min with subsequent reduction and alkylation taking a further 30 min each.
- 100% sequence coverage can be obtained for all the studied digestion protocols and low levels of sample preparation-induced PTMs were observed for the SMART Digest Kit approach
- SMART Digest allows for automated peptide mapping resulting in less sample handling, increased productivity, and improved reproducibility.

Instrumentation

- KingFisher Duo Prime purification system
- Vanquish Flex Binary UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer

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Peptide mapping analysis

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Growth Media Effects on Post-translational Modifications Investigated Through Peptide Mapping LC-MS/MS Analysis of Anti-IL8 Monoclonal Antibody

Giorgio Oliviero, Izabela Zaborowska, Craig Jakes, Sara Carillo, and Jonathan Bones, Characterisation and Comparability Laboratory, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

With the expansion of the biotherapeutics industry, there is a growing demand for cell culture media production and optimization. Media used for cell cultivation can affect cellular behavior with an associated potential influence on the yield and quality of the expressed therapeutic protein. Chemically defined media (CDM) formulation requires that all of the components are identified with exact concentrations and that no materials of human or animal origin are used in formulation. Depending on the requirements of a specific cell line, supplements with recombinant growth factors may be required. Thus, variability present in either the basal CDM or the supplements used to support the cell culture can affect the stability of a process, potentially affecting the yield or the quality of the produced biotherapeutic. Therefore, a key challenge in media development and optimization is not only the evaluation of cell growth, but also the evaluation of the resultant quality profile of the expressed biotherapeutic.

This application note characterizes proteins produced by Chinese hamster ovary (CHO) cell lines grown in different types of media. This study presents the benefits of using the recently developed Magnetic SMART Digest Kit to perform a peptide mapping-based comparability study of an anti-IL8 IgG1 grown using different CDMs. It also highlights the HRAM capabilities of the Q Exactive Plus mass spectrometer with the high-resolution chromatographic separation achieved using the Acclaim VANQUISH C18 column on the Vanquish Flex Binary UHPLC system.

Key points

- Quick and simple data preparation leading to highly reproducible characterization results for therapeutic mAbs
- Simple and rapid protein digestion for peptide mapping analysis of monoclonal antibodies (CHO-DP12) cultured in different types of media
- High reproducibility and ease-of-use of samples with less hands-on time and excellent recovery
- High confidence in results with excellent quality data, excellent peptide coverage, and identification of PTMs across all the samples

Instrumentation

- KingFisher Duo Prime purification system
- Vanquish Flex Binary UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- Nanodrop 2000 Spectrophotometer

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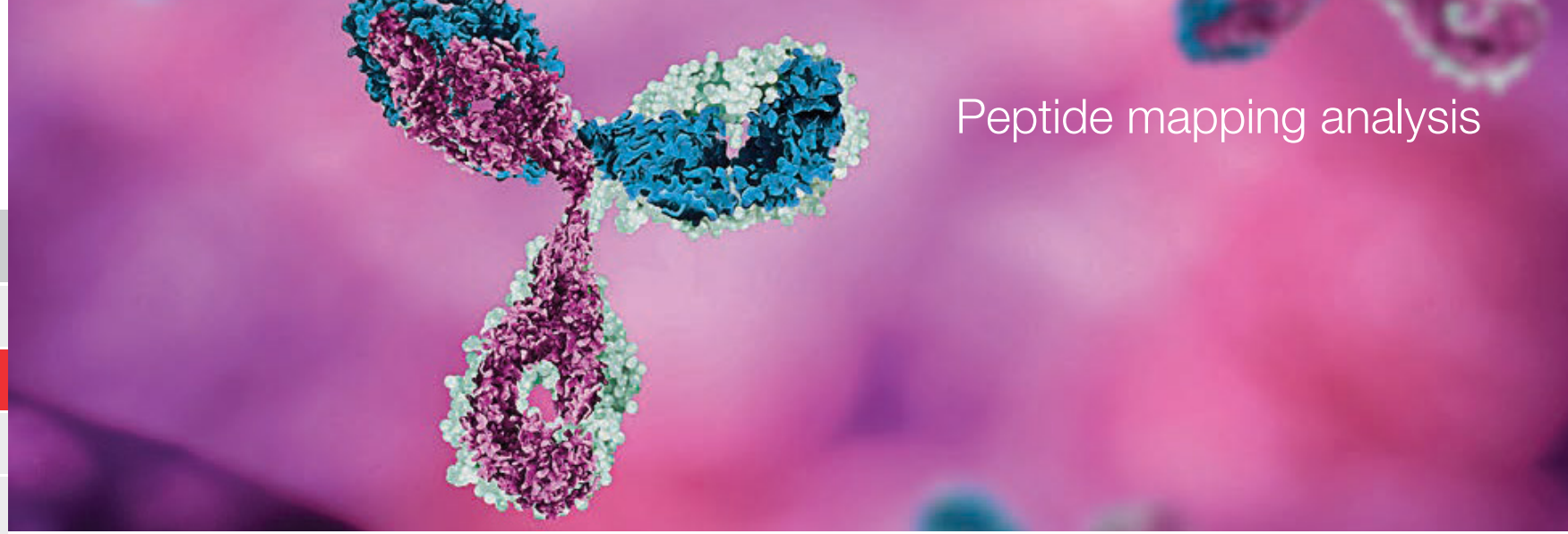

Peptide mapping analysis

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Investigating Process-related Post-translational Modifications in NISTmAb RM 8671 Using High-throughput Peptide Mapping Analysis

Silvia Millán, Craig Jakes, Noemí Dorival, Sara Carillo, Jonathan Bones, Characterisation and Comparability Laboratory, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

Monoclonal antibodies are a class of recombinant proteins that are susceptible to a variety of enzymatic or chemical modifications during expression, purification, and long-term storage. Monoclonal antibodies require close monitoring of their structural characterization and evaluation of their quality in each step, ensuring drug safety and efficacy.

In this application note, peptide mapping experiments were performed using the NISTmAb (NISTmAb RM 8671) provided by the National Institute of Standards and Technology (NIST). The NISTmAb was chosen because it is a well characterized, commercially available test material that is expected to greatly facilitate analytical development applications associated with the characterization of originator and follow-on biologics for the foreseeable future. After digestion with the SMART Digest kit, sequence coverage was assessed, as well as the identification and relative quantitation of specific PTMs: oxidation, glycosylation, and deamidation.

Key points

- A fast and simple protein digestion for peptide mapping analysis that results in low levels of sample preparation-induced post-translational modifications
- Time to complete digestion considerably shorter than traditional methods, making it possible to achieve an efficient digestion even in 30 min
- Workflow achieves 100% sequence coverage with high confidence
- Analytical separation achieves outstanding reproducibility with retention time precision RSD ≤ 0.2%

Instrumentation

- Vanquish Flex Binary UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- Nanodrop 2000 spectrophotometer

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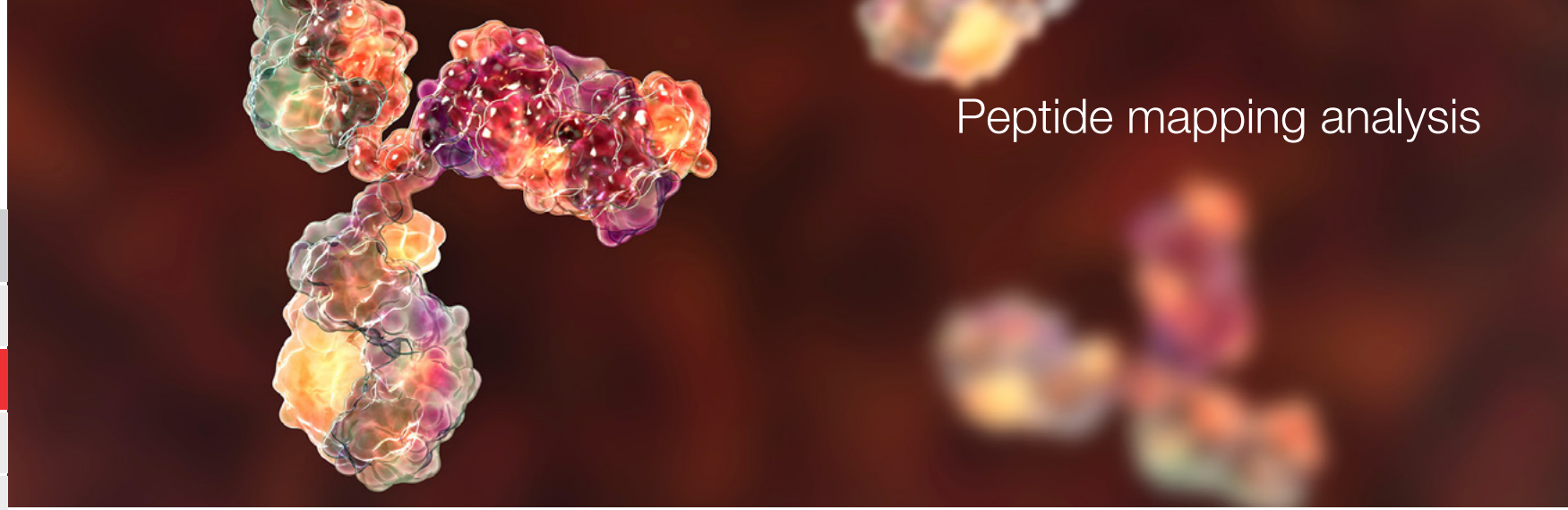

Automated biopharmaceutical protein digestion optimization for peptide mapping method development

Tom Buchanan, Amy J. Claydon, and Jennifer Sutton; Thermo Fisher Scientific, Hemel Hempstead, UK.

Overview

When peptide mapping is combined with high resolution accurate mass (HRAM) mass spectrometry (MS) it is a powerful analytical tool that can be used to characterize the primary structure of a biotherapeutic. Scientists are challenged with developing reproducible and robust analytical methods that are transferable and easily implemented by nonexperts. Traditional peptide and monitoring methods are notoriously difficult to validate due to the number of steps required to ensure a successful and reproducible protein digest. The aim of this application note is to simplify this process and develop methods that are easily validated for in-process testing, batch release, and stability testing within QC laboratories.

Here, an automated digestion time-course approach, using SMART Digest kits coupled with the KingFisher Duo Prime purification system is presented. The protocol facilitates determination of optimal incubation time to ensure complete digestion of a biotherapeutic during peptide mapping method optimization.

Key points

- Combining the SMART Digest magnetic kits with the KingFisher Duo Prime purification system provides an automated approach to protein digest method optimization.
- High quality, reproducible peptide mapping data eases the burden of method development, optimization, and validation.
- Immobilized trypsin in excess to sample—removes requirement to determine optimum enzyme to protease ratio.
- Precise termination of digestion—magnetic beads enable automated removal of immobilized trypsin by the KingFisher Duo Prime system.
- Downloadable Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) eWorkflow™ procedures and Thermo Scientific™ BindIt™ software time-course protocol to determine minimum optimal digest time.

Instrumentation

- KingFisher Duo Prime purification system
- Vanquish Horizon UHPLC System
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer with BioPharma option

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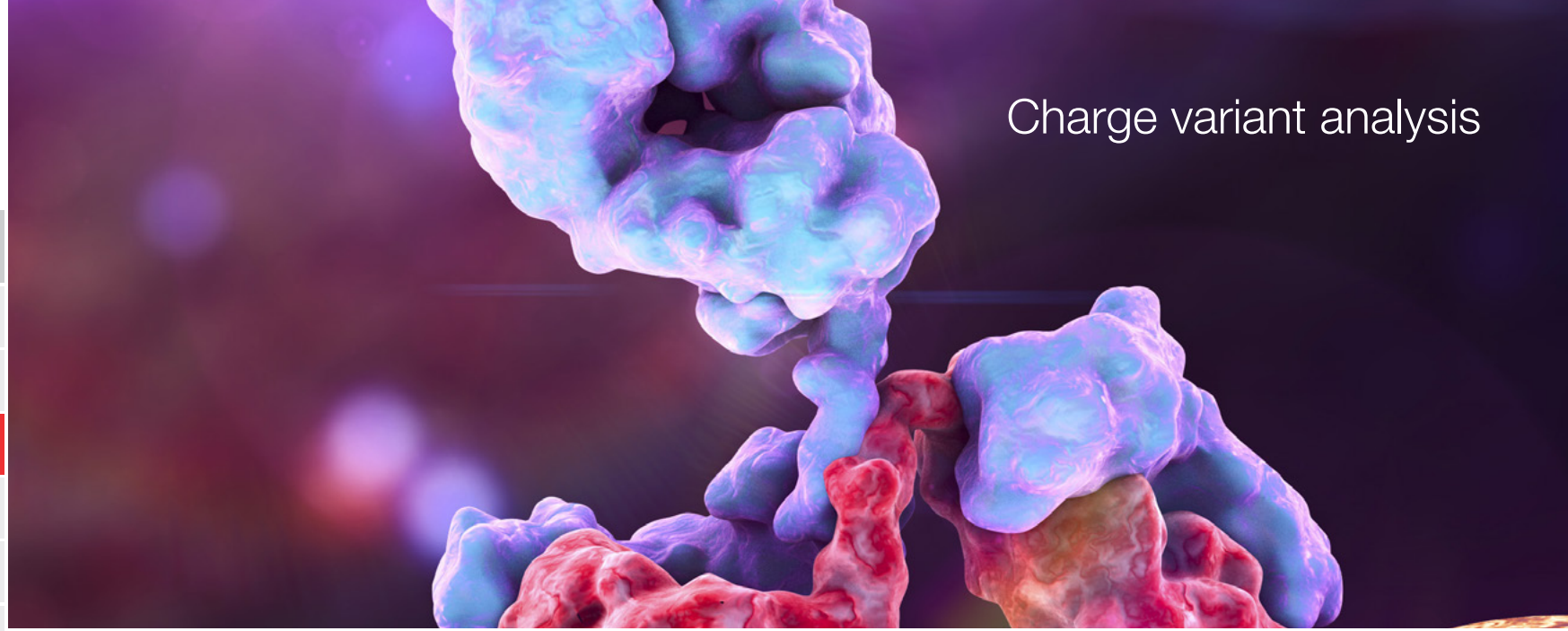

Charge variant analysis

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The characterization of monoclonal antibodies (mAbs) during biopharmaceutical development involves the identification, monitoring, and analysis of charge variants. Antibodies can exhibit changes in charge heterogeneity during production and purification caused by amino acid substitutions, glycosylation, and other post-translational or chemical modifications. Not only can these changes impact stability and activity, they can also cause adverse immunological reactions. Therefore it is critical to identify charge variants in development monitor them throughout manufacturing.

Ion-exchange chromatography is widely used for the characterization of therapeutic proteins and is considered a powerful reference technique for the qualitative and quantitative evaluation of charge heterogeneity. Numerous variants are commonly observed when mAbs are analyzed by charged-based separation techniques. These variants are generally referred to as acidic or basic species, compared with the main isoform, and are defined based on their retention times relative to the main peak. Acidic species are variants with lower pI that elute before the main peak by cation exchange (CEX), and basic species are variants with higher pI that elute after the main peak by CEX. For example, deamidation of asparagine residues and sialic acid content have been widely reported to contribute to the formation of acidic species. Basic species can be explained by modifications such as N-terminal glutamine, C-terminal lysine, and C-terminal amidation.

Analytical and biophysical methodologies must provide very detailed process and product information. Of critical importance for these methodologies are accuracy, precision, robustness, and suitability. In these application notes, pH gradient buffers are explored. The Thermo Scientific™ CX-1 pH gradient platform is shown to accelerate method development and facilitate method transfer to QA/QC. Although traditional charge variant analysis is powerful technique for revealing mAb heterogeneity, previously undetected isoforms require further analysis. In the following application notes, a method combining highly selective cation exchange chromatography-based charge variant analysis with on-line mass spectrometric (MS) detection is explored.

