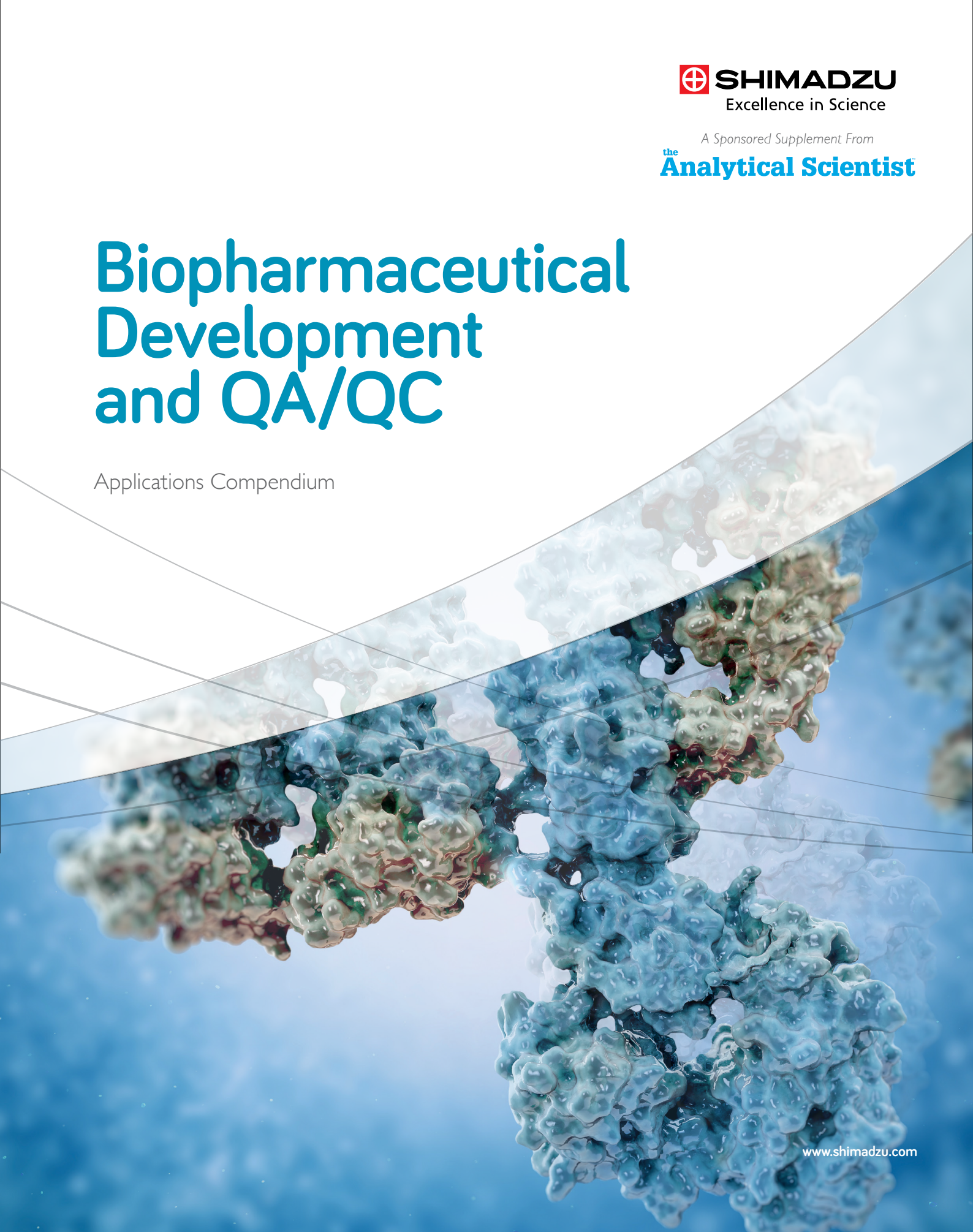
SHIMADZU
Excellence in Science

A Sponsored Supplement From
the Analytical Scientist

Biopharmaceutical Development and QA/QC

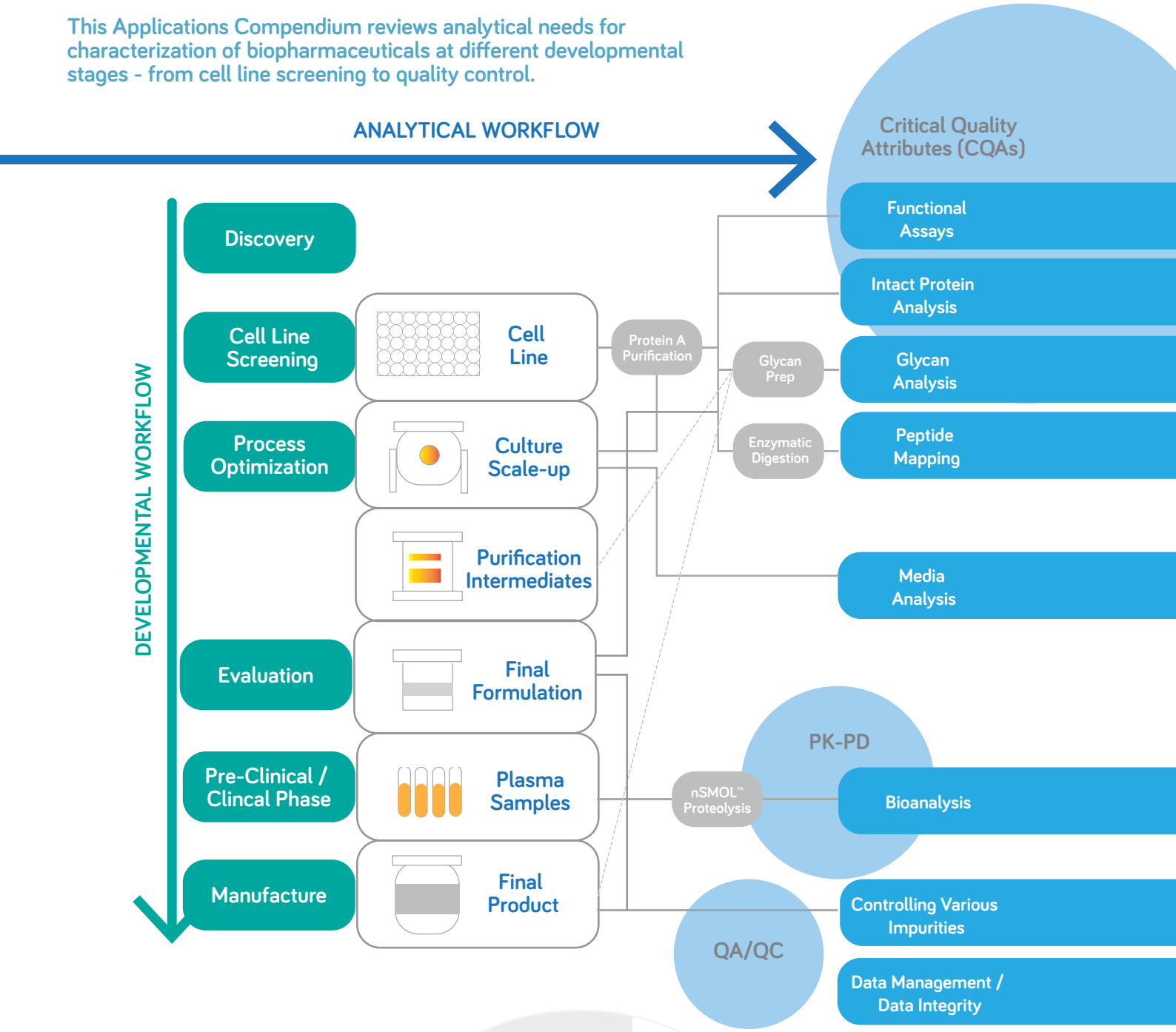
Applications Compendium



www.shimadzu.com

Introduction

This Applications Compendium reviews analytical needs for characterization of biopharmaceuticals at different developmental stages - from cell line screening to quality control.



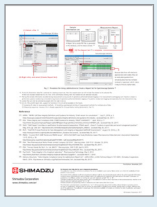
Intact Proteins Robust and reliable separation and measurement

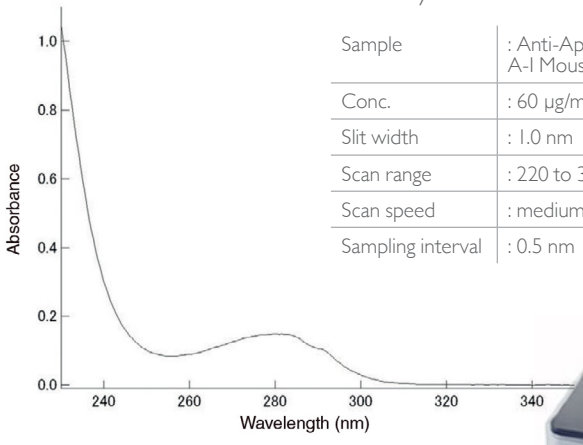
Due to the complexity of the cellular process controlling the synthesis and translocation of macromolecules, the efficiency at which host cells secrete the target protein into culture medium is largely dependent on individual cell line. Thus, at an early stage of biopharmaceutical development, screening is performed for selecting a cell line that is most suited as the vehicle for the product manufacturing. Here, analysis of intact proteins are performed to characterize few of the most important quality attributes for safety and efficacy. These attributes continue to be monitored throughout the developmental workflow, and thus require robust systems and data integrity compliance.

Titer & Protein Quantification

How much protein is produced under a given condition is a critical factor affecting manufacturing productivity and ultimately the long-term profitability of the final product. Shimadzu has developed reliable spectroscopic and chromatographic solutions for protein concentration measurements.

