BioPharma Applications Compendium

AGLENT APPLICATIONS FOR BIOPMARMACEUTICAL DISCOVERY, DEVELOPMENT AND QA/QC

The Measure of Confidence





Agilent Technologies

MAXIMIZE DEVELOPMENT AND ENSURE QUALITY

As you move from biopharmaceutical discovery into development, your success depends on encountering as few surprises as possible.

Enjoy the confidence of knowing the exact state of your biomolecule throughout the entire development process. Agilent solutions provide robust and reliable analytical methods that ensure the thorough characterization of biopharmaceuticals and complication-free transfer to quality control.

What's Inside?

This collection of biopharmaceutical applications will help you find the right solution for your analytical needs. From biopharmaceutical characterization to stability monitoring throughout production, Agilent has enabling solutions that build confidence and reliability in all your analytical measurements.

Table of Contents

Interactive file users: click on the titles below to access the appropriate information.

	Protein Analysis by Mass Spectrometry	4
	Aggregate and Fragment Analysis	5
	Charge Variant Analysis	6
	Glycoform and Glycan Analysis	7
1-7-7	Post-Translational Modifications and Degradation Analysis	8
	Peptide Mapping	9
	Biosimilars	10
	2D-LC, High-Throughput LC and Workflow Automation	11
	Automated Sample Preparation	12
	Host Cell and Process-Related Impurities	13
	Titer and Protein Quantitation	14
H ₁ N ¹ COOH OH COOH H ₂ N ¹ COOH H ₂ N ¹ COOH H ₂ N ¹ COOH	Raw Material and Media Analysis	15
0,0 0,0 0,0	Capillary Electrophoresis and Micro-Fluidic Electrophoresis of Protein	16
CATCAGTACGTTACAG	Nucleic Acid Analysis	17
	Compliance, QC and QbD	18
	Overview of Agilent Solutions	19

PROTEIN ANALYSIS BY MASS SPECTROMETRY

Confirmation of intact protein mass, major glycoforms, and other post-translational modifications (PTMs) are all critical measurements for characterizing therapeutic proteins and understanding their efficacy and stability. Mass spectrometry is the primary tool that enables all of these measurements on a single platform with high mass accuracy, specificity and sensitivity.

The application notes listed below detail highly accurate solutions we have developed for routine measurements of intact protein mass and common PTMs using Accurate-Mass Time-of-Flight (TOF) LC/MS and Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS platforms. See more about Agilent solutions for protein analysis by **mass spec**.

- Characterization of Monoclonal Antibodies Using Capillaray Electrophoresis-Electrospray Ionization-Mass Spectrometry (CE-ESI-MS).
 5991-5212EN
- Analysis of Monoclonal Antibody (mAb) Using Agilent 1290 Infinity LC System Coupled to Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight.
 5991-4266EN.
- Agilent MassHunter Easy Access
 Software for Lot-to-Lot Purification
 Analysis of a Model Therapeutic Protein.
 5991-3521EN
- Intact Protein Analysis Using an Agilent 6550 Q-TOF Mass Spectrometer. 5991-2116EN
- Analysis of Polyethylene Glycol (PEG) and a Mono and Di-PEGylated Therapeutic Protein Using HPLC and Q-TOF Mass Spectrometry.
 5991-1509EN

- Identification of Oxidation Sites on a Monoclonal Antibody Using an Agilent 1260 Infinity HPLC-Chip/MS System Coupled to an Accurate-Mass 6520 Q-TOF LC/MS.
 - 5990-8768EN
- Quantitation of Oxidation Sites on a Monoclonal Antibody Using an Agilent 1260 Infinity HPLC-Chip/MS System Coupled to an Accurate-Mass 6520 Q-TOF LC/MS.

5990-8769EN

- Characterization of Bacteriophage Derived Anti-Staphylococcal Protein (P128) from Production to Purification Using Agilent HPLC-Chip Q-TOF LC/MS System.
 5990-7952
- Primary Characterization of a Monoclonal Antibody Using Agilent HPLC-Chip Accurate-Mass LC/MS.
 5990-3445EN



AGGREGATE AND FRAGMENT ANALYSIS

Aggregates that form due to improper production, storage, or handling conditions can have important consequences for the safety and efficacy of biopharmaceuticals. The presence of aggregates is undesirable for two reasons: small aggregates can lead to immune reactions and particulates can cause adverse events upon administration.

The application notes listed below detail reliable solutions we have developed for routine protein sizing and the quantification of aggregates using electrophoresis, liquid chromatography, and size exclusion columns. See more about Agilent solutions for **aggregate and fragment analysis**.

- Determination of Protein Molecular Weight and Size Using the Agilent 1260 Infinity Multi-Detector Bio-SEC Solution with Advanced Light Scattering Detection.
 5991-3955EN
- Detailed Aggregation Characterization of Monoclonal Antibodies Using the Agilent 1260 Infinity Multi-Detector Bio-SEC Solution with Advanced Light Scattering Detection.
 5991-3954EN
- Agilent 1260 Infinity Multi-Detector GPC/SEC System.