To learn more, visit Thermo Scientific Charge Variant Analysis Learning Center

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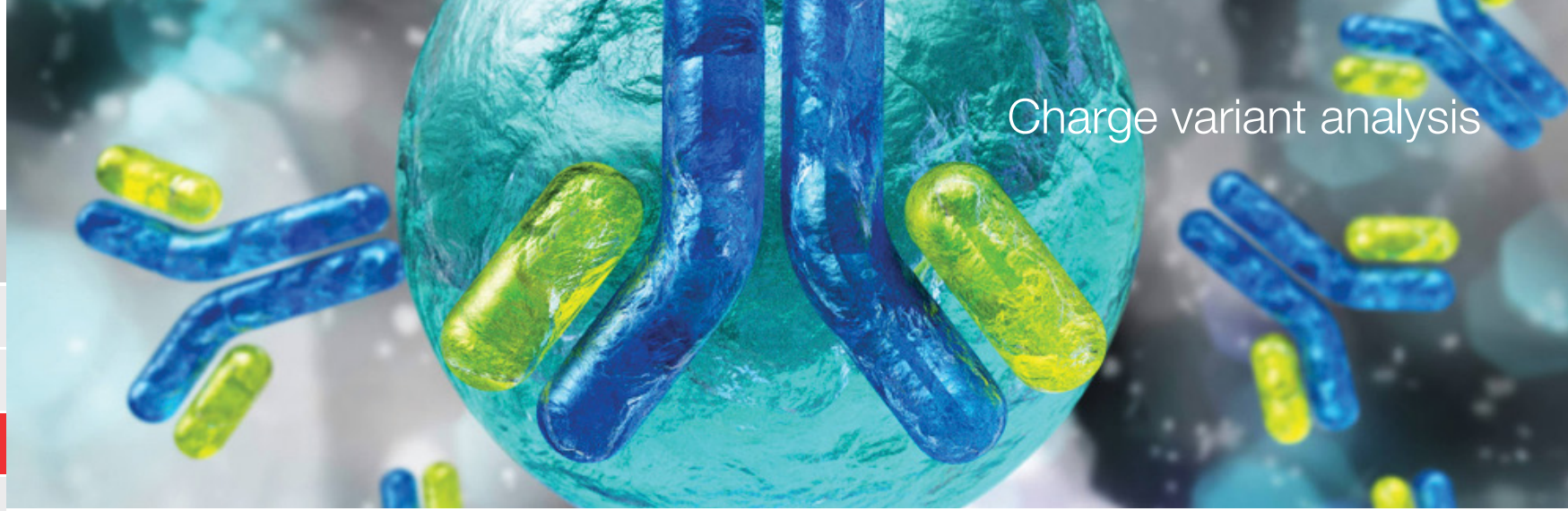

Charge variant analysis

A Global pH-gradient Based Charge Variant Analysis Directly Coupled to HRAM-MS (CVA-MS) for mAb Analysis

Florian Füssl,[1] Jonathan Bones,[1] Ken Cook,[2] and Kai Scheffler,[3] [1]National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland; [2]Thermo Fisher Scientific, Hemel Hempstead, UK; [3]Thermo Fisher Scientific, Germering, Germany.

Overview

The analysis of recombinant monoclonal antibodies and the study of their biological interactions are important in biopharmaceuticals. The structure of a mAb can be altered during the stages of production, purification, and storage from deglycosylation, deamidation, and truncation. These modifications impact critical attributes on the mAb that are essential for biosimilar comparability, safety, and efficacy.

In this application note, a method of charge variant analysis of mAbs with on-line MS detection is presented and shown to be superior to anything previously reported in terms of universal applicability and low adduction. Using this method, a comprehensive analysis of a monoclonal antibody can be achieved in a single injection with no sample preparation required. The accuracy of the deconvoluted masses using this method is better than results obtained from reversed-phase methods under denaturing conditions due to the chromatographic separation of the closely isobaric variants. This method should be useful for applications where speed and a more comprehensive characterization may be required.

Key points

- High information content with no sample preparation using native mass analytical methods for the simultaneous quantification of multiple mAb critical quality attributes
- Global applicability of the workflow using a Thermo Scientific™ MAbPac™ SCX-10 column
- SCX-10 column for mAb charge variant analysis and volatile buffers with pH-gradients to interface directly with high resolution mass spectrometry
- Ease of use of BioPharma Finder software

Instrumentation

- Vanquish Flex Binary UHPLC System
- Q Exactive Plus Hybrid Quadrupole Orbitrap mass spectrometer
- BioPharma Finder software

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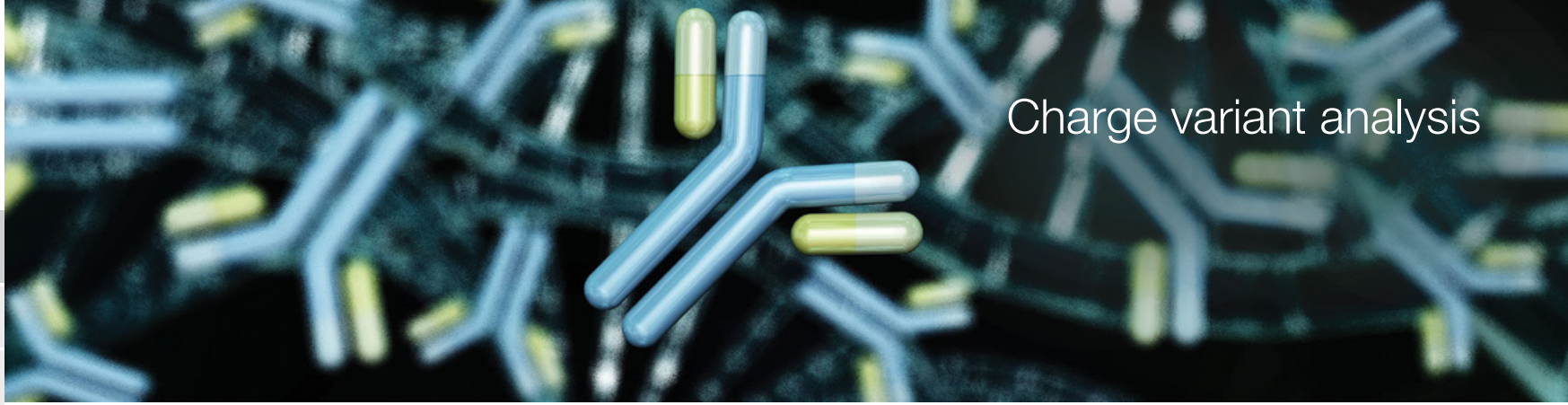

Charge variant analysis

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Degradation Pathways Analysis of Adalimumab Drug Product Performed Using Native Intact CVA-MS

Florian Füssl,[1] Sara Carillo,[1] Jonathan Bones,[1] Ken Cook,[2] and Kai Scheffler,[3] [1]National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland; [2]Thermo Fisher Scientific, Hemel Hempstead, UK; [3]Thermo Fisher Scientific, Germering, Germany.

Overview

Monoclonal antibodies have high specificity, low side effects, and are used to treat cancers, autoimmune, and inflammatory diseases. However, the heterogeneity of these biotherapeutics requires monitoring of critical attributes that affect product potency, stability, and biological activity. Furthermore, the steps from protein expression to finished product may generate aggregates or fragments formed by handling or storage. These degradation products need to be monitored and analyzed to ensure that their formations are minimized and consistent from batch to batch.

Degradation studies are used to investigate the potential modifications and degradation pathways under extreme conditions. They include high temperature, freeze-thaw cycles, agitation, high pH, low pH, light exposure, oxidation, and glycation. These stress factors reflect the conditions the drugs are exposed to during bioprocessing, packaging, shipping, and handling. Information-rich analytical methods, such as liquid phase separations with size exclusion, reversed-phase (RP) or ion-exchange chromatography, coupled with mass spectrometry can identify the variants and degradation products. This application note demonstrates global methods to identify and analyze adalimumab characterization and its degradation products under long-term storage conditions.

Key points

- High information content with no sample preparation using native mass analytical methods for the quantification of multiple mAb critical quality attributes and the identification of mAb degradation pathways that may have an impact on the protein activity and stability
- Global applicability of the workflow using a MAbPac SCX-10 column for mAb charge variant analysis with volatile buffer-aided pH gradients to allow direct coupling to high-resolution mass spectrometry
- High data quality obtained using a Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer with BioPharma Option for intact native high-resolution accurate-mass analysis with on-line charge variant separation of monoclonal antibodies
- Ease of use of BioPharma Finder software for intact mass analysis

Instrumentation

- Vanquish Flex Binary UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- BioPharma Finder software

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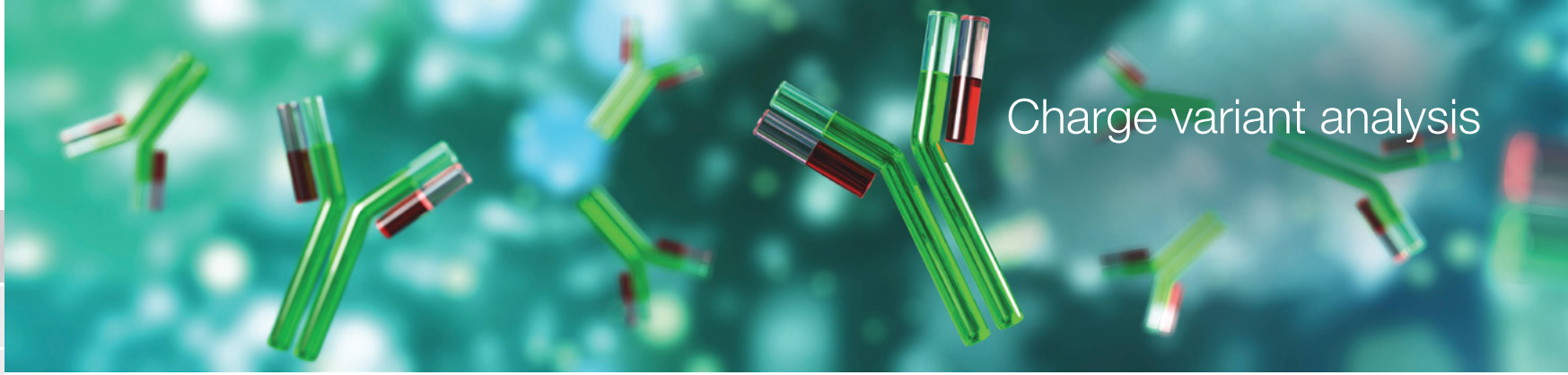

Charge variant analysis

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Evaluation and Application of Salt- and pH-based Ion-exchange Chromatography Gradients for Analysis of Therapeutic Monoclonal Antibodies

Amy Farrell, Craig Jakes, and Jonathan Bones, Characterisation and Comparability Laboratory, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

Monoclonal antibodies are heterogeneous biomolecules that undergo a wide range of enzymatic and chemical modifications during bioprocessing and storage. It is important to detect, characterize, and quantify mAb variants and modifications to ensure the safety and efficacy of mAbs for regulatory approval and clinical use. Charge-related microheterogeneity of mAbs is frequently observed following bioprocessing due to both enzymatic processes and non-enzymatic degradation of therapeutic proteins. Determination of a mAb charge variant profile provides information regarding commonly encountered product CQAs.

This application note demonstrates the use of a pH buffer system for the determination of charge variants in therapeutic monoclonal antibodies. Two approaches to the generation of a pH gradient are investigated. One approach uses quaternary pump mixing of an acid, a base, a salt solution, and water to produce a pH separation gradient. The second approach generates a pH gradient using a Thermo Scientific™ cation-exchange pH gradient buffer platform consisting of a low pH buffer at pH 5.6 (Thermo Scientific™ CX-1 pH Gradient Buffer A) and a high pH buffer at pH 10.2 (Thermo Scientific™ CX-1 pH Gradient Buffer B). Compared to the salt-based gradient systems, the CX-1 pH gradient buffer system demonstrates the ease of optimization and improved reproducibility for charge variant analysis.

Key points

- The CX-1 pH gradient buffers in combination with MAbPac SCX-10 analytical columns enable rapid method development for charge variants analysis of most mAb biotherapeutics.
- Use of a pH gradient for mAb charge variant analysis by IEX enables increased reproducibility of chromatography across different buffer preparations.
- This straightforward, easily optimized, widely applicable approach to charge variant analysis of therapeutic mAbs generated using the CX-1 pH gradient buffers has the potential to greatly expedite the characterization of mAb charge variants during drug development and routine quality control analyses.

Instrumentation

- Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system
- BioPharma Finder software

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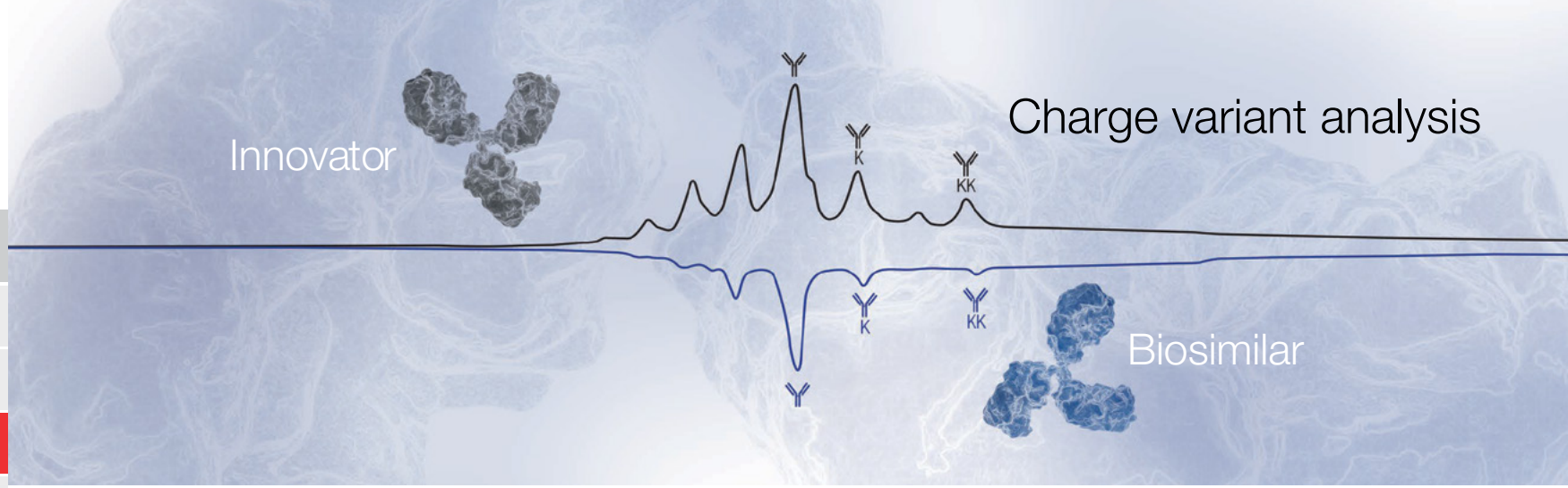

Charge variant analysis

Simple Charge Variant Profile Comparison of an Innovator Monoclonal Antibody and a Biosimilar Candidate

Silvia Millán, Anne Trappe, Amy Farrell, and Jonathan Bones, Characterisation and Comparability Laboratory, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

With a growing number of biosimilars entering the biopharmaceutical market, reliable analytical techniques are needed to evaluate the similarities and differences between the biosimilar and its reference product (innovator). Charge heterogeneity analysis is critical for mAb characterization as it provides valuable information regarding product quality and stability. Heterogeneity of mAbs can be caused by molecular adaptations such as C-terminal lysine modification, deamidation, and other PTMs. The number and type of ionizable amino acid groups dictates the overall number of charges on a protein at a given pH. Ion-exchange chromatography (IEX) is a widely used, powerful reference technique for the characterization of biosimilar proteins.