Technical report:
Data Integrity Compliance: An Innovative Solution for Molecular Spectroscopy



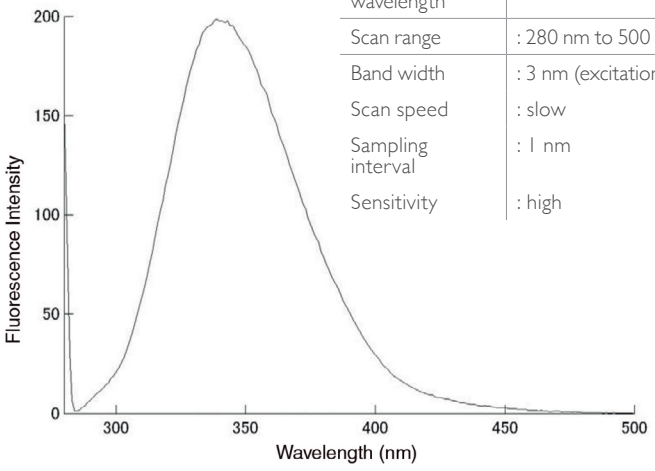


UV Analytical Conditions

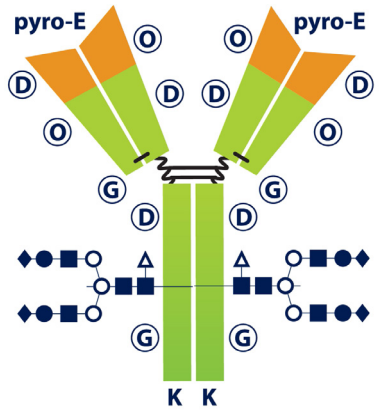
Sample	: Anti-Apolipoprotein A-I Mouse mAb (CALBIOCHEM)
Conc.	: 60 µg/mL
Slit width	: 1.0 nm
Scan range	: 220 to 350 nm
Scan speed	: medium
Sampling interval	: 0.5 nm

RF Analytical Conditions

Sample	: Anti-Apolipoprotein A-I Mouse mAb (CALBIOCHEM)
Conc.	: 60 µg/mL
Spectrum mode	: emission
Excitation wavelength	: 280 nm
Scan range	: 280 nm to 500 nm
Band width	: 3 nm (excitation/emission)
Scan speed	: slow
Sampling interval	: 1 nm
Sensitivity	: high



Intact Proteins Robust and reliable separation and measurement



CQAs observable at intact protein level	*Analytical method*
Aggregation, dimerization	Size-exclusion chromatography (SEC)
Deamidation, pyroglutamination	Charge-variant analysis (IEX)
Disulfide bond scrambling	Molecular weight measurement (LC-MS)
Major glycoforms	
Drug conjugation	Hydrophobic interaction chromatography (HIC)

Accurate Mass Analysis

Purified Protein → Mass Spectrometry

Accurate mass measurement helps to determine whether the correct protein sequence has been expressed with the expected post-translational modifications (PTMs). It also provides relative abundance of different proteins or PTMs present in the same sample. A high resolution and high sensitivity mass spectrometer will facilitate this analysis.

Shimadzu offers the best solution for a routine high-resolution accurate-mass analysis with the LCMS-9030, quadrupole time-of-flight mass spectrometer (Q-TOF LC-MS). It maintains its mass accuracy for days without requiring re-calibration or using internal standard.

Intact Proteins Robust and reliable separation and measurement

Aggregate and Fragment Analysis

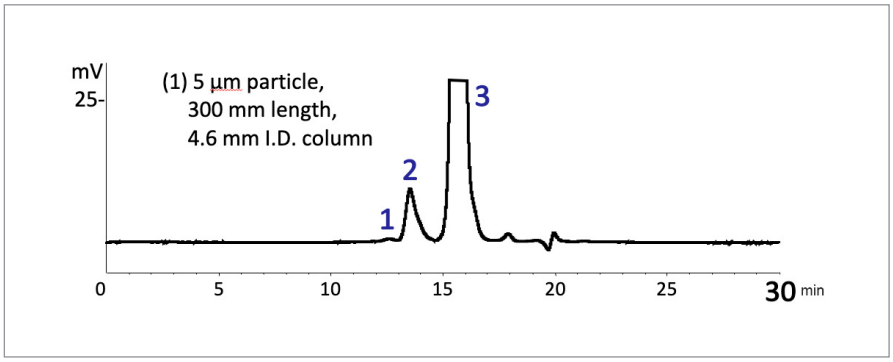
Purified Protein → HPLC (SEC)

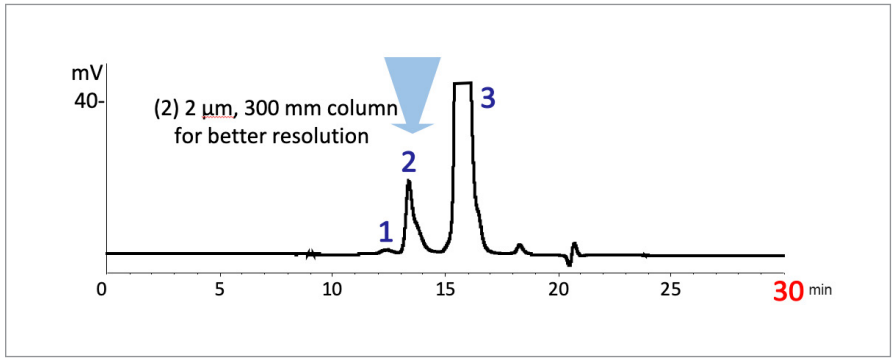
Protein aggregates are known to strongly induce immunogenic responses when administered, and aggregation is one of the most important quality attributes that must be monitored and managed at every step of process development and refinement. For example, in the initial stage of development, cell lines that have greater tendency to produce aggregates or degradants are screened out even if it showed a high secretion yield.