5991-2884EN

- Choosing the Right Calibration for the Agilent Bio SEC-3 Column.
 5991-2463EN
- Multiple Detector Approaches to Protein Aggregation by SEC.
 5991-1400EN

- Development and Partial Validation of a SEC Method for High-Resolution Separation and Quantification of Monoclonal Antibodies.
 5991-0835EN
- Optimum Pore Size for Characterizing Biomolecules with Agilent Bio SEC Columns.
 5990-9894EN
- Rapid UHPLC Analysis of Reduced Monoclonal Antibodies using an Agilent ZORBAX Rapid Resolution High Definition (RRHD) 300SB-C8 Column.
 5990-9631EN
- Precise Determination of Protein Molecular Weight Using the Separation of Recombinant Human Erythropoietin (rEPO) Using AgilentBio SEC-3.
 5990-9544EN

- Defining the Optimum Parameters for Efficient Size Separations of Proteins. 5990-8895EN
- Fast Separation of Monoclonal Antibody and Dimer by SEC with Agilent Bio SEC. 5990-8613EN
- Characterization of Monoclonal Antibodies on the Agilent 1260 Infinity Bio-Inert Quaternary LC by Size Exclusion Chromatography Using the Agilent BioSEC Columns.

5990-6414EN



CHARGE VARIANT ANALYSIS

Proteins can exhibit changes in charge heterogeneity during biopharmaceutical production and purification processes. These changes can not only impact stability, but also activity. In addition, they can cause adverse immunological reactions. The identification of charge variants in development, and their monitoring throughout manufacturing, is critical to the production of safe and effective drugs.

At Agilent Technologies, we have developed robust solutions for identifying and monitoring charge variants using our electrophoresis platforms, biochromatography systems, and ion exchange columns portfolio. See more about Agilent solutions for **charge variant analysis**.

- Separation of Native Monoclonal Antibodies and Identification of Charge Variants.
 5991-4969EN
 - 3991-4909EIN
- Reducing Cycle Time for Charge Variant Analysis of Monoclonal Antibodies – Alternating Column Regeneration Using an Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/ 10-port Valve.

5991-4722EN

 Simple Method Optimization in mAb Charge Variant Analysis using pH Gradients Generated from Buffer Advisor with Online pH and Conductivity Monitoring.

5991-3365EN

 Analysis of Intact and C-terminal Digested IgG1 on an Agilent Bio MAb 5 μm Column.

5991-0895EN

6

- pH Gradient Elution for Improved Separation of Monoclonal Antibody Charge Variants.
 5990-9629EN
- Proof of Performance: Analysis of Proteins by Anion Exchange Chromatography.
 5990-9614EN

- Faster Separations Using Agilent Weak Cation Exchange Columns.
 5990-9931EN
- High-resolution Analysis of Charge Heterogeneity in Monoclonal Antibodies Using pH-gradient Cation Exchange Chromatography - Agilent 1260 Infinity Bio-inert Quaternary LC System with Agilent Bio Columns.
 5991-1407EN
- Protein Separation with pH Gradients Using Composite Buffer Systems Calculated by the Agilent Buffer Advisor Software.
 5991-1408EN
- Monoclonal Antibody Charge Heterogeneity Analysis by Capillary Isoelectric Focusing on the Agilent 7100 Capillary Electrophoresis System.
 5991-1142EN

- Optimizing Protein Separation with Cation Exchange Chromatography using Agilent Buffer Advisor.
 5991-0565EN
- Characterize mAb Charged Variants by Cation-Exchange Chromatography. 5991-5273EN
- Characterize Fab and Fc Fragments by Cation-Exchange Chromatography. 5991-5274EN
- Monoclonal Antibody Charge Heterogeneity Analysis by Capillary Isolectric Focusing on Fluorocarbon Coated Capillaries
 5991-2885EN



GLYCOFORM AND GLYCAN ANALYSIS

More than 90 % of protein drugs are glycosylated. These glycoprotein biopharmaceuticals contain complex oligosaccharide moieties whose presence, absence, sites of attachment, and relative abundance profiles can have significant impact on the efficacy, pharmacokinetics, immunogenicity, folding, and stability of a drug.

The application notes below describe accurate solutions for characterizing glycan structure and monitoring glycan profiles using mAb-Glyco Chip technology, LC/MS, liquid chromatography systems, and electrophoresis. See more about Agilent solutions for glycan analysis.

- N-Glycan Analysis of mAbs and Other Glycoproteins with UHPLC and Fluorescence Detection.
 5991-5253EN
- Separation of IgG Glycopeptides using HILIC-LC/MS in Comparison to RP-LC/MS.

5991-4903EN

- Differential Analysis of Glycan Populations of Monoclonal Antibodies Using Agilent mAb-Glyco Chip and Mass Profiler Professional.
 5991-5068EN
- Sensitive and Reproducible Glycan Analysis of Human Immunoglobulin G – The Agilent 1260 Infinity Bio-inert Quaternary LC System with an Agilent AdvanceBio 2.7 μm Glycan Mapping Column and Fluorescence Detection.
 5991-4801EN
- N-Glycan Profiling Analysis of a Monoclonal Antibody Using UHPLC/FLD/Q-TOF.
 5991-5067EN
- Fast and Efficient HILIC Methods for Improved Analysis of Complex Glycan Structures.
 5991-4896EN
- A Novel HILIC Column for High Speed N-linked Glycan Analysis. 5991-4886EN

- Characterization of Glycosylation in the Fc Region of Therapeutic Recombinant Monoclonal Antibody. 5991-2323EN
- Glycopeptide Analysis of Antibodies by Capillary Electrophoresis and Q-TOF Mass Spectrometry.
 5990-7138EN
- High Resolution Glycopeptide Mapping of EPO Using an Agilent AdvanceBio Peptide Mapping Column.
 5991-1813EN
- Analysis of N-glycans from a Monoclonal Antibody by Capillary Electrophoresis and Mass Spectrometry.
 5991-1020EN
- N-Glycan analysis of monoclonal antibodies and other glycoproteins using UHPLC with fluorescence detection – Agilent 1260 Infinity Bio-inert Quaternary LC System with Agilent 1260 Infinity Fluorescence Detector.
 5990-9774EN

- The Agilent mAb-Glyco Chip Kit for Rapid and Fully Automated Characterization of N-linked Glycans from Monoclonal Antibodies.
 5990-6924EN
- Glycopeptide and Glycan Analysis of Monoclonal Antibodies Using a Microfluidic-based HPLC-Chip Coupled to an Agilent Accurate-Mass Q-TOF LC/MS.
 5990-5190EN

POST-TRANSLATIONAL MODIFICATIONS AND DEGRADATION ANALYSIS

Biopharmaceuticals are complex molecules that often require specific conditions to remain stable and intact. Throughout development these molecules are stressed to determine those stable conditions and to identify degradation products that may form during manufacturing or if the drug is improperly stored.