This application note demonstrates the effectiveness of a pH gradient/ion-exchange chromatography workflow approach in the characterization of different charge variants profiles of an innovator molecule (cetuximab) to its biosimilar. Furthermore, the ion-exchange assay is simple, reproducible, easily optimized and effectively resolves variants. This approach detects charge heterogeneity differences between an innovator and biosimilar mAb. The pH-based gradients are effective for simplified ion-exchange analysis of charge variants.

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The CX-1 pH gradient buffer method meets the speed and repeatability requirements for a platform method. Finally, this method provides excellent separation of charge variants from biotherapeutics with excellent retention time precision.

Key points

- Confident charge variant evaluation of biosimilars obtained in a single analysis
- Quick assay optimization: no need for mobile phase evaluation
- Reduced risk of method variability

Instrumentation

- Vanquish Flex Quaternary UHPLC system
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS)

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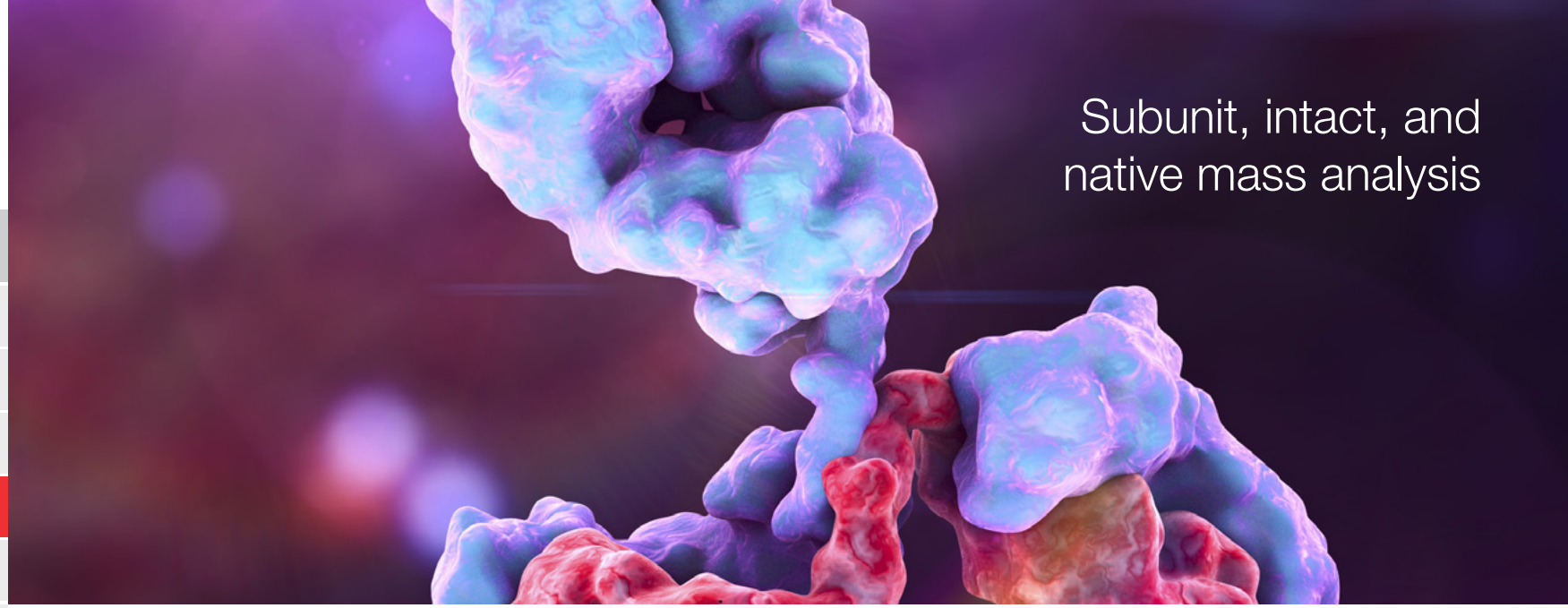

Subunit, intact, and native mass analysis

Biotherapeutic compounds, such as proteins and monoclonal antibodies (mAbs), as well as antibody-drug conjugates, complexes and assemblies, make up a majority of the rapidly growing biologics drug market. Full characterization of these therapeutics by mass spectrometry includes determination of protein sequences, and identification and relative quantitation of protein isoforms, including identification and localization of one or multiple post-translational modifications (PTMs). Traditional workflows for such analyses use a “bottom-up” approach, where proteins are digested into their peptide counterparts. However, complete sequence coverage is rarely attainable, and qualitative and quantitative information about protein isoforms, including those resulting from post-translational modifications, is often lost. These same research endeavors would, in many cases, also benefit from subunit, intact, and native mass spectrometric analyses.

Subunit, intact, and native protein characterization by mass spectrometry (MS) has emerged as a valuable technology that has numerous advantages over bottom up sequencing. These MS techniques, using high resolution accurate mass (HRAM) technologies, provide accurate information on various protein properties, such as intact molecular mass, glycosylation forms, amino acid sequence, post-translational modifications, and minor impurities due to sample processing and storage, as well as higher-order structural information, such as protein conformational changes upon modifications, noncovalent interactions between protein drugs and receptor proteins, and protein aggregation caused by misfolding. High-resolution mass spectrometry is essential for resolving co-eluting intact proteins as well as isotopic peaks of highly charged proteins for charge state determination and accurate mass determination. Additionally, due to the number of product ions generated during fragmentation of intact and large subunits of proteins, high-resolution mass analysis is required for accurate detection and assignment of product ions in the resultant complex MS/MS spectra. Orbitrap-based mass spectrometers are essential tools for routine intact and native biopharmaceutical analysis as demonstrated by a variety of examples below.

To learn more, visit Thermo Scientific Intact Protein Analysis Learning Center

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Subunit, intact, and native mass analysis

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Performance Evaluation of MAbPac RP Columns for Monoclonal Antibody IdeS Subunit Analysis

Meire Ribeiro da Silva, Sara Carillo, Craig Jakes, Jonathan Bones, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

Monoclonal antibodies are inherently complex and heterogeneous due to post-translational modifications. A method to evaluate mAb heterogeneity and simplify analysis is to analyze mAb variants and locate the modifications through the characterization of mAb fragments. Middle-up approaches can provide rapid confirmatory analysis with minimal sample handling.

In this application note, fast separation methods are presented for variants of trastuzumab subunits using MAbPac RP columns with reversed-phase liquid chromatography (RPLC) coupled to high-resolution mass spectrometry. The samples are digested with IdeS protease, cleaving the monoclonal antibody in the hinge region and generating two pairs of polypeptides from the heavy chain (Fd' and scFc) along with the light chain portion, after reduction of disulfide bonds. All analyses are performed on an ultra-high-resolution analytical platform consisting of a Vanquish Flex Quaternary or a Vanquish Horizon Binary UHPLC system hyphenated with a variable wavelength UV detector.

The results demonstrate rapid, efficient, and high-resolution methods for separating mAb fragments. The mAb scFc, Fc, and Fd' fragments are successfully separated using a MAbPac RP column with a 15 min gradient.

Key points

- Exceptionally high efficiency, high resolution for separation of mAb fragments
- Fast analysis
- Ideal for stability and QA/QC testing

Instrumentation

- Vanquish Flex Quaternary UHPLC system
- Vanquish Horizon UHPLC system

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Subunit, intact, and native mass analysis

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Comparing Biosimilars Using Intact Mass Analysis Under Denaturing and Native Conditions

Sara Carillo, Izabela Zaborowska, and Jonathan Bones, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

A biosimilar should have minimal variations from its reference product (innovator) in terms of physiochemical characterization data and clinical evaluation of pharmacological performance. Since a biotherapeutic is inherently heterogeneous, a biosimilar and its innovator may present the same variation, but in a different ratio that could potentially affect functions like antigen recognition, Fc binding, or product stability.

In this application note, comparisons between reference products and their respective biosimilars are performed with HRAM mass spectrometry. Using a high-resolution Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer, it is possible to obtain rapid information about glycosylation profiles and C-terminal lysine loss based on high-resolution intact mass analysis. To analyze monoclonal antibody variants, it is important that the mass spectrometer provides high-resolution data on the three most-used levels of analysis (peptide level, subunit, and intact protein analysis). The BioPharma option available on the Q Exactive Plus Orbitrap MS provides three modes of operation and a higher upper mass range, allowing increased flexibility across the three levels of analysis mentioned above. More importantly, this information is obtained without sample preparation that can cause modifications to the sample.

Download Application Note 21880

Key points

- Demonstrates benefits of a rapid intact mass analysis workflow for the analysis of product quality while minimizing potential artefacts that may be generated by sample preparation.
- Demonstrate the applicability of MAbPac RP and Thermo Scientific™ MAbPac™ SEC-1 columns for intact protein applications in the denatured and native form.
- Highlight the benefits of using a Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer with Biopharma Option and extended mass range for high-resolution accurate-mass MS analysis on different subunits of monoclonal antibodies.

Instrumentation

- Vanquish Flex Binary UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- Nanodrop 2000 Spectrophotometer ND-2000

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Subunit, intact, and native mass analysis

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IdeS-cleaved mAb Subunit Analysis with LC-HRAM-MS: A Quick and Accurate Comparison of Biosimilar and Originator Biotherapeutics

Sara Carillo, Craig Jakes, Izabela Zaborowska, and Jonathan Bones, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

Biosimilars are a rapidly growing class of human therapeutics that are creating a large demand for quick and reliable characterization methods to ensure drug quality, safety, and efficacy. The variations of a biosimilar from its reference product (originator) are due to several factors like the expression system, growing conditions, purification steps, and final formulations. It is important to monitor and quantify these variations to correlate them to any potential difference in *in vivo* activity. To detect these differences, full sequence coverage and information on PTMs are usually obtained with a bottom-up approach employing a combination of several LC-MS/MS datasets derived from different and orthogonal enzymatic digestions of the protein. Top-down or middle-up approaches have the potential to minimize sample handling and consequently artefacts and provide complementary information. In this application note, liquid chromatography hyphenated with high resolution, accurate mass spectrometry (LC-HRAM-MS) is used in a middle-up approach for the comparison of three commercially available mAb originators to their respective biosimilars. This method enables the characterization of mAbs variants using MabPac RP columns and the recording of structural data on glycoforms, lysine truncation, and other micro-variants present on the products.

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Key points

- Demonstrate the benefits of using a middle-up approach for biotherapeutics characterization
- Demonstrate the applicability of MAbPac RP columns for subunit analysis
- Highlight the benefits of using the Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer for high-resolution, accurate-mass MS analysis on different subunits of monoclonal antibodies to evaluate glycans and other micro-variants

Instrumentation

- Vanquish Flex Binary UHPLC System
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- Nanodrop 2000 Spectrophotometer
- BioPharma Finder software

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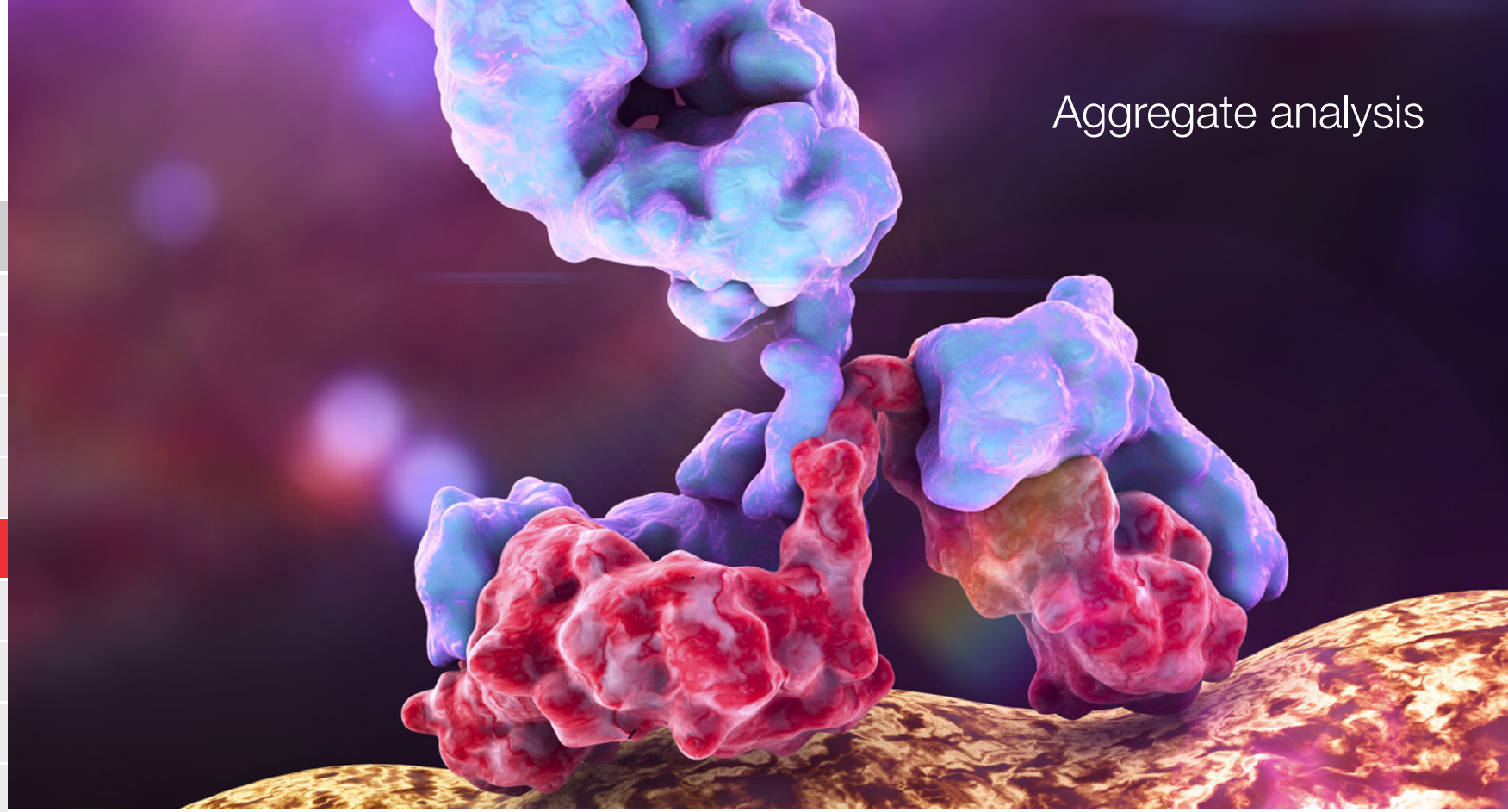

Aggregate analysis

The biopharmaceutical industry continues to develop mAb-based biotherapeutics in increasing numbers, leading to a need for rapid and robust high-throughput analyses. Due to the complexity of these biotherapeutics, there are several critical quality attributes (CQAs) that need to be measured and controlled to guarantee their safety and efficacy. Aggregation of biotherapeutic proteins can result in incorrect drug dosage and an undesired immune response, and therefore must be monitored throughout the production process and during storage of formulated biotherapeutics.

Size-exclusion chromatography (SEC) is considered a gold standard for monitoring the formation and level of mAb aggregates and fragments and is probably one of the most frequently performed analyses in QC laboratories. Although standard UHPLC systems can quickly analyze samples, they can only use one stationary phase at a given time. As a result, analysts are limited in the number of injections that a chromatography system can perform, leading to less efficient sample analysis with additional costs. Furthermore, handling and storage can cause unintentional size variants of the biotherapeutic.