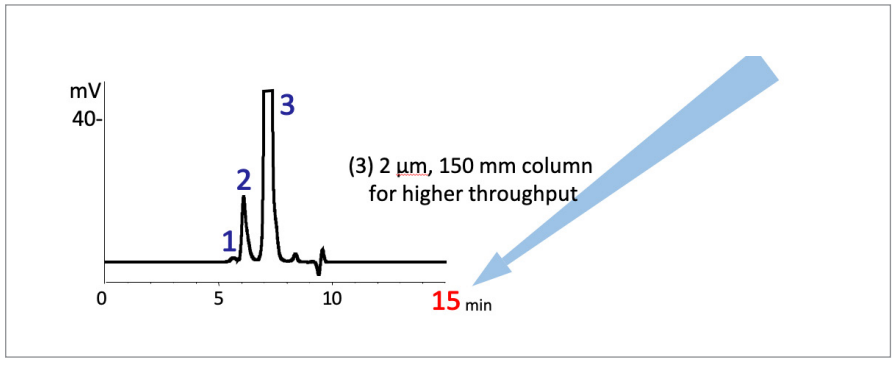
Size-exclusion chromatography (SEC) is the method of choice for separating the major monomer fraction from size variants occurring by either aggregation or degradation. Shimadzu supplies the range of SEC columns that are useful particularly in increasing the throughput needed for the screening analysis.

Size-exclusion chromatography of mAb
Column: Shim-pack Bio Diol-300
Flow rate: 0.2 mL/min
Sample: Humanized monoclonal IgG1

Here, the chromatograms compare the SEC separation profile of mAb acquired by Shim-pack Bio Diol-300 columns of different dimension at a constant flow rate. Reduction of particle size (5 µm to 2 µm) resulted in increased peak resolution, which in turn gave the room to reduce the analysis time by using a shorter column (30 minutes to 15 minutes).







Column	*N (3)*	*Rs (1,2)*	*Rs (2,3)*
(1) 5 µm, 300 × 4.6 mm	8,500	0.88	2.67
(2) 2 µm, 300 × 4.6 mm	16,200	1.17	4.15
(3) 2 µm, 150 × 4.6 mm	8,700	0.85	2.75

Intact Proteins Robust and reliable separation and measurement

Charge Variant Analysis

Purified Protein → HPLC (IEX)

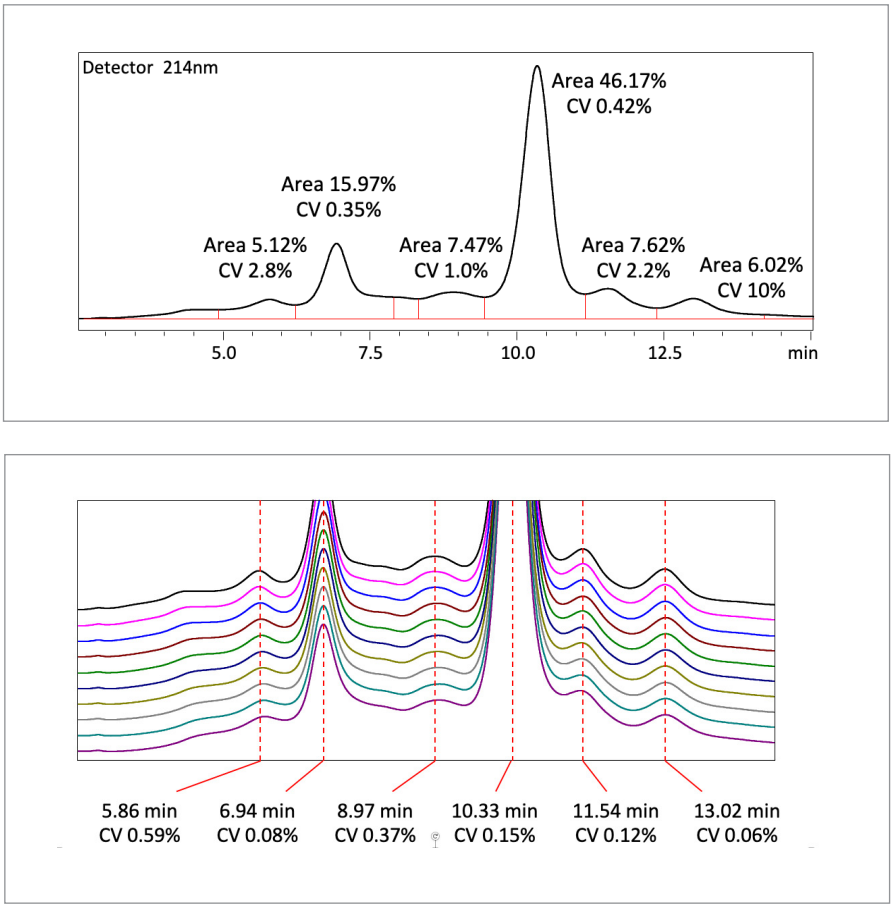
Modification, isomerization or cleavage occurring on a charged amino acid residue alters the overall charge of the protein, affecting its structure, binding affinity and stability. Hence charge-related heterogeneity is an important quality attribute of mAb.