The application notes below describe reliable solutions for identifying degradation products, and monitoring them throughout production and formulation, using liquid chromatography systems, a wide variety of column chemistries, and electrophoresis. See more about Agilent solutions for **PTMs**.

- N-Terminal Site-Specific PEGylation and Analytical-Scale Purification of PEG Lysozyme Agilent 1260 Infinity Bio-Inert Quaternary LC with Agilent BioHPLC Columns.
 5991-2883EN
- Disulfide Linkage Analysis of IgG1 using an Agilent 1260 Infinity Bio-inert LC System with an Agilent ZORBAX RRHD Diphenyl sub-2 µm Column.
 5991-1694EN
- Reversed-Phase Optimization for Ultra-Fast Profiling of Intact and Reduced Monoclonal Antibodies Using ZORBAX Rapid Resolution High Definition 300SB-C3 Column.

5990-9667EN

- Ultra High Speed and High Resolution Separations of Reduced and Intact Monoclonal Antibodies with Agilent ZORBAX RRHD Sub-2 µm 300 Diphenyl UHPLC Column.
 5990-9668EN
- Analysis of PEGylated proteins using the Agilent 2100 Bioanalyzer. 5990-9593EN
- Rapid UHPLC Analysis of Reduced Monoclonal Antibodies using an Agilent ZORBAX Rapid Resolution High Definition (RRHD) 300SB-C8 Column.
 5990-9631EN

- Reproducible integrity and purity testing of antibodies with P200 ScreenTape.
 5990-9052EN
- Fast Separation of Recombinant Human Erythropoietin Using Reversed Phased Agilent ZORBAX RRHD 300SB-C18, 1.8 µm.
 5990-9248EN
- Reversed-Phase Separation of Intact Monoclonal Antibodies Using Agilent ZORBAX Rapid Resolution High Definition 300SB-C8 1.8 μm Column.
 5990-9016EN
- Identification of Oxidation Sites on a Monoclonal Antibody Using an Agilent 1260 Infinity HPLC-Chip/MS System Coupled to an Accurate-Mass 6520 Q-TOF LC/MS.
 5990-8768EN

- Quantitation of Oxidation Sites on a Monoclonal Antibody Using an Agilent 1260 Infinity HPLC-Chip/MS System Coupled to an Accurate-Mass 6520 Q-TOF LC/MS.
 5990-8769EN
- Analysis of Oxidized Insulin Chains using Reversed Phase Agilent ZORBAX RRHD 300SB-C18.

5990-7988EN

 Speed up the LC Analysis of Notoginsenoside R1 and Ginsenosides Rg1, Re, and Rb1 in Compound TCM Using the Agilent 1290 Infinity LC system and ZORBAX RRHD 1.8 µm Column.
 5990-5861EN



PEPTIDE MAPPING

Comprehensive protein characterization is crucial for the quality control of biotherapeutics. It includes powerful methods that detect and monitor single amino acid changes, modifications, and degradation products.

The application notes below detail a comprehensive portfolio of measurement solutions for the highly accurate confirmation of protein sequence, identification of modifications, and routine protein fingerprint monitoring for QA/ QC, using liquid chromatography systems and columns, capillary electrophoresis, and LC/MS systems. See more about Agilent solutions for **peptide mapping analysis**.

- Analysis of Monoclonal Antibody Digests with the Agilent 1290 Infinity 2D-LC Solution – Part 2: HILIC × RPLC-MS.
 5991-4530EN
- Fast and Efficient Peptide Mapping of a Monoclonal Antibody: UHPLC Performance with Superficially Porous Particles.
 5991-3585EN
- High Resolution and Rapid Peptide Mapping of Monoclonal Antibody Using an Agilent 1290 Infinity UHPLC and an Agilent 6550 iFunnel Q-TOF LC/MS System.
 5991-3600EN
- Analysis of Monoclonal Antibody Digests with the Agilent 1290 Infinity 2D-LC Solution. Published September 2013 5991-2880EN
- CE/MS and LC/MS Synergy Complementary Solutions for Peptide Mapping.
 5991-2583EN
- CE/MS/MS as an Orthogonal Technique for Sensitive and Easy Quantification of Peptides in Complex Matrixes.
 5991-2729EN
- Peptide Mapping of Glycoprotein Erythropoietin by HILIC LC/MS and RP-LC/MS.
 5991-2085EN

9

 High Resolution Glycopeptide Mapping of EPO Using an Agilent AdvanceBio Peptide Mapping Column.
 5991-1813EN