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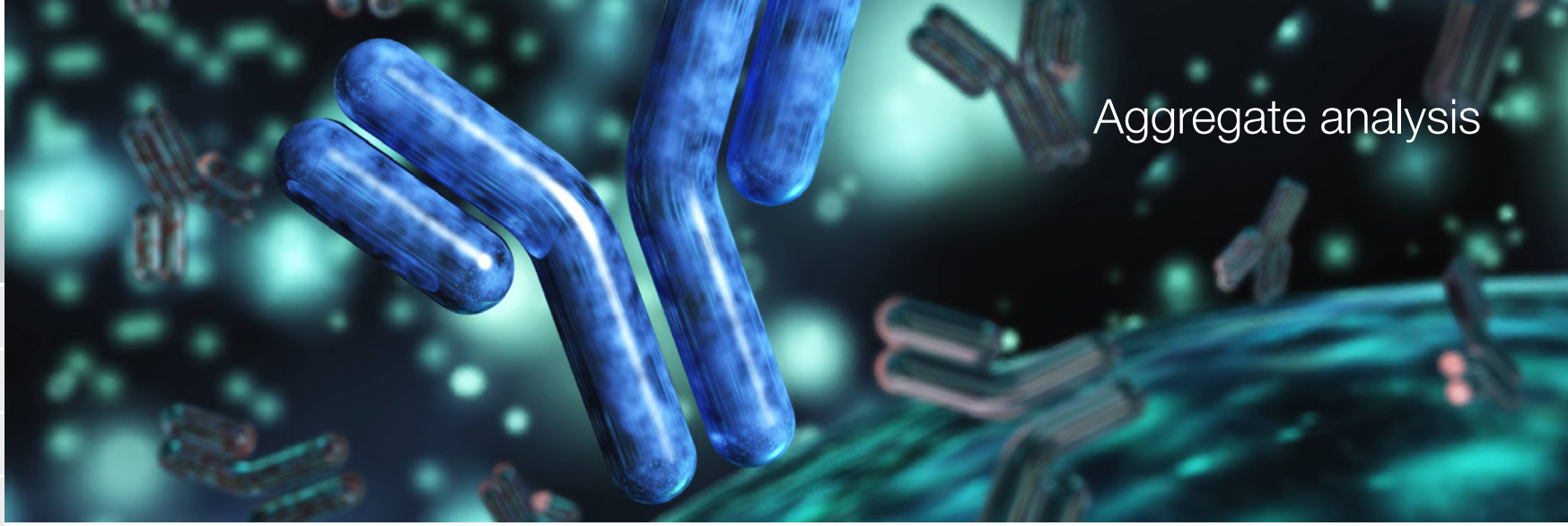

Aggregate analysis

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High-throughput Protein Aggregate Analysis of Monoclonal Antibodies Using a Novel Dual-channel UHPLC Instrument

Nicola McGillicuddy,[1] Amy Farrell,[1] Sara Carillo,[1] Martin Samonig,[2] Jonathan Bones,[1]
[1]National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland and
[2]Thermo Fisher Scientific, Germering, Germany.

Overview

There is an increasing need for rapid and robust high-throughput analysis of mAbs. Thus, standardized chromatographic methods with excellent reproducibility are essential for sample analysis in quality control laboratories. Size-exclusion chromatography (SEC) is considered a gold standard for monitoring mAb aggregates and is probably one of the most frequently performed analyses in QC laboratories. Although standard UHPLC systems can quickly analyze samples, only one stationary phase can be used at a given time. As a result, analysts are limited regarding the number of injections that a chromatography system can perform, leading to less efficient sample analysis with additional costs. Furthermore, handling and storage can cause unintentional size variants of the biotherapeutic that are potentially harmful for human health.

In this application note, the Thermo Scientific™ Vanquish™ Flex Duo UHPLC system for Dual LC is used for the high-throughput analysis of a mAb. One hundred injections of bevacizumab were performed on two identical MAbPac SEC-1 size exclusion columns. These results were also obtained on a standard Vanquish Flex Quaternary UHPLC system for direct comparison.

Key points

- Dual LC workflow demonstrates the ability to employ two chromatography channels (columns, pumps, and detectors) simultaneously with no loss in data quality.
- Higher number of sample injections can be performed during analysis time resulting in efficient sample analysis and overall lower costs.
- Separate software modules for each LC system result in easy data processing.

Instrumentation

- Vanquish Flex Duo UHPLC system for Dual LC
- Vanquish Flex Quaternary UHPLC system

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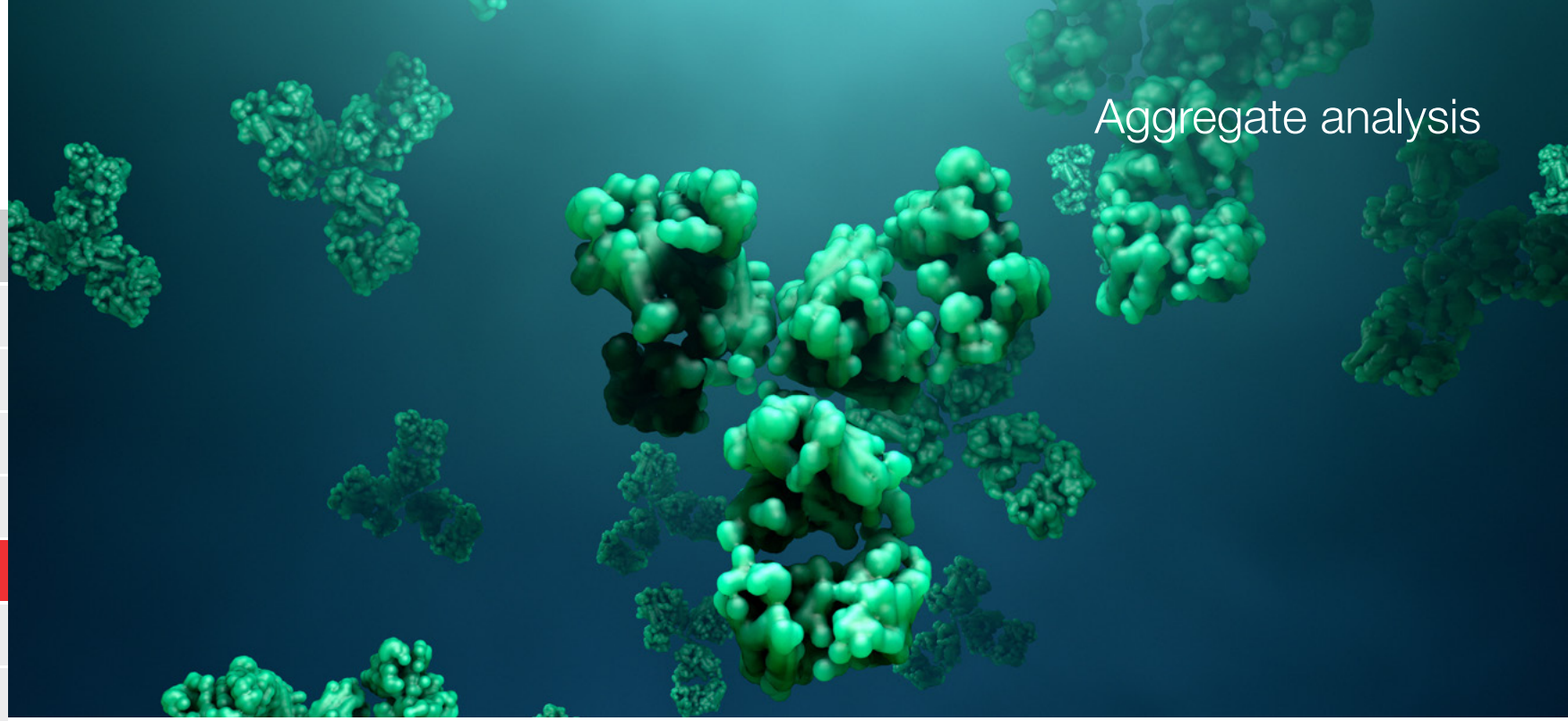

A Universal Chromatography Method for Aggregate Analysis of Monoclonal Antibodies

Amy Farrell,[1] Jonathan Bones,[1] and Ken Cook,[2] [1]National Institute for Bioprocessing Research and Trail (NIBRT), [2]Thermo Fisher Scientific, Hemel Hempstead, UK.

Overview

The biopharmaceutical industry continues to develop mAb biotherapeutics in increasing numbers. Due to the complexity of mAbs, CQAs need to be measured and controlled to guarantee the safety and efficacy of the biotherapeutics. The presence of aggregates in a formulated drug product must be assessed to avoid potential issues.

The aim of this application note is to perform aggregate analysis of five biotherapeutic mAbs using the MAbPac SEC-1 column coupled to the Vanquish Flex Quaternary UHPLC system. A single globally applicable SEC method is performed for the bevacizumab, cetuximab, infliximab, rituximab, and trastuzumab samples.

Key points

- Demonstrates a single globally applicable SEC chromatography method for five mAb samples
- Shows peak symmetry (implying limited secondary interaction with the column)
- The method maintains the required monomer and dimer resolution

Instrumentation

- Vanquish Flex Quaternary UHPLC system
- Chromeleon CDS

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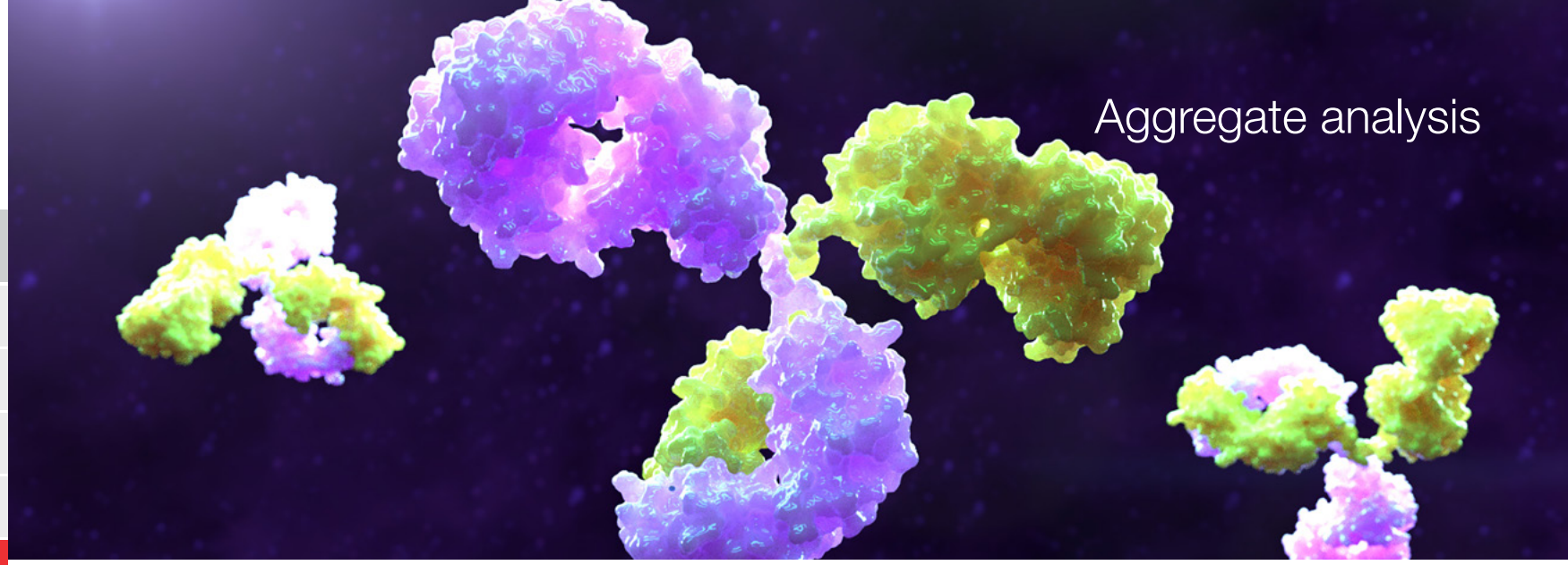

Lifetime Stability of Size-Exclusion Chromatography Columns for Protein Aggregate Analysis

Amy Farrell,[1] Craig Jakes,[1] Alexander Ley,[2] Mauro De Pra,[2] Frank Steiner,[2] and Jonathan Bones,[1] [1]The National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland, [2]Thermo Fisher Scientific, Germering, Germany.

Overview

During monoclonal antibody expression, purification, formulation, and storage, the mAbs undergo various degradation processes, which may alter the safety, efficacy, and quality profile of the drug product. Consequently, CQAs must be monitored throughout drug development and production to ensure the biotherapeutic is suitable for clinical use. Aggregation can result from partial unfolding or other types of conformational changes in protein structure to form dimers, trimers, and other higher order structures. Due to high costs of drug development and production of mAbs, reliable, long-use consumables and equipment for CQA evaluation are needed.

The aim of this application note is to demonstrate the long-term stability of the MAbPac SEC-1 column for monoclonal antibody aggregate analysis using the Vanquish Flex Quaternary UHPLC system. Consistent retention time, excellent peak symmetry, and exceptional column efficiency are demonstrated using The MAbPac SEC-1 column coupled to a Vanquish Flex Quaternary UHPLC system for aggregate analysis of mAbs. The MAbPac SEC-1 column lifetime stability determined far exceeds other commercially available SEC columns, without requirements for guard columns.

Key points

- Columns for analysis of mAb aggregates with exceptionally long lifetime
- A biocompatible UHPLC system capable of operating with high salt content and potentially corrosive mobile phases
- A UHPLC solution for mAb aggregate analysis that operates continuously for several weeks

Instrumentation

- Vanquish Flex Quaternary UHPLC system
- Chromeleon CDS

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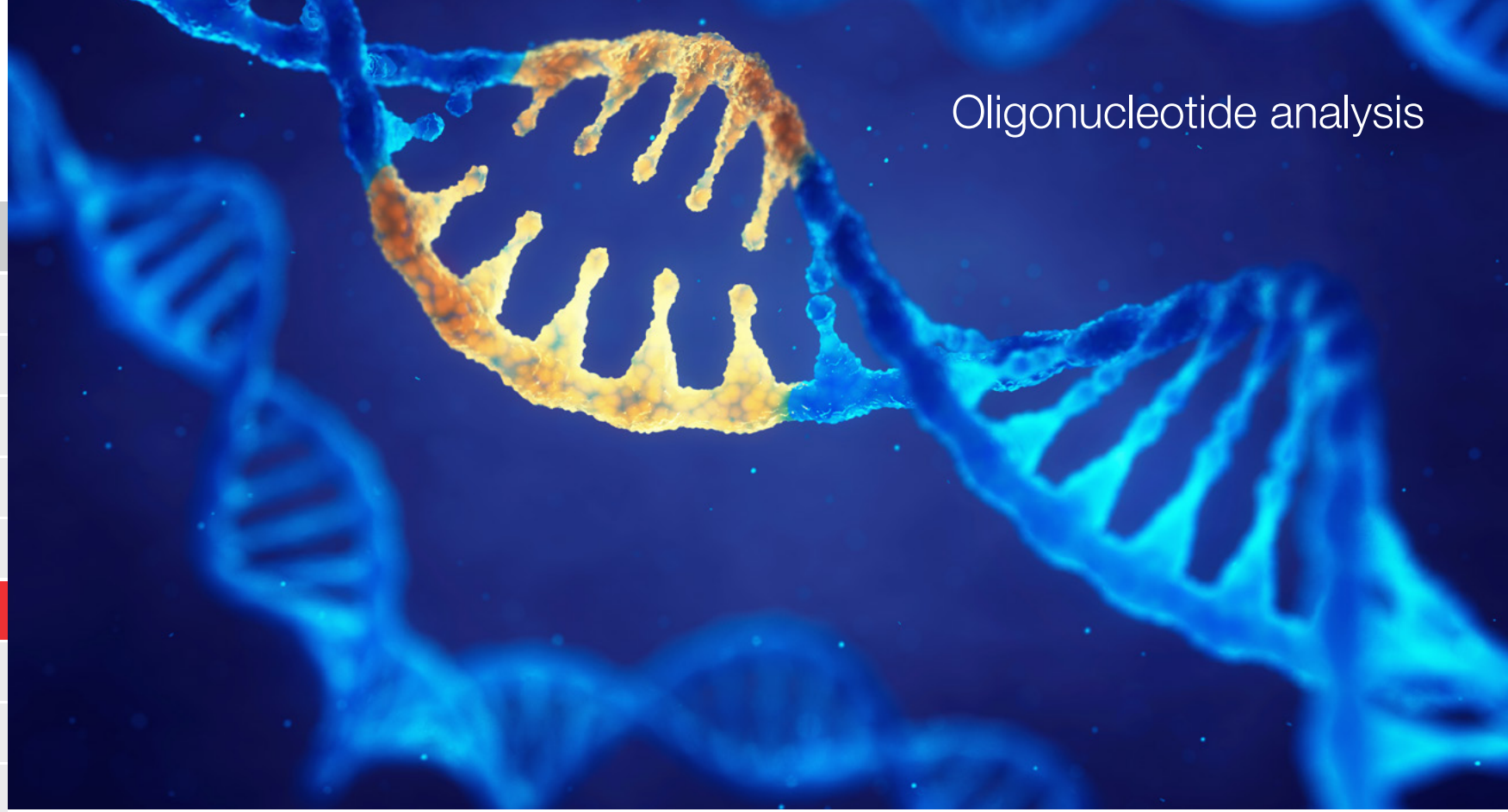

Oligonucleotide analysis

Monitoring product quality of oligonucleotide synthesis requires confident confirmation of oligonucleotide mass and rough quantification of yield and impurity levels using a rapid and efficient method. Quantification of yield can easily be performed by UV detection because DNA molecular properties facilitate strong absorption at 260 nm.