The profile of charge variants can be evaluated by ion exchange chromatography (IEX), and this is repeatedly monitored throughout the developmental workflow.

Repeatability of elution profile strongly depends on the robustness of the HPLC system to sustain precision and accuracy in the presence of strong salts needed for IEX.

Here, mAb sample was separated by cation-exchange chromatography and repeatability of 10 consecutive injections was evaluated. The results shown demonstrate that the system precisely sustains the baseline and elution profile in the presence of 0.2 M NaCl.

Cation-exchange chromatography of mAb
HPLC System : Prominence-i
Mobile phase : 0.02 M sodium phosphate and 0.2 M NaCl
Flow rate : 1.0 mL/min
Sample : mAb (Trastuzumab)



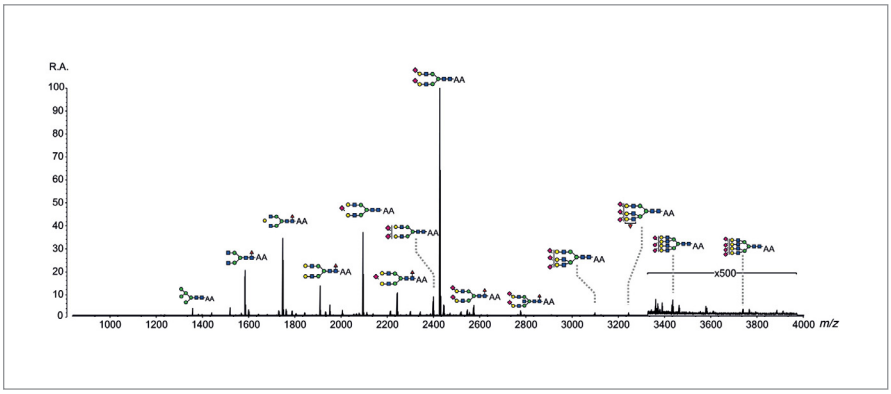
(top) A representative separation profile showing the percentages of peak areas, indicative of product purity, with %CV.
(bottom) Chromatograms of 10 repeat injections were overlaid with base shift, showing the exceptional repeatability of retention times.

Glycans Addressing both fast screening and full profiling

Glycosylation is one of the most common post-translational modification of proteins. The presence of the glycan moiety and its structural profile must be monitored, since it affects not only the effector function, but also ADME and immunogenicity.

Quick Screening of Glycan Profile

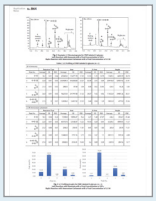
Acquiring the glycan structure profile at an early stage of biotherapeutic development helps eliminate any molecules expressing high levels of unfavorable glycan species, e.g. fucosylation. This is taken into account during cell line selection to reduce long-term risk. MALDI-TOF MS methodology offers a simple, high-throughput option for this purpose.



MALDI MS Spectrum of 2-AA labeled N-glycan prepared from a human plasma sample. The peak profile can be directly converted to and interpreted as the glycan expression profile.

Purified Protein → Enzymatically Release Glycan → Label and Purify → MALDI-TOF MS

Application News:
Evaluating Glycans in Biopharmaceuticals 1- side-reactions that occur during sample preparation for O-glycan analysis.



Application News:
Evaluating Glycans in Biopharmaceuticals 2 - differences and benefits of various sample preparation methods for N-glycans.

Application News:
Analysis of Glycopeptides of Monoclonal Antibody Using High-Resolution MALDI-TOF MS

Peer-reviewed journal article:
Differentiation of Sialyl Linkage Isomers by One-Pot Sialic Acid Derivatization for Mass Spectrometry-Based Glycan Profiling. Nishikaze T, Tsumoto H, Sekiya S, Iwamoto S, Miura Y, Tanaka K. Anal Chem. 2017 Feb 21;89(4):2353-2360

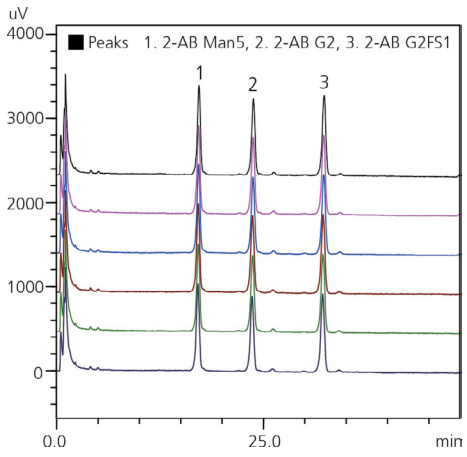
Application News:
Characterization of Glycan Binding Sites of O-Linked Glycopeptides Using High-Resolution MALDI-TOF MS

Glycans Addressing both fast screening and full profiling

Full Characterization and Monitoring

At later stages of biotherapeutics development, full characterization of glycan profile is required in order to define the reference glycan profile of a product for later QA/QC. High-resolution chromatography is needed for in-depth characterization, in order to separate and identify structural isomers that pose a health risk, e.g. alpha-1,3 galactose. Retention time gives reliable identification, and reproducibility of chromatography is the key driver for method selection.