- Simultaneous Quantitation and Confirmation of Peptides with Triggered MRM Acquisition.
 5990-8912EN
- Optimizing Performance of the Agilent 1290 Infinity LC System Using 1-mm id Columns. Enhancing UHPLC Separation for Peptide Analysis. 5991-0734EN
- QA/QC of Monoclonal Antibodies: High-Resolution Peptide Mapping Using the Agilent 1260 Infinity Bio-Inert Quaternary LC and Agilent 1290 Infinity LC Systems.
 5991-0524EN
- Physicochemical characterization of a therapeutic protein by peptide mapping, SEC and IEX using the Agilent 1260 Infinity Bio-inert Quaternary LC system.
 5990-6192EN
- Fast, efficient HPLC purification of peptides from solid-phase synthesis.
 5989-8306EN

- Peptide Mapping of a Monoclonal Antibody using a Microfluidic-based HPLC-Chip coupled to an Agilent Accurate-Mass Q-TOF LC/MS
 5990-4587EN
- Tryptic digest analysis using the Agilent 1290 Infinity LC System.
 5990-4031EN
- Peptide and protein analysis by capillary HPLC – Optimization of chromatographic and instrument parameters.
 5988-8628EN
- Micropreparative Capillary Zone Electrophoresis—tryptic digest analysis of recombinant GroES.
 5990-3384EN
- Peptide Mapping and Analysis Using Capillary Electrophoresis.
 5989-9807EN

PLAYR PLAYROSM AYROSMNNF SMNNFGHS NNFGHSY

BIOSIMILARS

The promise for biosimilars in health care is vast, and recent developments have already begun to tap their therapeutic potential. However, the complexity of these molecules, as well as their sensitivity to environmental factors, necessitates stringent protocols for establishing their structural identity and ensuring their integrity throughout the discovery, development, and manufacture process.

Effective methodologies for producing biosimilars draw on established tools used in the production of related innovator drugs. These techniques include extensive structural characterization via a host of chromatographic methods and mass spectrometry. Advanced software tools can greatly enhance the biosimilar characterization workflow, enabling faster and more confident confirmation of structural similarities between biologics.

- Molecular Characterization of Biotherapeutics: The Agilent 1260 Infinity Multi-detector Bio-SEC Solution with Advanced Scattering Light Detection.
 5991-5220EN
- Separation of Native Monoclonal Antibodies and Identification of Charge Variants: Teamwork of the Agilent 3100 OFFGEL Fractionator, Agilent 2100 Bioanalzyer and Agilent LC/MS System.
 5991-4969EN
- Comparison of Biosimilar and Innovator Monoclonal Antibody Rituximab Using the Agilent 1260 Infinity Bio-inert LC System and Agilent OpenLAB Match Compare Software.
 5991-4920EN

"Prepping Biosimilars for a Big Play", produced in cooperation with Genetic Engineering News. 5991-5493EN



2D-LC, HIGH-THROUGHPUT LC AND WORKFLOW AUTOMATION

Biologics, specifically recombinant proteins, are highly complex molecules that require specific purification techniques, complex sample preparation and often long analytical methods to achieve resolution and detection. High-throughput platforms and automated workflow development are two ways to combine these various steps of the workflow to improve efficiency, increase throughput and ensure precision.

The application notes below demonstrate a variety of Agilent solutions that support high-throughput workflows and enable increased workflow automation using liquid chromatography systems, columns, 2D-LC software, LC/MS and customized method development LC platforms. See more about Agilent solutions for **2D-LC and workflow automation**.

- Online 2D-LC Analysis of Complex N-Glycans in Biopharmaceuticals Using the Agilent 1290 Infinity 2D-LC Solution 5991-5349EN
- Top-down and Bottom-up Proteomics with the Agilent 1290 Infinity 2D-LC Solution Using DAD and Q-TOF LC/MS. 5991-5179EN
- Analysis of Monoclonal Antibody Digests with the Agilent 1290 Infinity 2D-LC Solution.
 5991-2880EN
- Reducing Cycle Time for Charge Variant Analysis of Monoclonal Antibodies – Alternating Column Regeneration Using an Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/ 10-port Valve.

5991-4722EN

 Reducing Cycle Time for Affinity Removal of High-Abundant Proteins in Human Plasma - Alternating Column Regeneration Using an Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port Valve and an Agilent 1290 Infinity Flexible Cube.
 5991-4721EN

- Reducing Cycle Time for Quantification of Human IgG Using the Agilent Bio-Monolith Protein A HPLC Column -Alternating Column Regeneration Using an Agilent 1200 Infinity Quick-Change Bio-inert 2-position/10-port Valve and the 1290 Infinity Flexible Cube.
 5991-4723EN
- Analysis of Monoclonal Antibody Digests with the Agilent 1290 Infinity 2D-LC Solution - Part 2: HILIC × RPLC-MS.
 5991-4530EN
- Heart-Cut 2D-LC/MS Approach for Pharmaceutical Impurity Identification Using an Agilent 6540 Q-TOF LC/MS System.
 5991-1873EN

- Combining Small-Scale Purification and Analysis of Monoclonal Antibodies on One Instrument.
 5991-1195EN
- Peak-based fraction collection of proteins with the Agilent 1260 Infinity Bio-inert Quaternary LC. Versatile purification and re-analysis using automated column switching.

5991-0990EN

 Detection of Impurities by Heart Cutting Using the Agilent 1290 Infinity 2D-LC Solution.

5991-0834EN

 Agilent 1260 Infinity Method Development Solution. 5990-6863EN



AUTOMATED SAMPLE PREPARATION

Automating biologic sample preparation enable quantitation and sample preparation in the same workflow, reduce errors and increase walkaway time with reliable, automated processes and increases throughput using existing chemistry or customized workflows. For recombinant proteins, these automated sample preparation steps may include capture steps, glycan removal and labeling, and protein digestion for peptide mapping.