Strong anion exchange columns, such as the Thermo Scientific™ DNAPac™ PA200, are commonly used for high resolution analysis and purification of synthetic oligonucleotides. In addition, the Thermo Scientific™ DNAPac™ RP columns offers superior reversed-phase separations using a unique chemistry designed for small or large oligonucleotides using LC-UV or LC-MS. This column provides excellent performance under a broad range of pH, temperature, and mobile phase compositions. Adding a mass spectrometer to the oligonucleotide product quality monitoring allows for non-ambiguous identification and confirmation of oligomer identity and present impurities.

In the following examples, the versatility of the DNAPac RP Column is explored as well as the addition of the ISQ EM single quadrupole mass spectrometer in the quality control setting for mass confirmation and impurity level estimation.

To learn more, visit Thermo Scientific Oligonucleotide Analysis Learning Center

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Oligonucleotide analysis

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Oligonucleotide Characterization for Quality Control and Increased Productivity by Single Quadrupole Mass Spectrometer with Extended Mass Range

Katherine Lovejoy, Sylvia Grosse, Mauro de Pra, Martin Samonig, Frank Steiner, Thermo Fisher Scientific, Germering, Germany.

Overview

Quality control of oligonucleotide synthesis requires confirmation of mass and rough quantification of yield and impurity levels using a rapid and efficient method. Quantification of yield can be performed by UV detection because DNA molecular properties facilitate strong absorption at 260 nm. Rough estimation of impurities requires a mass spectrometer, as aborted sequences (N-1) are not usually chromatographically separated from complete sequences (N) during a quick QC method. A mass spectrometer also allows for non-ambiguous confirmation of oligomer identity.

Mass spectrometry is often considered to be complex and too difficult to use in routine quality control applications. The Thermo Scientific™ ISQ EM Single Quadrupole mass spectrometer was developed for operation by chromatographers. Its full integration into Chromeleon CDS and the Thermo Scientific™ AutoSpray smart method setup make LC-MS operation and data analysis straightforward and intuitive.

The orthogonal source design provides high levels of instrument robustness, even for challenging conditions of ion pairing chromatography commonly used to ensure retention of DNA oligomers. This application note describes quality control of oligonucleotides, including mass confirmation and impurity level estimation by mass spectrometry, as well as quantification by UV.

Key points

- Expanding a UV quality control method with MS provides mass confirmation and impurity identification.
- Use of common DNA ion pairing reagents facilitates transfer of existing QC methods.

Instrumentation

- Vanquish Flex Binary UHPLC system
- ISQ EM Single Quadrupole mass spectrometer

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Oligonucleotide analysis

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Separation of Large Double-stranded DNA (dsDNA) Fragments Using a Wide-pore Reversed-phase Chromatography Column

Julia Baek, Jim Thayer, Shanhua Lin, Xiaodong Liu, Thermo Fisher Scientific, Sunnyvale, CA.

Overview

Restriction nuclease DNA fragments are typically purified prior to subsequent use. Agarose and polyacrylamide gels are common methods to separate and recover dsDNA fragments. This is followed by manual excision of the target dsDNA size from the gel and extraction of the DNA from the excised gel. The method is laborious, time consuming, and produces low dsDNA yields. HPLC provides reliably higher yields, allows direct DNA quantitation, and can be automated using fraction collection.

The DNAPac RP column is based on a 4 µm, wide-pore polymer resin, which is stable at high pH and high temperatures. The wide-pore resin (1000–2000 Å) separates large dsDNA molecules as well as single-stranded oligonucleotides. This application note demonstrates the separation of DNA fragments generated from restriction enzymes and DNA ladder components, with automated purification and collection of the PCR product using ion-paired reversed-phase chromatography on the DNAPac RP column.

Key points

- The combination of the DNAPac RP column and Vanquish Flex UHPLC system delivers rapid, high-resolution dsDNA fragment separation up to 10,000 base pairs.
- Elution of the dsDNA fragments is essentially proportional to fragment length (in base pairs).
- Thermo Scientific™ UltiMate™ 3000 system equipped with the WPS-3000 TBFC Well Plate Autosampler configured for Fraction Collection facilitates the purification and recovery of dsDNA fragments.

Instrumentation

- Vanquish Flex Binary UHPLC system
- UltiMate 3000 system

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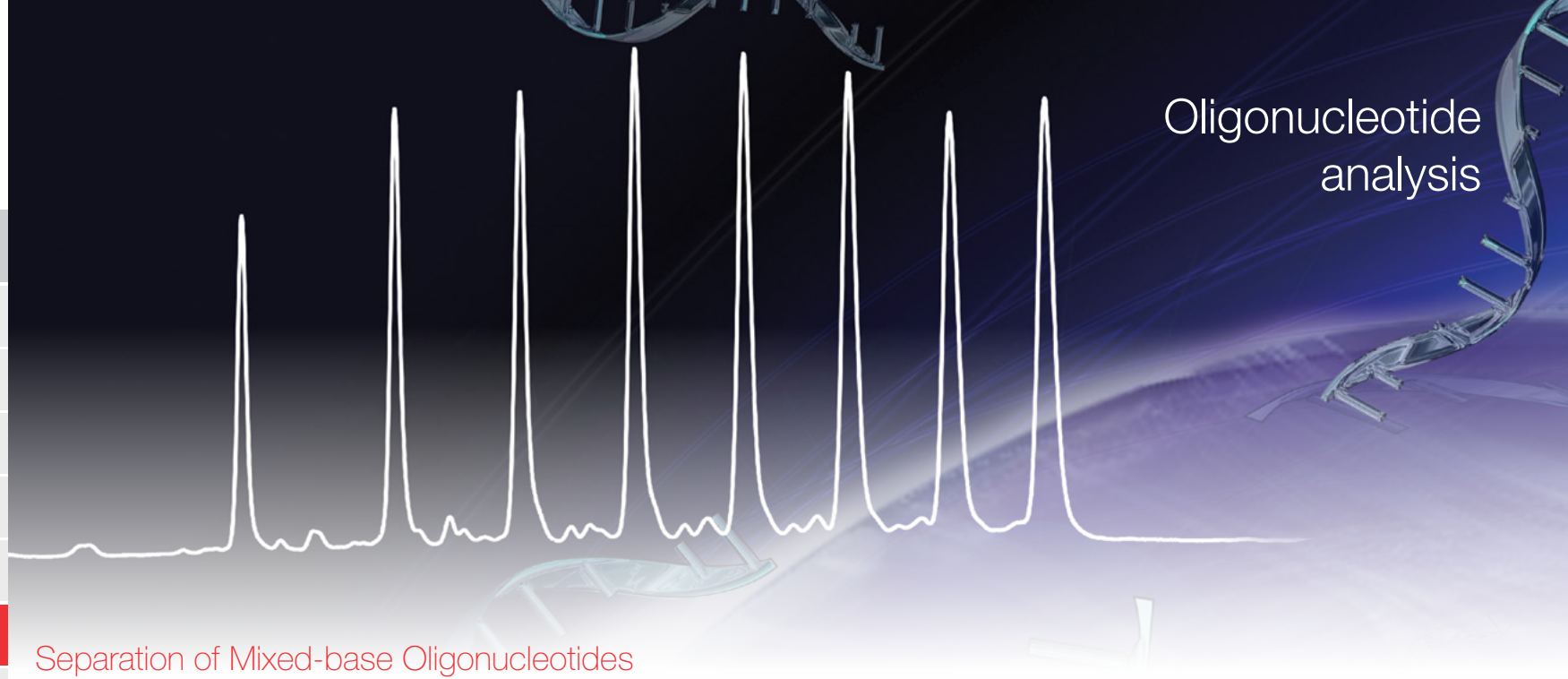
Oligonucleotide analysis



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Separation of Mixed-base Oligonucleotides Using a High-resolution, Reversed-phase Chromatography Column

Julia Baek, Jim Thayer, Shanhua Lin, Xiaodong Liu, Thermo Fisher Scientific, Sunnyvale, CA, USA.

Overview

Synthetic oligonucleotides (ONs) are used as primers for polymerase chain reactions (PCR) and DNA sequencing, probes to visualize a specific DNA or RNA, tools to study gene function, and biopharmaceutical drugs. ONs are most often synthesized using solid-phase chemistry, which consists of many sequential coupling reactions. Although the yield of each reaction is high, it results with accumulation of minor reaction failures in truncated ONs and to the target ON. These truncated ONs and other impurities (e.g., deprotection failures) must be removed for most molecular biology applications and to ensure efficacy and safety of therapeutic agents. Analyses of synthetic ONs are most commonly performed using ion-pair reversed phase chromatography (IP-RP). IP-RP utilizes the ionic interaction between the analyte and the ion-pair reagent, which also interacts with the stationary phase via its hydrophobic moiety. IP-RP provides high-resolution separation of failure sequences and can be directly coupled to mass spectrometry for identification of target ON and impurities.

Download Application Note 21476

This application note demonstrates the impact of different chromatography conditions to achieve fast and high-resolution separation of mixed-base oligonucleotides using a porous reversed-phase chromatography column.

Key points

- The DNAPac RP column delivers fast, high-resolution separation of mixed-base ONs on a stable polymeric stationary phase.
- The mixed-base ONs separate using different flow rates, gradient curves, temperatures, and ion-pair reagents can be adjusted to improve resolution and/or reduce analysis time.

Instrumentation

- Vanquish Flex Binary UHPLC system
- Chromeleon CDS

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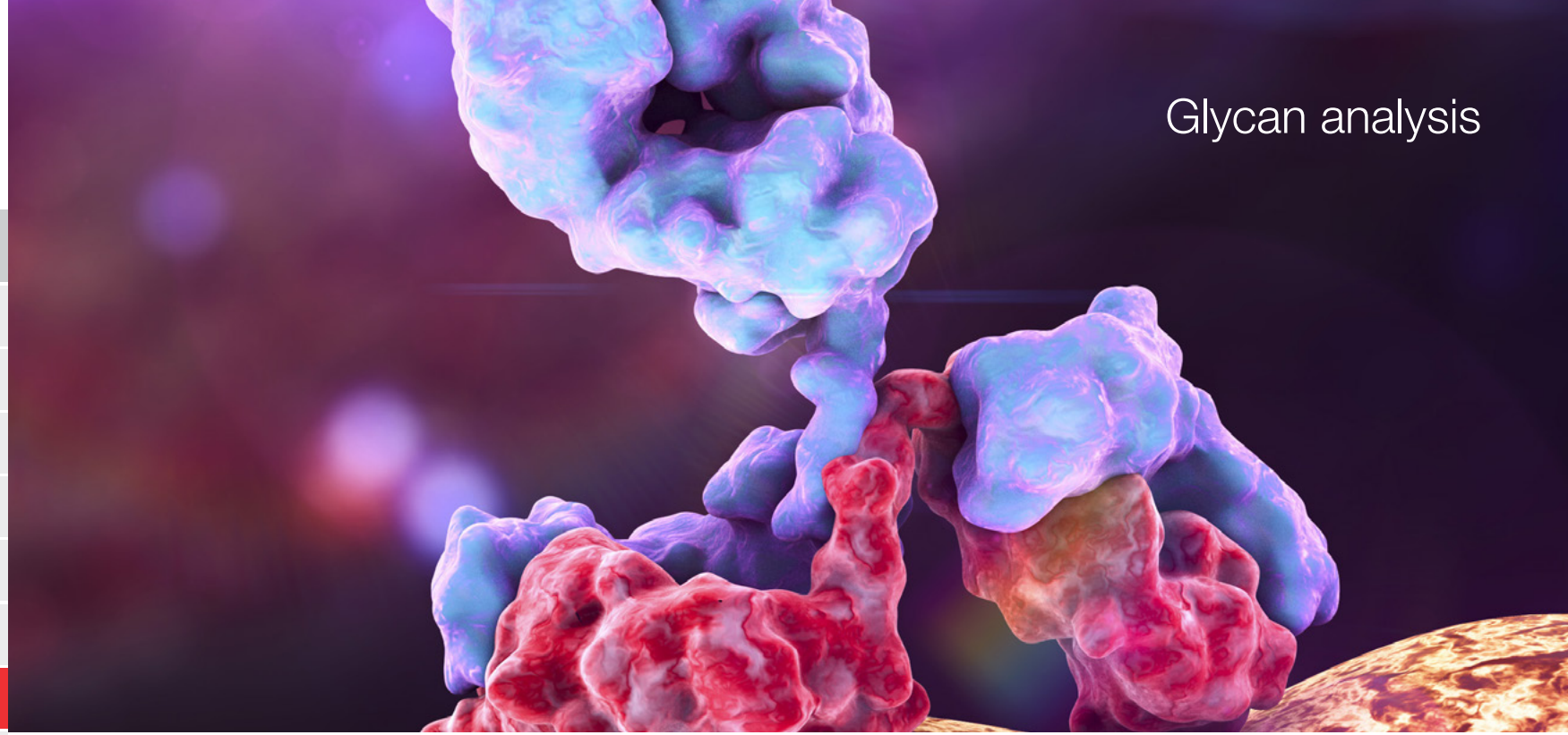
Glycan analysis



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In biotherapeutics, recombinant monoclonal antibodies (mAbs) have gained significant importance in diagnostic and therapeutic applications in recent years. The general structural features of mAbs, such as assembly of light and heavy chains via disulfide bridges, are commonly known. These molecules contain complex oligosaccharide moieties whose sites of attachment, presence or absence, and relative profiles can have a significant impact on therapeutic efficacy, pharmacokinetics, immunogenicity, folding, and stability of a biologic drug. Thorough characterization is necessary to verify the correctness of the overall molecule and provide a reproducible, safe, and effective biological drug compound. Mass spectrometry (MS) has emerged as one of the most powerful tools for the structural elucidation of glycans. This is due to its sensitivity of detection and its ability to analyze complex mixtures. The complex branching and isomeric nature of glycans pose significant analytical challenges to the identification of these structures. In addition to the characterization of the sugar sequence, the analysis must elucidate branching, linkages between monosaccharide units, and the location of possible sulfate or phosphate groups. To ensure comprehensive structural elucidation and structural isomer differentiation, a combination of high mass resolution, accurate mass, sensitivity, multistage fragmentation, and multiple fragmentation techniques are required.

The following application notes demonstrate the power of Thermo Scientific™ Orbitrap™ mass spectrometers for glycan and glycopeptide analysis. An example of the robustness, reproducibility, accuracy, and precision of quantification of N-glycans using a Thermo Scientific™ Accucore™ 150 Amide HILIC column with the Vanquish Horizon UHPLC integrated biocompatible system is also presented.