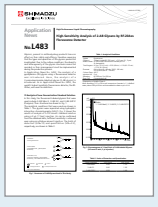
Purified Protein → Enzymatically Release Glycan → Label and Purify → HPLC LC/MS

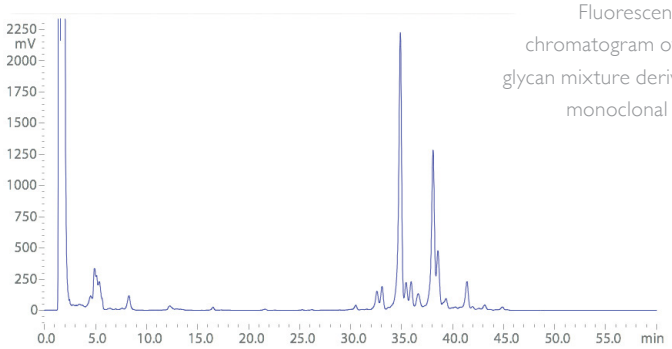


Glycan standard	R.T. %RSD	Area %RSD
2-AB Man5	0.273	0.743
2-AB G2	0.245	0.684
2-AB G2FS1	0.196	0.589

Reproducibility of HILIC mode glycan separation, tested with 2-AB labeled glycan standard (40 fmol each)

Application News:
High-Sensitivity Analysis of 2-AB Glycans by RF-20Axs Florescence Detector





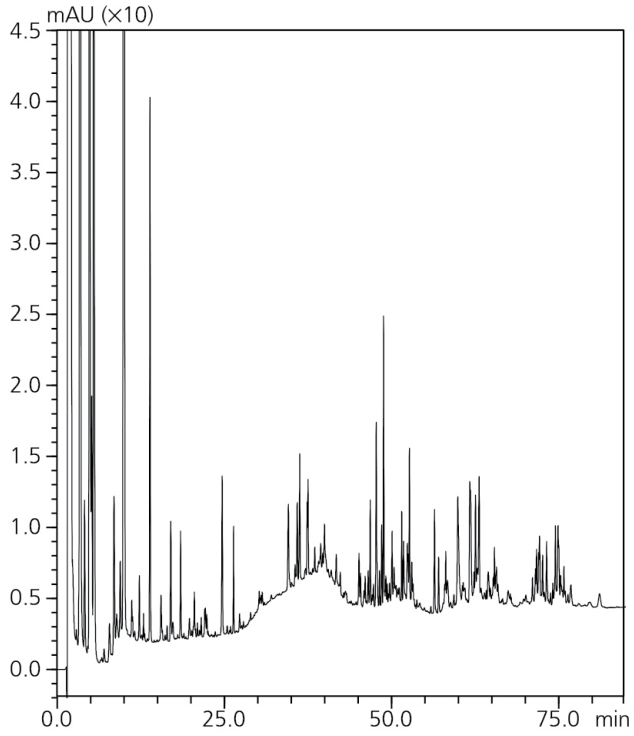
Fluorescence HPLC chromatogram of labelled glycan mixture derived from monoclonal antibody sample.

Peptides In-depth characterization of primary structure

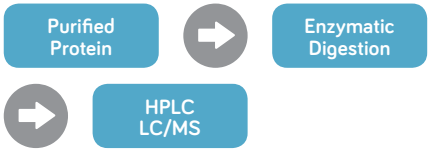
During process development and before entering clinical trials, the primary structure of a recombinant protein must be confirmed, and all potential PTMs must be characterized to assess the associated risk.

Peptide mapping is the fundamental technique for this purpose, whereby the recombinant protein is enzymatically digested into peptide fragments that are chromatographically separated to give a fingerprint of the primary structure. When coupled to mass spectrometry, peptide mapping can also give precise identification of various PTMs, including oxidation, deamidation, disulfide bond scrambling, C-terminal lysine truncation and N-terminal pyroglutamination.

Shimadzu offers a comprehensive portfolio of solutions for the highly accurate confirmation of protein sequence, identification of modifications, and routine protein fingerprint monitoring for QA/QC, using Part 11 compliant liquid chromatography and LC/MS systems.