The application notes below demonstrate the utility of the Agilent AssayMAP platform, an open access, walkaway automation solution specifically designed for biomolecule sample preparation. It is based on the powerful combination of miniaturized, packed-bed chromatography cartridges, the state-of-the-art Bravo Automated Liquid Handling Platform, and a simple, application-based user interface that creates an open access environment for both novice and experienced automation users. See more about Agilent solutions for **automated sample prep**.

- Automation of Sample Preparation for Accurate and Scalable Quantification and Characterization of Biotherapeutic Proteins Using the Agilent AssayMAP Bravo Platform.
 5991-4872EN
- Workflow Automation for LC/MS: In-Solution Protein Digestion, Peptide Cleanup, and Strong Cation-Exchange Fractionation of Peptides Enabled by AssayMAP Technology.
 5991-3602EN
- Automation for LC/MS Sample Preparation: High Throughput In-Solution Digestion and Peptide Cleanup Enabled by the Agilent AssayMAP Bravo Platform.
 5991-2957EN
- Brochure: Agilent AssayMAP Solutions for N-Glycan Sample Preparation using ProZyme Glyko-prep.
 5991-1140EN

- Purification of Antibodies from Cell Culture Supernatant Using the Agilent AssayMAP Bravo Platform.
 5990-9247EN
- High Throughput Purification of Human IgG Using the Agilent Bravo for Protein Purification and AssayMAP Protein A Cartridgess.
 5990-7203EN



HOST CELL AND PROCESS-RELATED IMPURITIES

Bacterial and mammalian cells are commonly used as host systems for the production of human therapeutic proteins. An important part of the purification process is the removal of host cell material, such as DNA or proteins, which can pose a safety concern if administered along with the therapeutic compound. The production of biopharmaceuticals also includes the use of purification buffers, solvents, bioreactors, and various apparatus which are made of plastic, stainless steel, and other metals. The final biopharmaceutical product must be free of all potential process related impurities present in buffers and solvents during manufacturing, as well as those that leach from the hardware used to produce and purify drugs.

The application notes below demonstrate reliable solutions for the accurate measurement of host cell DNA using our qPCR system and identification and quantitation of host cell proteins using LC/MS. Agilent also provides a comprehensive portfolio of solutions for determining process related impurities such as metals and residual solvents using ICP-OES, ICP-MS, GC headspace analyzers, and GC/MS platforms. See more about Agilent solutions for host cell and **process-related impurities analysis**.

- Impurity Detection with a New Light Emitting Diode Induced Fluorescence Detector Coupled to the Agilent 7100 Capillary Electrophoresis System.
 5991-4043EN
- Agilent Solutions for Removal and Monitoring of Genomic DNA from Monoclonal Antibody Preparations.
 5991-3527EN
- Detecting Residual CHO Host Cell DNA Using the Agilent Mx3005P QPCR System.
 - 5990-9519EN
- Fast impurity profiling of synthetic oligonucleotides with the Agilent 1290 Infinity LC System and Agilent 6530 Accurate-Mass QTOF LC/MS.
 5990-5825EN

- Validating the Agilent 7700x ICP-MS for the Determination of Elemental Impurities in Pharmaceutical Ingredients According to Draft USP General Chapters <232>/<233>.
 5990-9365EN
- Proposed new ICH and USP methods for elemental impurities: The application of ICP-MS and ICP-OES for pharmaceutical analysis
 5991-9382EN
- Determination of Contaminant Bacterial Host Cell Proteins in Recombinant Proteins Expressed in E.coli by LC-QQQ View poster

TITER AND PROTEIN QUANTITATION

The ability to accurately quantify a biopharmaceutical is critical to the development of a high yield (titer) fermentation and purification process in manufacturing. The concentration of a biopharmaceutical is also important for determining a safe and effective formulation and dose.

At Agilent Technologies, we have developed reliable solutions for routine titer and concentration measurement using our UV/Vis systems, AssayMAP for Bravo, and liquid chromatography systems and columns. See more about Agilent solutions for titer and protein **quant analysis**.

- mAb Titer Analysis with the Agilent Bio-Monolith Protein A Column. 5991-5135EN
- Cell Culture Optimization Using an Agilent Bio-Monolith Protein A Column and LC/MS.
 5991-5125EN
- Cell Clone Selection Using the Agilent Bio-Monolith Protein A Column and LC/MS.

5991-5124EN

- Agilent Bio-Monolith Protein A Monitors Monoclonal Antibody Titer from Cell Cultures.
 5991-2990EN
- Superior Linearity and Reproducibility Using the Agilent Cary 60 for Routine Measurements of Micro Volumes.
 5990-9688EN

- Purification of antibodies from cell culture supernatant using the Agilent AssayMAP Bravo platform.
 5990-9247EN
- Rapid Analysis of Adenoviruse Type 5 Particles with Bio-Monolith Anion-Exchange HPLC Columns to Support the Development of a High-Titre Manufacturing Platform.
 5990-5524EN
- Protein Sizing and Quantitation with the Agilent Protein 80 and Protein 230 Kits on the Agilent 2100 Bioanalyzer.
 5990-5721EN
- Quantification Strategies Using the High Sensitivity Protein 250 Assay for the Agilent 2100 Bioanalyzer.
 5989-8941EN



RAW MATERIAL AND MEDIA ANALYSIS

Since every protein or peptide has a unique amino acid sequence and composition, amino acid analysis is used from drug discovery through manufacturing to demonstrate batch-to-batch consistency, for protein characterization, and for cell culture monitoring.