To learn more, visit Thermo Scientific Glycan Analysis Learning Center

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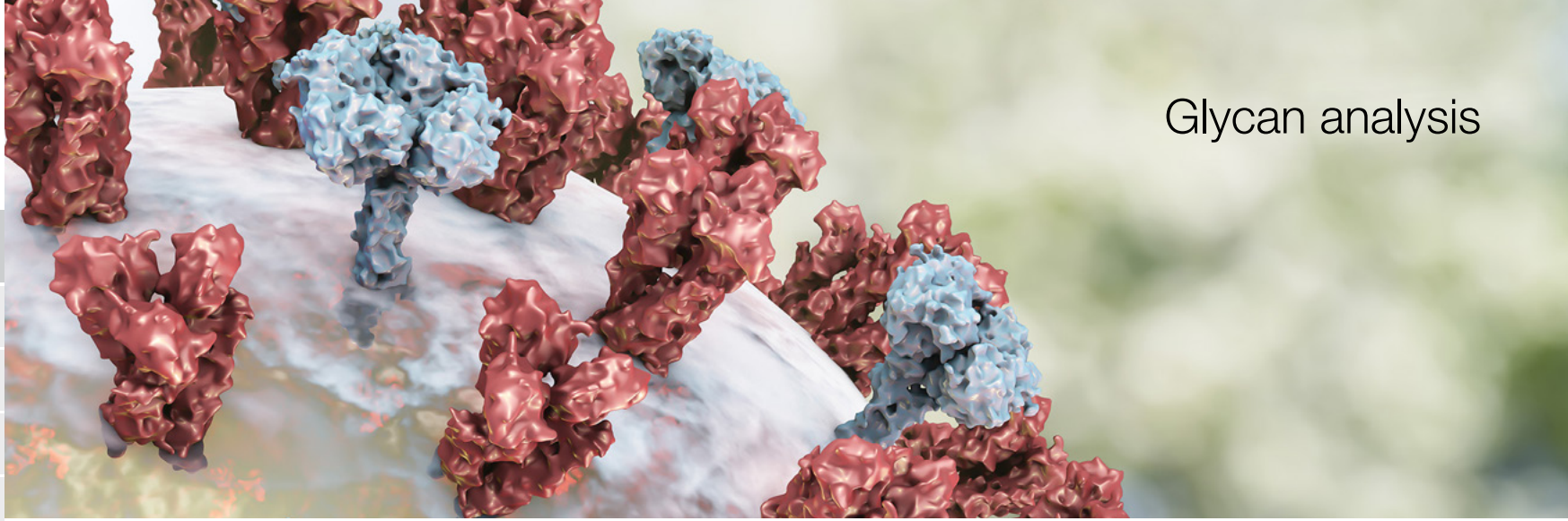

Glycan analysis

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Improved Profiling of Sialylated *N*-linked Glycans by Ion Chromatography-Orbitrap Mass Spectrometry

Sachin Patil and Jeff Rohrer, Thermo Fisher Scientific, Sunnyvale, CA.

Overview

Glycoproteins are central to several important biological functions. With increased understanding of the biological significance of carbohydrates, large numbers of glycoprotein therapeutics are being developed for various pathological conditions. Given the interest in glycoproteins as therapeutics, there have been attempts to use high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) to create a glycan database. HPAE-PAD is very effective for separating sialylated glycans, but there are opportunities to increase resolution and improve glycan identification. The coupling of a HPAE chromatography system to a Thermo Scientific™ Orbitrap™ mass spectrometer can harvest synergy between these two techniques to facilitate rapid structure determination.

This application note presents an improved approach to further increase coverage in glycan analysis using HPAE-PAD coupled to an HRAM mass spectrometer. This application note also evaluates how changing elution conditions can resolve different glycan structures using HPAE coupled to a HRAM mass spectrometer.

Key points

- Demonstrates a HPAE-MS method for improved glycan profiling.
- Highlights the use of Thermo Scientific™ Dionex™ CarboPac™ PA200 column for high-efficiency, high-resolution glycan separation.

Instrumentation

- Thermo Scientific™ Dionex™ ICS-5000+ High-Pressure Ion Chromatography (HPIC™) system
- Chromeleon CDS
- Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ mass spectrometer with HESI-II probe
- Thermo Scientific™ Dionex™ AS-AP autosampler (P/N 074926) with cooling tray option (recommended)
- Thermo Scientific™ Dionex™ ERD 500 desalter (P/N 085089)

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Glycan analysis

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Subunits Analysis Approach for the Determination of Fucosylation Levels in Monoclonal Antibodies Using LC-HRAM-MS

Craig Jakes, Sara Carillo, Izabela Zaborowska, and Jonathan Bones, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland.

Overview

N-glycans on the Fc region of a mAb can affect stability and interfere with the intended mechanism of action of a drug. It has been demonstrated that when the mAb presents an afucosylated chitobiose core, the affinity of the mAb for the FcγRIII expressed on natural killer cells is enhanced leading to an antibody-dependent cellular cytotoxicity (ADCC). In antibody-drug development, much effort is made to enhance this feature, and an accurate quantification of glycan core fucosylation is often required.

In this application note, LC-HRAM-MS is used in a middle-up approach for the quantification of core afucosylation on three mAbs. The samples are digested with IdeS and EndoS enzymes in non-reducing conditions for analysis. The results demonstrate the benefits of using middle-up analytical methods for the simultaneous quantification of mAb core afucosylation and high-mannose type glycans, which affect efficacy and drug clearance. This study also demonstrates the applicability of the Q Exactive Biopharma platform equipped with MabPac RP columns resulting in quick and highly accurate data sets. BioPharma Finder software allows for quick deconvolution of the spectra and confident identification of the subunits and their variations.

Key points

- Demonstrate the benefits of using middle-up analytical methods for the simultaneous quantification of mAb core afucosylation and high-mannose type glycans, which affect efficacy and drug clearance
- Demonstrate the applicability of MAbPac RP columns for mAbs subunits analysis
- Highlight the benefits of using the Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer for high-resolution, accurate-mass MS analysis on different domains of monoclonal antibodies

Instrumentation

- Vanquish Flex Binary UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- Nanodrop 2000 Spectrophotometer
- BioPharma Finder software

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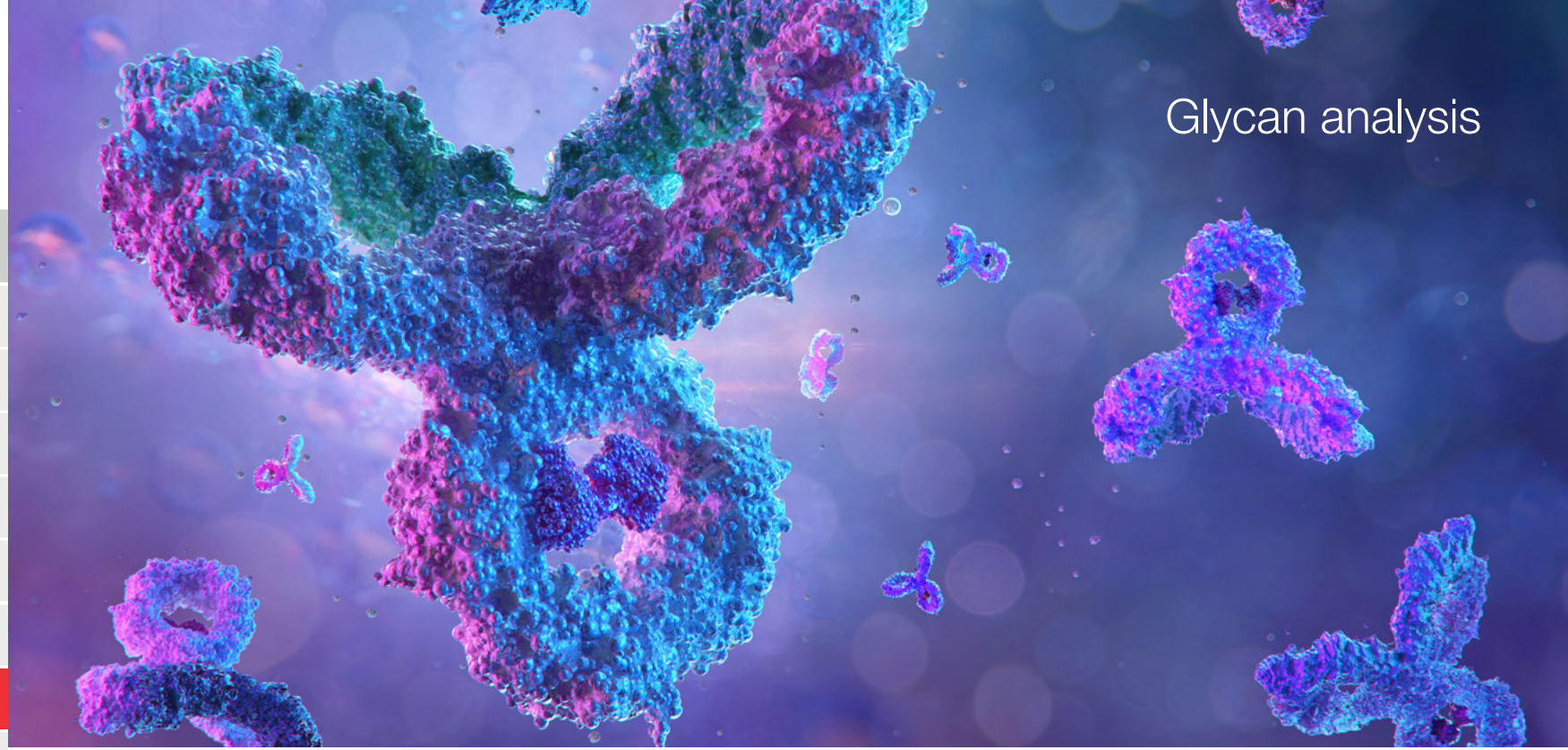

Glycan analysis

HILIC – An Alternative Separation Technique for Glycopeptides

Alexander Ley, Martin Samonig, and Mauro De Pra, Thermo Fisher Scientific, Germering, Germany.

Overview

Glycosylation affects the potency and efficacy of biotherapeutics. During optimization of the production process, the levels of critical glycosylations are monitored and used as feedback to facilitate development, in order to achieve a given glycan pattern. After enzymatic digestion, peptide mapping is typically run by reversed-phase (RP) chromatography. However, unlike RP chromatography which provides very low retention of glycopeptides, hydrophilic interaction liquid chromatography (HILIC) offers significantly more retention for glycopeptides than peptides.

In this application note, an approach to efficiently separate peptides and glycopeptides of a monoclonal antibody in a single run is demonstrated. This work demonstrates suitability of the Vanquish Flex UHPLC system and Accucore 150 Amide HILIC column for efficient and reliable separation of glycopeptides.

Key points

- Demonstrates an approach to efficiently separate peptides and glycopeptides in a single run.
- Showcases the high application flexibility of the Vanquish Flex Quarternary UHPLC system.
- Demonstrates the reproducibility using the SMART Digest kit for digestion followed by the Accucore 150 Amide HILIC column.

Instrumentation

- Vanquish Flex Binary UHPLC system
- Q Exactive HF Hybrid Quadrupole-Orbitrap mass spectrometer

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Glycan analysis

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Accurate and Precise Quantification of mAb-released *N*-glycans with an Amide HILIC Column

Xin Zhang, Xuefei Sun, Thermo Fisher Scientific, Sunnyvale, CA, USA.

Overview

Glycosylation is one of the key CQAs of mAb-based biotherapeutics. Glycosylation changes can impact a biological drug's safety, efficacy, clearance, and immunogenicity, making it necessary to accurately detect changes. Robust, information-rich, and reproducible methods for glycan analysis must be included in regulatory filings for glycoprotein-based biotherapeutics to ensure accuracy and consistency. Current glycan analysis methods involve sample preparation, followed by high-performance capillary electrophoresis (HPCE) or HPLC separation.

The aim of this application note is to demonstrate the robustness, reproducibility, accuracy, and precision of *N*-glycans quantitation using an Accucore 150 Amide HILIC column with the Vanquish Horizon UHPLC integrated biocompatible system. This platform accurately and precisely quantifies *N*-glycans of human IgG, illustrating an impressive correlation with a fully validated manufacturing quantification method.

Key points

- Proven intra- and inter-column reproducibility in retention time, backpressure, and peak shape with overall run-to-run % RSD < 2% and lot-to-lot % RSD < 7%
- Solid core particle results in moderate backpressures with UHPLC efficiencies
- Accurate and precise quantification of human IgG N-glycans with absolute average % bias at 3%
- Excellent correlation (R^2 > 0.99 in Deming regression) with a fully validated manufacturing quantification method

Instrumentation

- Vanquish Horizon UHPLC system

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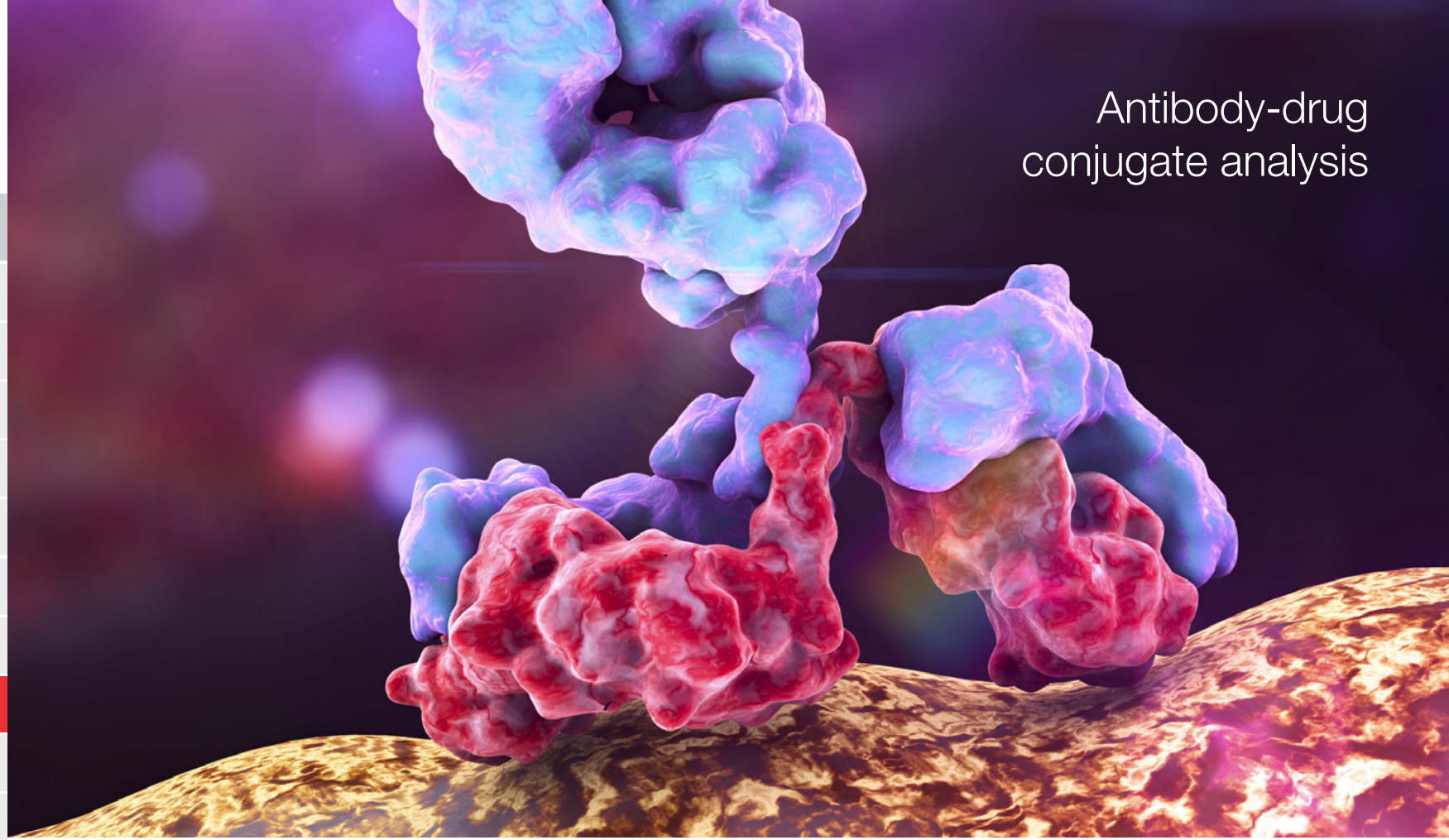

Antibody-drug conjugate analysis

The effective targeting of MAb's towards infected cells has given rise to the use of antibody-drug conjugates (ADC) where a toxic small drug molecule is linked to the antibody and direct the toxic payload to the required area of treatment. Combining the targeting capabilities of a mAb with a potent cancer-killing drug allows sensitive discrimination between healthy and diseased cells. Development of these biotherapeutics is more difficult than development of mAbs alone due to the potency of the conjugated drug, and extra molecular complexity requiring the control of more product attributes..