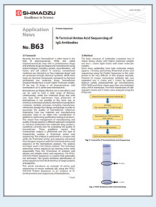


Chromatogram of IgG Tryptic Digest

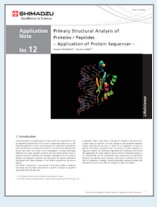


Application News:
Peptide Mapping of Antibody Drugs by Nexera-i

Application News:
N-Terminal Amino Acid Sequencing of IgG Antibodies



Application News:
Primary Structure Analysis of Proteins / Peptides Using Protein Sequencer



Culture Media Comprehensive analysis made fast and easy

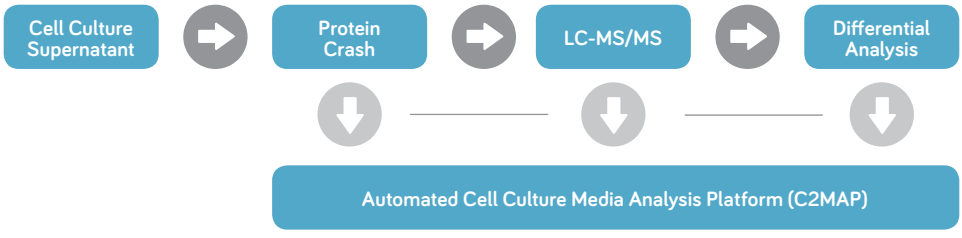
The increasing interest in biopharmaceuticals has resulted in significant growth in the cell culture market, as scientists demand optimal media to ensure the viability of their work.

Cell culture media must contain a precise balance of components, such as glucose, glutamine, nucleic acid, vitamins, and other biologically important compounds and primary metabolites. Ensuring that cell culture media have the optimal formulation for growth and are free of impurities is vital to the success of biopharmaceutical development. The quality of the product depends on the quality of the media. Moreover, cell culture media is necessary in calculating product yield and cost of manufacturing.

Cell culture products are used in biopharmaceutical research throughout the drug development process, from discovery to development and manufacturing. Biopharmaceutical companies, cell culture media manufacturers, biosimilar manufacturers and stem cell researchers all rely on precisely controlled cell culture media.

To meet this demand, Shimadzu developed the triple quadrupole mass spectrometer LCMS-8060 and Cell Culture Profiling Method Package and C2MAP to make comprehensive cell culture analysis fast, easy and effective.

A Complete Analyzer Solution



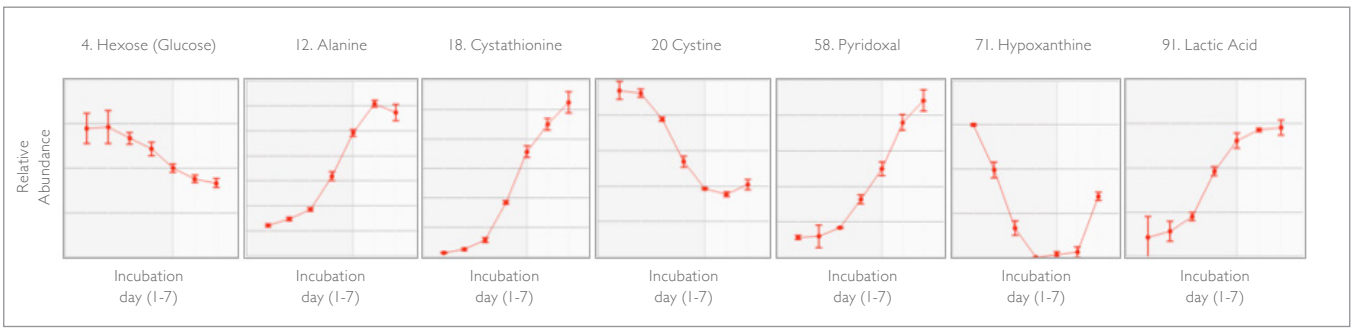
Culture Media Comprehensive analysis made fast and easy

Compounds analyzed by C2MAP

An experimental workflow can be both prospective (understanding the time-course change of culture medium components to find better composition for growth) or retrospective (comparing cell growth and composition of different culture medium products or lots).