The application notes below describe optimized solutions that have been developed to provide the data you need to quantify and categorize the amino acids that make up your biopharmaceuticals using liquid chromatography and high-resolution reversed-phase columns. See more about Agilent solutions for raw **material analysis**.



CAPILLARY ELECTROPHORESIS AND MICRO-FLUIDIC ELECTROPHORESIS OF PROTEINS

Electrophoresis of proteins and peptides provides separation based on isoelectric point or charge states, size or a combination of these attributes. Capillary electrophoresis (CE) is commonly used for intact protein separation based on charge states and for glycan analysis. CE can also be combined with mass spectrometry, and is often used as an orthogonal technique to LC/MS. Simple electrophoresis techniques like SDS-PAGE are commonly used for protein stability testing and QC, at-line monitoring of protein expression, and in protein purification optimization and monitoring.

The application notes below demonstrated the use of the Agilent 7100 Capillary Electrophoresis system for protein characterization and for protein modification analysis when combined with time-of-flight mass spectrometry. The 2200 TapeStation and 2100 Bioanalyzer are proposed as replacement platforms for SDS-PAGE gels for monitoring protein fate throughout process development. See more about Agilent solutions for **CE/MS**.

- Characterization of Small Immunoconjugates (< 40 kDa) Using Capillary Electrophoresis – Mass Spectrometry.
 5991-4433EN
- Impurity Detection with a New Light Emitting Diode Induced Fluorescence Detector Coupled to the Agilent 7100 Capillary Electrophoresis System.
 5991-4043EN
- Agilent Solutions for Removal and Monitoring of Genomic DNA from Monoclonal Antibody Preparations.
 5991-3527EN
- CE/MS and LC/MS Synergy Complementary Solutions for Peptide Mapping.
 5991-2583EN
- Monoclonal Antibody Charge Heterogeneity Analysis by Capillary Isoelectric Focusing on Fluorocarbon Coated Capillaries.
 5991-2885EN
- CE/MS/MS as an Orthogonal Technique for Sensitive and Easy Quantification of Peptides in Complex Matrixes.
 5991-2729EN

- Identification and Quantification of Oxidation Sites on Monoclonal Antibodies Using Capillary Electrophoresis and Quadrupole Time-of-Flight Mass Spectrometry.
 5991-2728EN
- Analysis of N-glycans from a Monoclonal Antibody by Capillary Electrophoresis and Mass Spectrometry.
 5991-1020EN
- Monoclonal Antibody Charge Heterogeneity Analysis by Capillary Isoelectric Focusing on the Agilent 7100 Capillary Electrophoresis System.
 5991-1142EN
- Performance of commercially available gels for protein characterization by capillary gel electrophoresis with UV detection on the Agilent 7100 CE System 5990-7976EN
- Monitoring Protein Fate during
 Purification with the Agilent 2100
 Bioanalyzer.
 - 5990-6153EN
- Glycopeptide Analysis of Antibodies by Capillary Electrophoresis and Q-TOF Mass Spectrometry.
 5990-7138EN



NUCLEIC ACID ANALYSIS

Synthetic oligonucleotides have emerged as promising therapeutic agents for the treatment of a variety of diseases, including viral infections and cancer. Several classes of nucleic acids, such as antisense oligonucleotides, small interfering RNAs (siRNAs), and aptamers are being investigated for therapeutic applications. Impurities arising from the incomplete capping of coupling reactions, product-related impurities, impurities in the starting materials, and post-synthesis processing must be monitored, identified, and removed.

The application notes below detail our comprehensive portfolio of solutions for identifying and monitoring impurities throughout the oligonucleotide synthesis process using LC/MS, liquid chromatography systems, and column chemistries.

- Polar Modified Stationary Phases are Ideal for the Analysis of Nucleotides. 5991-2058EN
- High Performance DNA Oligonucleotide Purification Using Agilent TOP-DNA. 5990-9006EN
- Improved Column Lifetime with Thermally Stable Polymer Columns for Oligonucleotide Ion-Pair RP HPLC.
 5990-7764EN
- High Resolution Separations of Oligonucleotides Using PL-SAX Strong Anion-Exchange HPLC Columns.
 5990-8297EN
- Use Temperature to Enhance
 Oligonucleotide Mass Transfer and
 Improve Resolution in Ion-Pair RP HPLC.
 5990-7765EN
- Fast Impurity Profiling of Synthetic Oligonucleotides with the Agilent 1290 Infinity LC System and Agilent 6530 Accurate-Mass QTOF LC/MS.
 5990-5825EN
- Oligonucleotides—Analysis of Antisense Therapeutics. 5990-3383EN



COMPLIANCE, QC AND QbD

Good manufacturing practice (GMP) quality control testing includes detailed information concerning the composition of raw materials, vitamins, media analysis, metal cations, and process- and product-related impurities. A comprehensive characterization profile is important for quality control of biopharmaceuticals, as it enables the establishment of specifications and acceptance criteria.

Traditional empirical approaches to method development can fail to meet desired separations during validation and transfer in manufacturing. Quality by Design (QbD) promotes better understanding of the product and manufacturing process starting with product development, essentially building quality criteria into the manufacturing process at each step. With QbD, the impact and interactions between critical method variables are understood using a Design of Experiments (DOE) approach, which incorporates statistical multi-variate analysis and modeling.

Agilent provides a comprehensive portfolio of solutions for Biopharma manufacturing and quality control using liquid chromatography, mass spectrometry, spectroscopy, columns, consumables, and software to support manufacturing, compliance, and QbD.