Our powerful technologies, including specific UHPLC systems, mass spectrometry, and LC columns designed for detailed ADC characterization, make separation and characterization of these novel molecules easier. The following examples demonstrate the full characterization of various ADCs using both the LC-MS platform with the Vanquish Flex UHPLC coupled to the Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer system and the ZipChip™ system coupled to the Q Exactive Plus.

To learn more, visit Thermo Scientific Antibody-Drug Conjugate Learning Center

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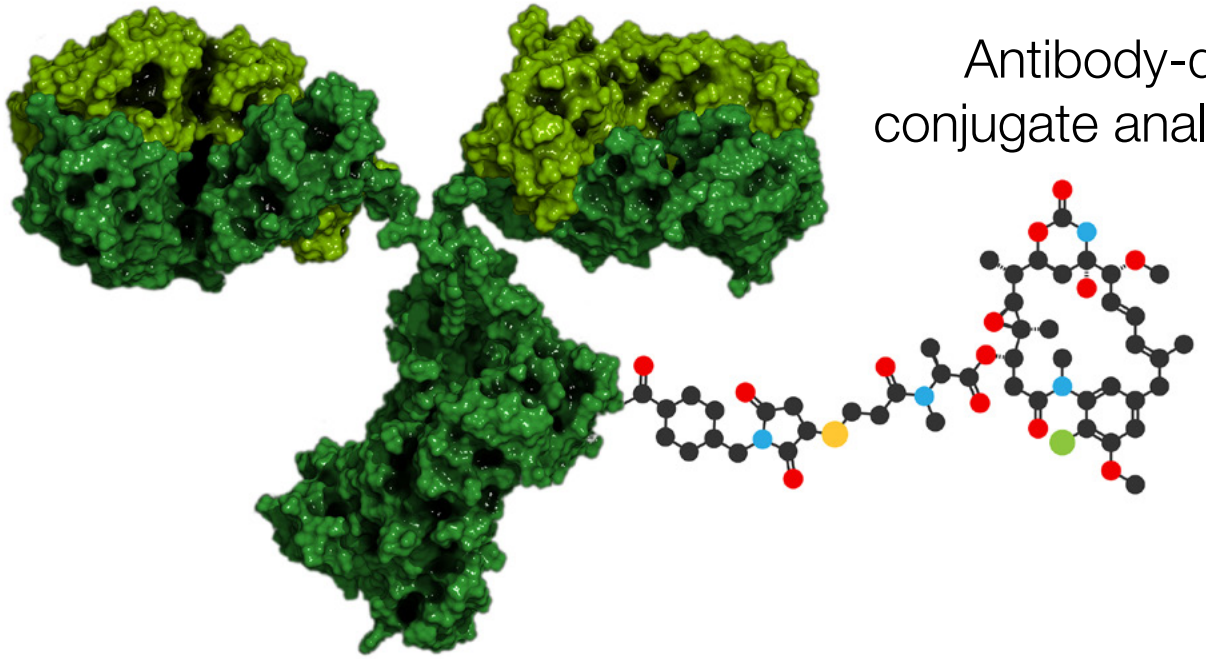
Antibody-drug conjugate analysis



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Complete Characterization of a Lysine-linked Antibody Drug Conjugate by Native LC-MS Intact Mass Analysis and Peptide Mapping

Aaron O. Bailey,[1] Stephane Houel,[1] Kai Scheffler,[2] Eugen Damoc,[3] Jennifer Sutton,[1] and Jonathan L. Josephs,[1] [1]Thermo Fisher Scientific, San Jose, CA, USA, [2]Thermo Fisher Scientific, Dreieich, Germany, [3]Thermo Fisher Scientific, Bremen, Germany.

Overview

Antibody-drug conjugates (ADCs) join a disease-specific mAb (usually cancer-specific) with one or more cytotoxic small molecules. Monoclonal antibodies are used to deliver highly toxic compounds directly to cancer cells minimizing non-specific toxicity and patient morbidity.

This application note demonstrates the complete characterization of the lysine-linked ADC trastuzumab emtansine using both intact mass and peptide mapping approaches with a single benchtop mass spectrometer. The use of an LC-MS platform with the Vanquish Flex Binary UHPLC System and Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer system flawlessly separates and characterizes the complex ADC and its different DAR species forms.

Key points

- Demonstrates critical quality attribute monitoring including drug-to-antibody ratio, glycosylation pattern, and conjugation site localization.
- Showcases peptide mapping application of sample digestion using SMART digest and peptide separation obtained with Thermo Scientific™ Acclaim™ VANQUISH™ C18 RP column.
- Demonstrates the use of size exclusion chromatography with the MAbPac SEC-1 column and native, MS-friendly mobile phases for spectra at higher mass-to-charge and reduced charge state values.

Instrumentation

- Vanquish Flex Binary UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer

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Antibody-drug conjugate analysis

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Quick Screening of Intact Antibody and Antibody Drug Conjugates with Integrated Microfluidic Capillary Electrophoresis and Mass Spectrometry

Chien-Hsun Alex Chen,[1] Stephane Houel,[1] Terry Zhang,[1] Brian J. Agnew,[2] Shanhua Lin,[3] Kai Zhou,[1] Jonathan Josephs,[1] Aran Paulus,[1] and Andreas F. R. Huhmer,[1]
[1]Thermo Fisher Scientific, San Jose, CA, USA, [2]Thermo Fisher Scientific, Eugene, OR, USA, [3]Thermo Fisher Scientific, Sunnyvale, CA, USA.

Overview

Monoclonal antibodies and antibody-drug conjugates are two important categories of drugs in biopharmaceuticals. During drug development and manufacturing, undesired mutations and *in vitro* modifications may introduce sample heterogeneity, causing changes to the protein structure impacting the safety and efficacy of the biotherapeutic. Therefore, the ability to quickly detect and assess any variability in the protein that might occur during drug development is attractive. Multiple analytical methods such as HPLC, capillary electrophoresis (CE), and mass spectrometry have been used separately or together to characterize mAbs and ADCs.

In this application note, a CE-MS method can be achieved by coupling the ZipChip™ (908 Devices) system to the Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer to rapidly analyze the heterogeneity of mAb and ADC samples within three minutes.

Key points

- Demonstrates the high resolution accurate mass (HRAM) capabilities of the Q Exactive MS with BioPharma option for the detection of very large biomolecules with mass range up to *m/z* 8,000.
- This application describes the combination of CE using the ZipChip™ system and the Q Exactive MS with BioPharma option in one platform and demonstrates its ability for quick screening of biopharmaceutical drug heterogeneity.

Instrumentation

- Q Exactive Plus Hybrid Quadrupole-Orbitrap MS with BioPharma option
- BioPharma Finder software
- ZipChip™ HR chip
- ZipChip™ system

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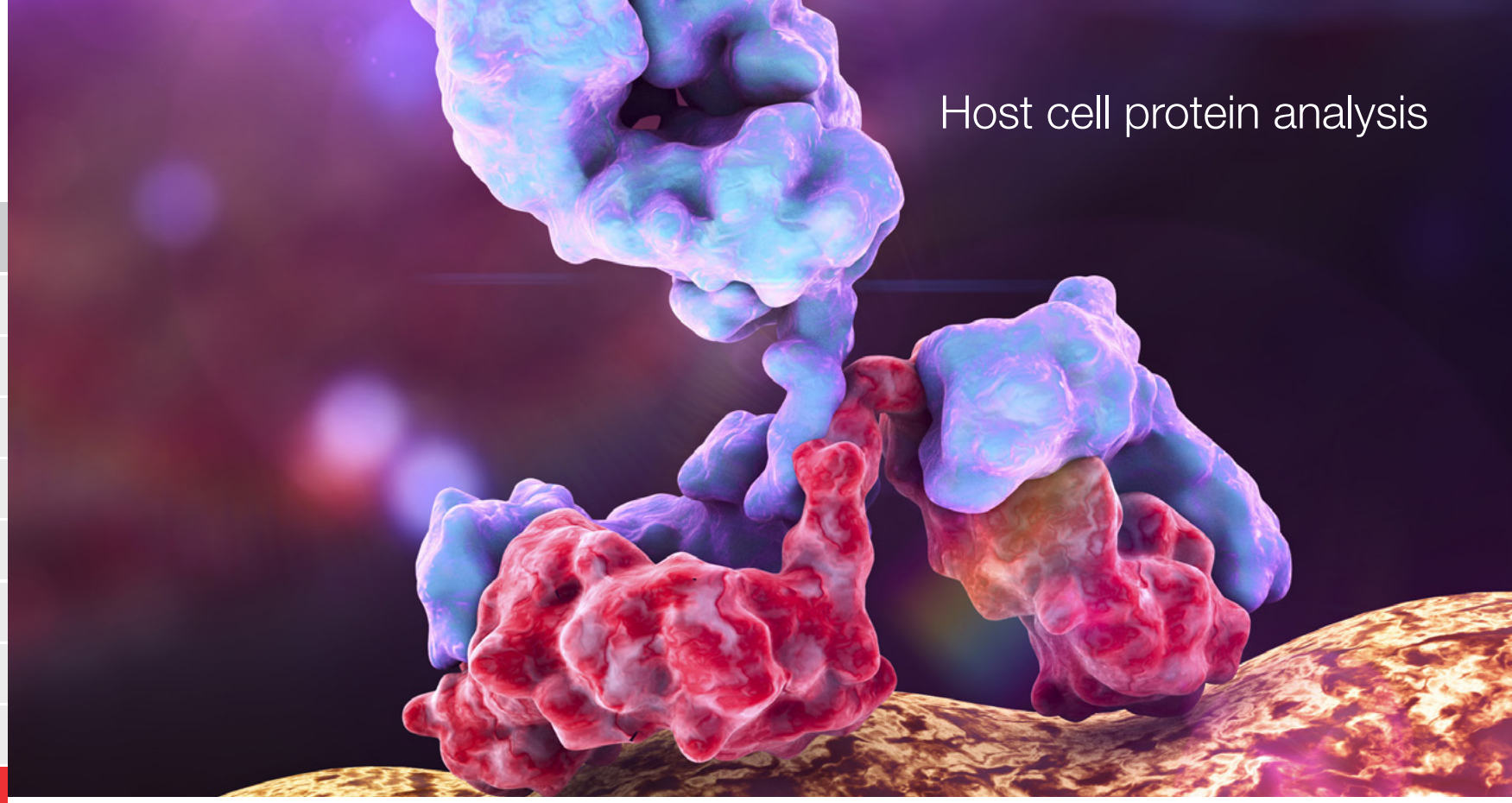

Host cell protein analysis

Host cell proteins (HCPs) are low-level, process-related protein impurities in drug products derived from the host organism during biotherapeutic manufacturing. During expression of a recombinant protein drug, host cell systems can express many endogenous proteins. Purification of the drug from these HCP contaminants can be challenging, with low-level contamination remaining after purification. The detection of HCPs in biotherapeutic proteins is an important analytical requirement because HCPs can present potential safety risks or impact product stability. HCP analysis is challenging because extensive dynamic range is required to be able to detect low ppm concentrations of residual HCPs. Also, to be widely adopted by the biopharmaceutical industry, the analytical solution must be extremely robust and relatively fast.

In the following application notes, UHPLC system is coupled to an HRAM mass spectrometer to identify and quantify multiple HCPs in a single chromatographic separation, allowing for HCP monitoring throughout the purification process. A non-denaturing protein digest using Magnetic SMART Digest kit with the automation of the KingFisher Duo Prime system improved HCP analysis by increasing throughput, reducing the sample dynamic range, and reducing complexity.

To learn more, visit Thermo Scientific Host Cell Protein Analysis Learning Center

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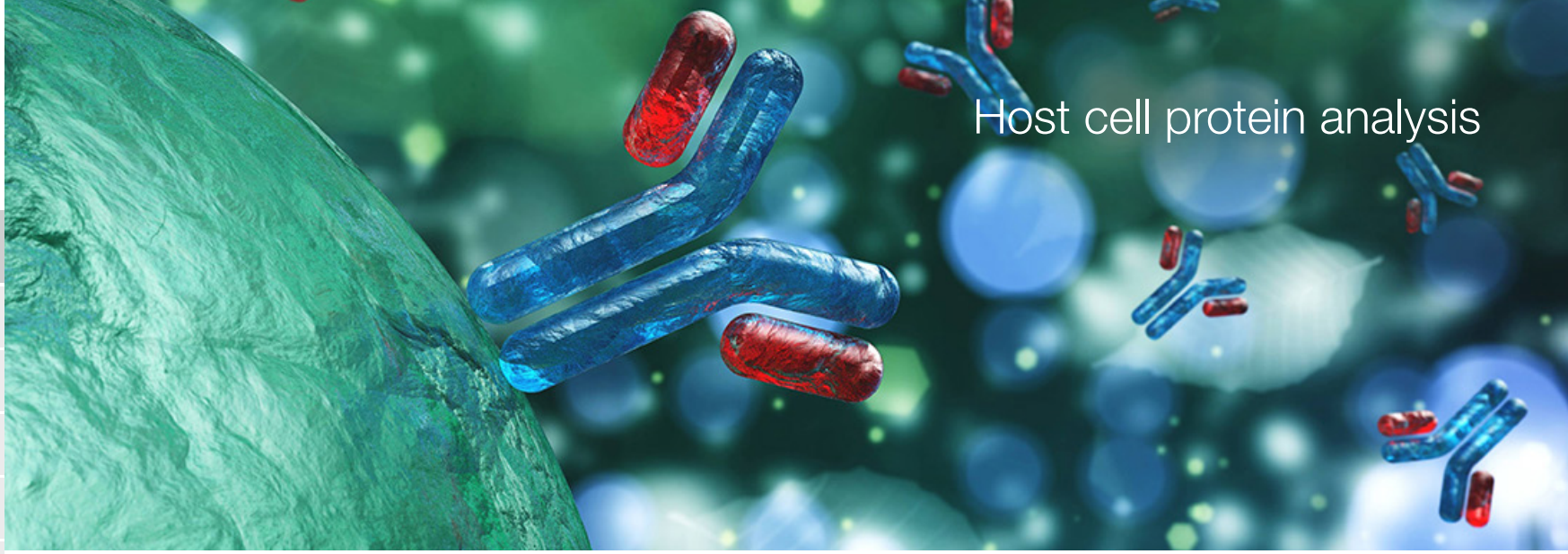

Host cell protein analysis

Residual Host Cell Protein Analysis of NISTmAb: From Simplified Sample Preparation to Reliable Results

Amy Claydon, Phil Widdowson, and Andrew Williamson.

Overview

Recombinant biotherapeutics are produced using non-human host cells, such as Chinese hamster ovary cell lines. During cell growth and harvest, host cell proteins (HCPs) are released that can detrimentally affect drug product safety and efficacy. These HCPs must be removed post-harvest through a series of purification steps. Consequently, HCP levels in mAb biotherapeutics must be determined prior to drug release. The current regulatory gold-standard for HCP analysis is enzyme-linked immunosorbent assay (ELISA). However, developing a new HCP ELISA requires suitable anti-HCP reagents. In addition, an ELISA does not have the capacity for individual protein identification or quantification.