- Compliance for Biopharma Laboratories: A Primer, authored by Ludwig Huber. 5990-7001EN
- Quality-by-Design Approach to Stability Indicating Method Development for Linagliptin Drug Product.
 5991-3834EN



OVERVIEW OF AGILENT SOLUTIONS FOR BIOPHARMA

Together: It's how we work with you to get great results for biopharmaceutical analysis.

The applications contained in this guide share specific details about various biopharmaceutical analyses. These next few pages provide highlights of the multi-product solutions Agilent recommends for various biopharma workflows.

PROTEIN ANALYSIS BY MASS SPECTROMETRY

The Agilent 1290 Infinity Binary/Quaternary LC System coupled with an Agilent 6230 TOF and 6500 Series Q-TOF provides fast and superior separation power, high mass accuracy, combined with exceptional sensitivity. Intelligent System Emulation Technology ensures you get consistent method transfer from instrument to instrument, around the world.

A wide choice of Agilent biocolumns support your complete protein characterization: The AdvanceBio family, including ZORBAX Rapid Resolution High Definition (RRHD) 300Å, Poroshell 300 and AdvanceBio Peptide Mapping Columns provide fast, UHPLC reversedphase analysis.

MassHunter BioConfirm software provides valuable tools to accelerate your understanding of the proteins you are studying. Learn more about Agilent solutions for **mass spectrometry** of proteins.



Agilent 1290 Infinity Binary/Quaternary LC System and Agilent 6500 Series Q-TOF



BioConfirm Software



Agilent ZORBAX RRHD 300Å Columns, 1.8 µm, and Poroshell 300 Columns, 5 µm

AGGREGATE AND FRAGMENT ANALYSIS

Agilent 1260 Infinity Multi-Detector Bio-SEC Solution provides the flexibility a light scattering detector to provide absolute molecular weight data. Light scattering also increases the area of aggregate peaks.

Agilent Bio SEC-3 and Bio SEC-5 column with smaller 3 µm and traditional 5 µm particles provide fast, reliable and accurate performance for aggregates, degradants and fragments.

Learn more about Agilent solutions for aggregate and fragment analysis.



Agilent 1260 Infinity Multi-Detector Bio-SEC Solution



Agilent Bio SEC columns

CHARGE VARIANT ANALYSIS

Agilent 1260 Infinity Bio-inert Quaternary LC System with Buffer Advisor simplifies method development and makes it easier to find the optimum charge variant separations, by facilitating the mixing of buffers from four stock solutions at a pH accuracy and precision better than 0.1 pH units. Buffer Advisor software is a system-independent solution that eliminates the need to prepare and titrate multiple buffers for pH scouting studies.

Agilent Bio MAb columns have particles, coating and bonding that are optimized for weak cation mAb separations and are resistant to high pressures, promoting higher resolution and faster separations. The unique hydrophilic coating eliminates most nonspecific interactions. The complete BioIEX family of columns are ideal for all proteins.

Agilent 7100 CE Systems are also excellent for analyzing charge heterogeneity of MAbs, as an alternative to HPLC. See p. 31 for more information.

Learn more about Agilent solutions for charge variant analysis.



Agilent 1260 Infinity Bio-inert Quaternary LC System with Buffer Advisor and Agilent Bio MAb columns

GLYCOFORM, GLYCOPEPTIDE AND GLYCAN ANALYSIS

Agilent provides a range of solutions for glycan analysis suitable for a few samples or many samples with a choice of full automation, as well as options for fast analysis by LC and LC/MS.

AdvanceBio Glycan Mapping columns, standards and kits are available for N-glycan profiling of a few or many samples by U/HPLC with UV or MS detection.

AssayMAP provides automation solutions for n-glycan profiling (see automated sample prep)

The MAb-Glyco Chip/MS Kit integrates chip-based deglycosylation, glycan separation, and glycan transfer with high-sensitivity TOF/QTOF detection.

Our single-source CE/MS Solution combines electrophoretic separation and mass spectrometry resolution for glycan analysis.

The ZORBAX RRHD 300-HILIC is a UHPLC column that has been shown to enhance glycopeptides separations.

More Information: View the webinar, "Workflow Solutions for High Speed Glycan Analysis".

Learn more about Agilent solutions for **glycoform**, **glycopeptide and glycan analysis**.





Strategies for intact glycoform profiling, glycopeptide and glycosylation site identification and release glycan analysis

Agilent AdvanceBio Glycan Mapping columns

POST-TRANSLATIONAL MODIFICATIONS AND DEGRADATION ANALYSIS

Agilent 1260 Infinity Bio-inert Quaternary LC System for true UHPLC performance for shorter analysis time and better resolution

AdvanceBio RP-mAb columns are a great option for analysis of intact, digested, and fragmented mAbs, enabling fast, high resolution separations with a 450A pore size and 3 bonded phase choices.

Poroshell 300 is good for separation of larger proteins (> 50 Da)

AdvanceBio Peptide Mapping Columns feature an optimized 120Å pore size with superficially porous 2.7 µm particles. They are specially tested with a challenging peptide mix to ensure reliable peptide mapping performance.

ZORBAX Rapid Resolution High Definition (RRHD) columns family includes four phases: SB-C18, SB-C8, SB-C3 and the unique SB-Diphenyl phase, for orthogonal separations that enhance your understanding of the protein you are analyzing.

Learn more about Agilent solutions for **post-translational modifications and degradation analysis**.







Agilent AdvanceBio RP-mAb, ZORBAX RRHD 300Å Columns, 1.8 µm, and Poroshell 300 Columns, 5 µm

PEPTIDE MAPPING

The Agilent 1290 Infinity II LC system coupled with 6500 Series Q-TOF LC/MS provide the power range and performance you require to optimize peptide mapping. CE/MS can also be used for peptide mapping. See more about CE/MS on p. 31.