This application note explores methods to increase the identification and characterization of HCPs. A non-denaturing protein digest takes advantage of the native monoclonal antibody's resistance to proteolytic digestion, reducing intrasample dynamic range and increasing the likelihood of HCP identification. Ultra-high-pressure liquid chromatography combined with high-resolution accurate-mass mass spectrometry can identify and quantitate multiple host cell proteins in a single chromatographic separation.

Download Application Note 73412

Key points

- Non-denaturing protein digestion with SMART Digest kit reduces intrasample dynamic range and increases host cell protein (HCP) identifications.
- Efficient and reproducible chromatographic separation with Acclaim VANQUISH C18, 2.1 × 250 mm, column.
- High sensitivity detection over large dynamic range, down to sub-1 ppm, using Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer.
- High-resolution accurate-mass (HRAM) MS data combined with Thermo Scientific™ Proteome Discoverer™ software provides confident HCP identifications.

Instrumentation

- KingFisher Duo Prime purification system
- Vanquish Horizon UHPLC system
- Orbitrap Exploris 480 mass spectrometer
- Thermo Scientific™ Proteome Discoverer software
- BioPharma Finder software

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Host cell protein analysis

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Easy, Fast and Reproducible Analysis of Host Cell Protein in Monoclonal Antibody Preparations

Giorgio Oliviero,[1] Ken Cook,[2] Kai Scheffler,[3] Florian Füssl,[1] Jonathan Bones,[1] [1]National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland, [2]Thermo Fisher Scientific, Hemel Hempstead, UK, [3]Thermo Fisher Scientific, Germering, Germany.

Overview

As the biopharmaceutical industry expands, the global demand for mAbs and recombinant proteins continues to increase. Monoclonal antibodies are produced by recombinant DNA-technology using a non-human host cell (e.g., Chinese hamster ovary cells). Most HCPs are removed during chromatographic purification steps, such as protein A/G affinity chromatography. However, some HCPs are present at very low concentrations in the final drug product. They can interfere with the product activity and/or stability and can compromise patient safety. The most popular methods for HCP detection are ELISAs and protein gel blots. These analytical techniques use a semi-quantitative approach that targets only a small set of expected proteins. This bears the risk of missing unexpected or unknown proteins that may still be present in the final drug product. LC-MS/MS is a powerful and sensitive method that can perform both quantitative and qualitative analyses of HCP impurities. In this application note, we present an LC-MS/MS approach for the determination of HCPs in a mAb drug product using the same workflow employed for peptide mapping analysis, without compromising chromatographic resolution or MS sensitivity.

Key points

- Improved reproducibility and reduced sample preparation time with automated digestion for host cell protein (HCP) analysis.
- Easy HCP analysis using the same workflow optimized for standard peptide mapping analysis of monoclonal antibodies.
- High confidence in results with excellent data quality using a Acclaim VANQUISH C18, 2.1 × 250 mm column for HCP and peptide mapping analysis.
- Detection of low abundance HCP proteins using automated magnetic bead digestion technology that facilitates excellent recovery of samples with less sample handling, increased reproducibility, and improved reliability of data.

Instrumentation

- KingFisher Duo Prime purification system
- Vanquish Flex Binary UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- Nanodrop 2000 Spectrophotometer

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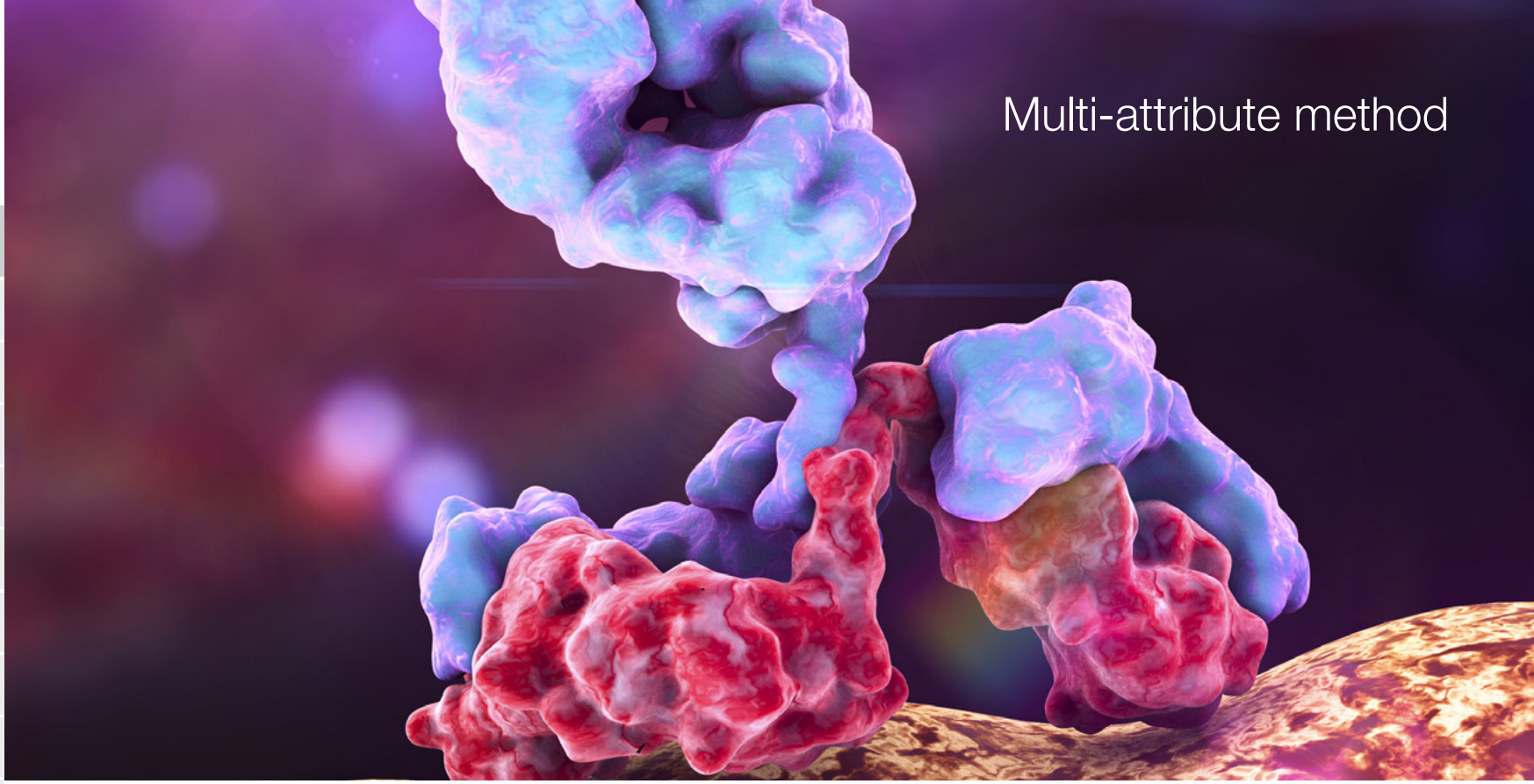

Multi-attribute method

Small modifications in protein sequence can impact the safety and biological activity of a biopharmaceutical product. By directly measuring potential critical quality attributes (CQA), information can be derived for the optimization of production processes ultimately affecting product quality. Evaluating and tracking product quality attributes is crucial to ensure quality, safety, and efficacy of biotherapeutics. Traditional chromatography methods are profile-based and are often not capable of identifying and quantifying potential residue-specific CQAs. The Multi-Attribute Method (MAM) is a mass spectrometry-based peptide mapping method that provides molecular level details in a single analysis. The number of individual tests (e.g. CEX, CE-SDS, HILIC, ELISA) for specific potential CQAs can be decreased considerably using an appropriately developed MAM.

The following application note demonstrates the optimization and application of the Thermo Scientific™ HR Multi-Attribute Method (HR MAM) as a complete workflow to monitor CQAs of the NISTmAb standard, including glycosylation, deamidation, isomerization, succinimide formation, oxidation, C-terminal lysine truncation, N-terminal pyroglutamate, and glycation, under normal and stressed conditions. This application also showcases the capability of the HR MAM workflow for new peak detection (NPD) using spiked and stressed samples. The high-quality data generated by the combination of high-resolution chromatography and HRAM MS is seamlessly interrogated by a powerful software workstream to provide the maximum confidence in securing product quality.

To learn more, visit Thermo Scientific HR MAM Workflow

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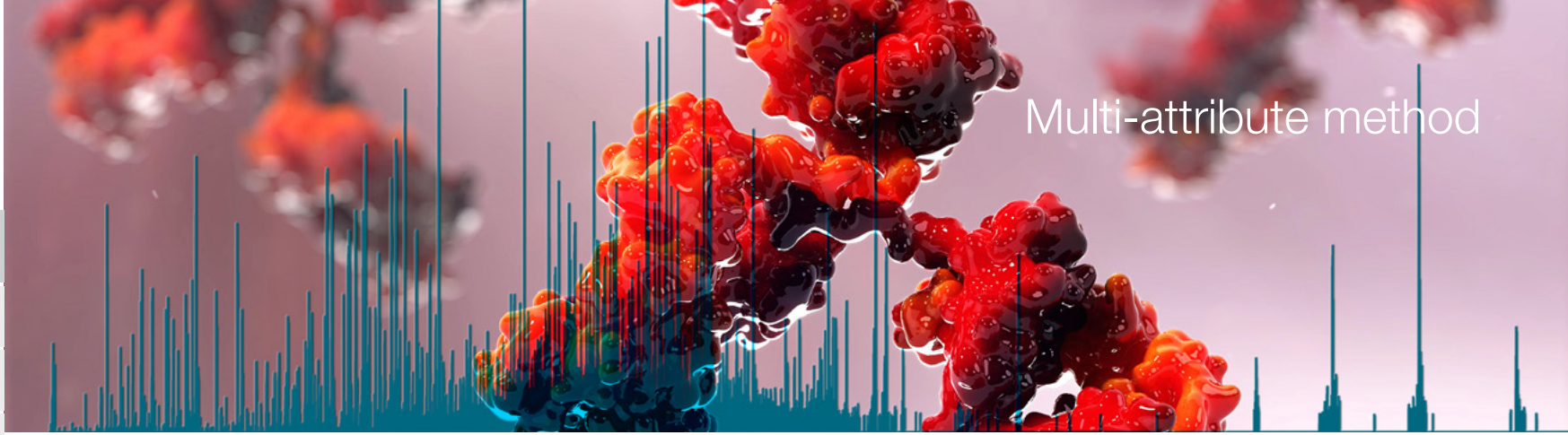

Multi-attribute method

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A High-resolution Accurate Mass Multi-attribute Method for Critical Quality Attribute Monitoring and New Peak Detection

Haichuan Liu,[1] Royston Quintyn,[2] John Rontree,[3] [1]Thermo Fisher Scientific, San Jose, CA, USA, [2]Thermo Fisher Scientific, West Palm Beach, FL, USA, [3]Thermo Fisher Scientific, Hemel Hempstead, UK.

Overview

Since the multi-attribute method (MAM) was introduced, it has gained popularity and acceptance in the biopharmaceutical industry, featuring as a hot topic in many recent conferences. With Quality by Design principles in mind, MAM could replace several traditional techniques used in QC for the lot release of drugs. Taking advantage of the HRAM capabilities of Orbitrap-based MS detection, MAM can simultaneously identify, quantify, and monitor product quality attributes of therapeutic proteins. Furthermore, MAM can identify impurities with new peak detection (NPD) when comparing to the reference product.

This study focuses on the optimization and application of the Thermo Scientific™ HR Multi-Attribute Method as a complete workflow to monitor CQAs of the NISTmAb standard, including glycosylation, deamidation, isomerization, succinimide formation, oxidation, C-terminal lysine truncation, N-terminal pyroglutamate, and glycation, under normal and stressed conditions. In addition, it demonstrates the capability of the HR MAM workflow for NPD using spiked and stressed samples.

Key points

- Demonstrates the use of the complete HR MAM workflow to monitor CQAs of the NIST mAb standard.
- Showcases the use of HRAM, allowing for the resolution of species that would otherwise be chromatographically overlapping.
- Seamless transition from BioPharma Finder software to Chromeleon CDS enables CQA monitoring and NPD within a compliant GMP environment.

Instrumentation

- Vanquish Horizon UHPLC system
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer
- BioPharma Finder software Peptide Mapping Only
- Chromeleon CDS

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New key application briefs on Thermo Scientific Orbitrap Exploris 240 mass spectrometer

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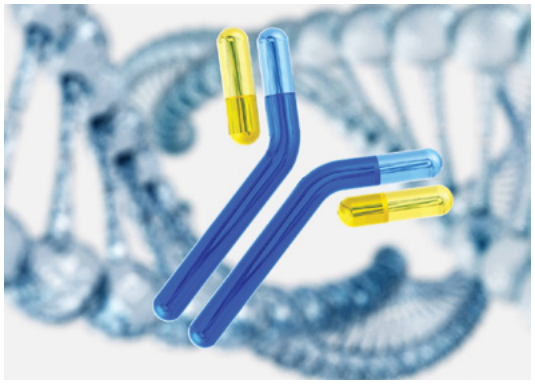

Confident peptide mapping and disulfide bond analysis of an IgG2 monoclonal antibody

Key points

- Complete sequence coverage peptide map of monoclonal antibody
- Confident detection of inter- and intrachain disulfide bonds
- Sensitive detection of low-level post-translational modifications with excellent mass accuracy

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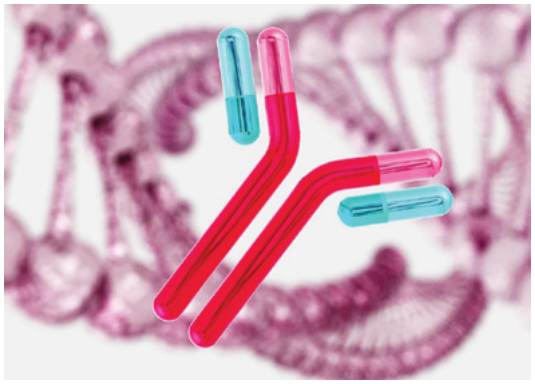

Characterization of IgG1 monoclonal antibody (mAb) oxidation variants at intact, subunit and peptide levels

Key points

- Confident site-specific identification and localization of oxidation hotspots
- System versatility enables the analysis of protein biotherapeutics at the intact protein, subunit and peptide levels using standard conditions and simple set-up routines
- Exceptional spectral clarity enables robust deconvolution and simplified spectral interpretation

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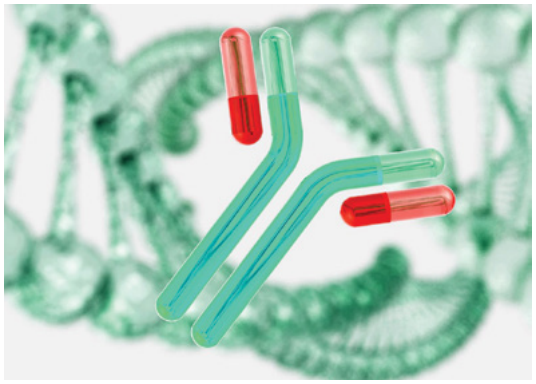

Sensitive profiling of IgG1 monoclonal antibody variants under native conditions

Key points

- Operational simplicity for mass spectrometer setup and acquisition under native conditions using standardized tune conditions
- Simplified data interpretation from exceptional spectral clarity and confident mass accuracy
- Exceptional sensitivity and mass accuracy for intact mass analysis under native conditions allowing confident analysis from low sample loading

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We hope you have enjoyed this collection of application notes that offer a comprehensive toolbox of technologies for comprehensive biopharmaceutical characterization. Further application notes are in development and if you are interested in viewing these as they become available, we would like to direct you to the Thermo Fisher Pharma and BioPharma Learning Center where new documents will be posted.

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