Agilent AdvanceBio Peptide Mapping Columns feature an optimized 120Å pore size with superficially porous 2.7 µm particles. They are specially tested with a challenging peptide mix to ensure reliable peptide mapping performance.

Agilent AssayMAP Peptide Sample Prep Solution (see "automated sample preparation") provides the opportunity to dramatically increase peptide mapping throughput.

Agilent MassHunter BioConfirm Software intelligently maps peptide digest LC/MS data onto protein sequences to reveal sequence coverage and localize modifications.

Learn more about Agilent solutions for peptide mapping.



Agilent 1290 Inifinity II LC system and Agilent 6500 Series Q-TOF



Agilent AdvanceBio Peptide Mapping Columns

2D-LC, HIGH THROUGHPUT LC AND WORKFLOW AUTOMATION

The Agilent 1290 Infinity II 2D-LC Solution simplifies method set-up with flexible hardware and software that add a new dimension to your analysis. The solution incorporates powerful features such as peak-triggered operations and innovative valve technology for heart-cutting analysis.

Pair your 2D-LC system with columns from the wide selection of Agilent biocolumns in reversed phase, IEX, SEC and Affinity techniques, offered in a range of particle sizes and configurations.

Agilent 1290 Infinity II Method Development Solution comes ready to run and is designed for automated access to more than 1000 sets of unique LC separation conditions.

Learn more about Agilent solutions for high-throughput workflows.



Agilent 1290 Infinity II 2D-LC Solution



Multiple Heart-Cutting enables users to store peaks from the first dimension to analyze them subsequently in the second dimension. The online status monitoring illustrates you the status of your cuts and 2D analysis at any time of the run.



Agilent 1200 Infinity Series Method Development Solution

AUTOMATED SAMPLE PREPARATION

The AssayMAP Technology Solution enables efficient N-glycan sample preparation using GlykoPrep-plus and the 1260 Infinity Bio-inert Quaternary LC System.

Agilent AssayMAP Peptide Sample Prep Solution provides improved reproducibility, increased throughput, less hands-on-time and faster method development for peptide mapping.

Learn more about Agilent solutions for **automated protein sample preparation**.



PROCESS-RELATED IMPURITIES

Agilent's ICP-MS and ICP-OES instruments are ideally suited for trace level analysis of elemental impurities according to ICH Q3D and USP <232>/<233>. **5990-9382EN**.

The Agilent 7900 ICP-MS provides best sensitivity for pharmaceuticals of all dosage routes, including oral, inhalational and intravenous. It offers automated method set up and instrument optimization, ultra-high matrix introduction (UHMI) and the widest dynamic range, up to 11 orders magnitude for sub ng/L to % levels. **5991-5314EN**

The Agilent 5100 ICP-OES is an ideal solution for labs that are cost conscious and focused on high sample throughput of oral dosage route drug products or raw material analysis. **5991-5403EN**

Learn more about Agilent solutions for host cell and process-related impurities.



Agilent 7900 ICP-MS



Agilent 5100 ICP-OES

Protein Analysis by ICP-000

The Agilent 8800 ICP-QQQ is the world's first triple quadrupole ICP-MS with MS/MS capabilities. Ion transmission to the ORS cell is selectively controlled by the first quadrupole (Q1), enabling unsurpassed interference removal through controlled chemical reactions.

Learn more here



Agilent 8800 ICP-000

TITER AND PROTEIN QUANTITATION

Agilent 1260 Infinity Bio-inert Quaternary LC system – 100% bio-inert: All capillaries and fittings throughout the autosampler, column compartment and detectors are completely metal-free so that the bio-molecules in your sample come in contact only with ceramics or PEEK.

Agilent BioMonolith Protein A Columns provide fast titer analysis over a wide linear range for accurate quantitation.

Agilent Cary 60 UV-Vis spectrophotometer provides superior linearity and reproducibility for the analysis of micro volumes of DNA, RNA and other proteins.

Learn more about Agilent solutions for titer and protein quantitation.



Agilent 1260 Infinity Bio-Inert Quaternary LC System



Agilent BioMonolith Protein A Columns

AMINO ACIDS AND MEDIA ANALYSIS

Agilent 1200 Infinity Series provides the power range and performance you require for amino-acid analysis.

Agilent ZORBAX Amino Acid Analysis (AAA) Columns provide high resolution separations for amino acids.

Learn more about Agilent solutions for **amino acid and media analysis**.



Agilent ZORBAX Amino Acid Analysis (AAA) Columns

CE AND MICRO-FLUIDIC ELECTROPHORESIS OF PROTEINS AND PEPTIDES

Agilent 7100 CE/MS System with MassHunter Software

Agilent 7100 CE and CE/MS Systems allows you to amplify your findings with an orthogonal approach that is the ideal complement to chromatographic LC/MS analysis, great for intact protein analysis, peptides out of protein digests and their modifications, e.g. with Glycans.

The Bioanalyzer 2100 system provides advantages in quantitation, resolution, time and data handling for analysis of DNA, RNA and proteins.

More information: Download the Agilent CE/MS Brochure at http://www.chem.agilent.com/Library/brochures/5991-1511EN.pdf



Agilent 7100 CE/MS System with MassHunter Software

OTHER GREAT RESOURCES FOR BIOPHARMA

Agilent strives to provide you with information and tools to support your biopharma workflows. Check out the "Get Bio Guide" site at **www.agilent.com/chem/getbioguides** for primers on the major techniques for biopharma, column selection guides, and white papers with tips for method development. You can select the resources you'd like to receive in print or download them.



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