List of Compunds

No.	Compound Name	Class.
1	2-Isopropylmalic acid	IS
2	Gluconic acid	Carbohydrate
3	Glucosamine	Carbohydrate
4	Hexose (Glucose)	Carbohydrate
5	Sucrose	Carbohydrate
6	Threonic acid	Carbohydrate
7	2-Aminoadipic acid	Amino acid
8	4-Aminobutyric acid	Amino acid
9	4-Hydroxyproline	Amino acid
10	5-Glutamylcysteine	Amino acid
11	5-Oxoproline	Amino acid
12	Alanine	Amino acid
13	Alanyl-glutamine	Amino acid
14	Arginine	Amino acid
15	Asparagine	Amino acid
16	Aspartic acid	Amino acid
17	Citrulline	Amino acid
18	Cystathionine	Amino acid
19	Cysteine	Amino acid
20	Cystine	Amino acid
21	Glutamic acid	Amino acid
22	Glutamine	Amino acid
23	Glutathione	Amino acid
24	Glycine	Amino acid
25	Glycyl-glutamine	Amino acid
26	Histidine	Amino acid
27	Isoleucine	Amino acid
28	Kynurenine	Amino acid
29	Leucine	Amino acid
30	Lysine Amino	acid
31	Methionine	Amino acid
32	Methionine sulfoxide	Amino acid
33	N-Acetylaspartic acid	Amino acid
34	N-Acetylcysteine	Amino acid
35	Ornithine	Amino acid
36	Oxidized glutathione	Amino acid
37	Phenylalanine	Amino acid
38	Pipecolic acid	Amino acid
39	Proline	Amino acid
40	Serine	Amino acid
41	Threonine	Amino acid
42	Tryptophan	Amino acid
43	Tyrosine	Amino acid
44	Valine	Amino acid
45	4-Aminobenzoic acid	Vitamin
46	Ascorbic acid	Vitamin
47	Ascorbic acid 2-phosphat	Vitamin
48	Biotin	Vitamin
49	Choline	Vitamin
50	Cyanocobalamin	Vitamin
51	Ergocalciferol	Vitamin
52	Folic acid	Vitamin
53	Folinic acid	Vitamin
54	Lipoic acid	Vitamin
55	Niacinamide	Vitamin
56	Nicotinic acid	Vitamin
57	Pantothenic acid	Vitamin
58	Pyridoxal	Vitamin
59	Pyridoxine	Vitamin
60	Riboflavin	Vitamin
61	Tocopherol acetate	Vitamin
62	Adenine	Nucleic acid
63	Adenosine	Nucleic acid
64	Adenosine monophosphate	Nucleic acid
65	Cytidine	Nucleic acid
66	Cytidine monophosphate	Nucleic acid
67	Deoxycytidine	Nucleic acid
68	Guanine	Nucleic acid
69	Guanosine	Nucleic acid
70	Guanosine monophosphate	Nucleic acid
71	Hypoxanthine	Nucleic acid
72	Inosine	Nucleic acid
73	Thymidine	Nucleic acid
74	Thymine	Nucleic acid
75	Uracil	Nucleic acid
76	Uric acid	Nucleic acid
77	Uridine	Nucleic acid
78	Xanthine	Nucleic acid
79	Xanthosine	Nucleic acid
80	Penicillin G	Antibiotics
81	2-Aminoethanol	Other
82	2-Ketoisovaleric acid	Other
83	3-Methyl-2-oxovaleric acid	Other
84	4-Hydroxyphenyllactic acid	Other
85	Citric acid	Other
86	Ethylenediamine	Other
87	Fumaric acid	Other
88	Glyceric acid	Other
89	Histamine	Other
90	Isocitric acid	Other
91	Lactic acid	Other
92	Malic acid	Other
93	O-Phosphoethanolamine	Other
94	Putrescine	Other
95	Pyruvic acid	Other
96	Succinic acid	Other

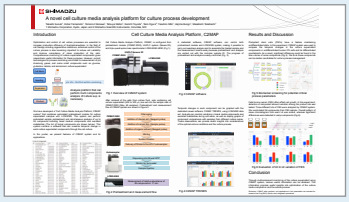


Representative results of a time-course experiment demonstrate consumption and accumulation of critical media components.
C2MAP TRENDS™ Software automatically outputs the tiled panel of time-course charts for each of the 95 compounds analysed (see list above), delivering users an intuitive glance at how the components change over time.

Culture Media Comprehensive analysis made fast and easy

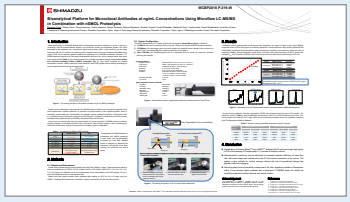
Primer eBook:
LC-MS/MS Makes Cell Culture Media Analysis Fast, Easy and Effective

Poster Presentation (WCBP 2018):
A Novel Cell Culture Media Analysis Platform for Culture Process Development

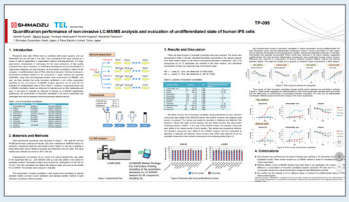


Application News:
Simultaneous Analysis of Culture Supernatant of Mammalian Cells Using Triple Quadrupole LC/MS/MS

Poster Presentation (ASMS 2017):
Using LC-MS/MS to Simultaneously Determine 95 Compounds in Mammalian Cell Culture Supernatants

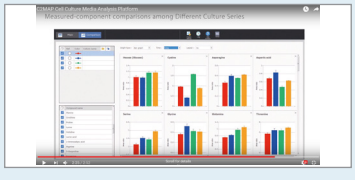


Poster (ASMS 2018):
Non-invasive LC-MS/MS analysis for evaluation of undifferentiated state of human iPS cells



Application News:
A Compilation on the Application of Cell Culture Supernatant and Medium Component Analysis

Video:
C2MAP Cell Culture Media Analysis Platform



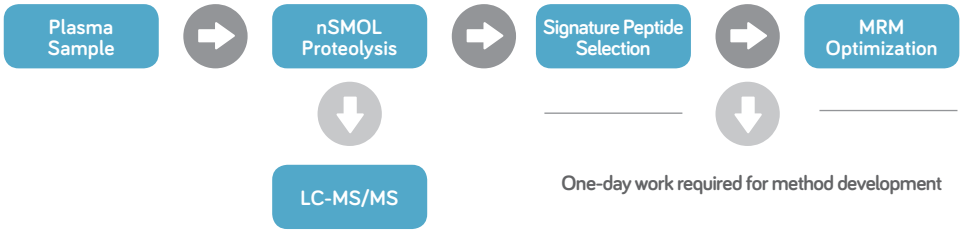
Video:
Advancing Cell Culture Media Analysis and Monitoring by LC-MS/MS

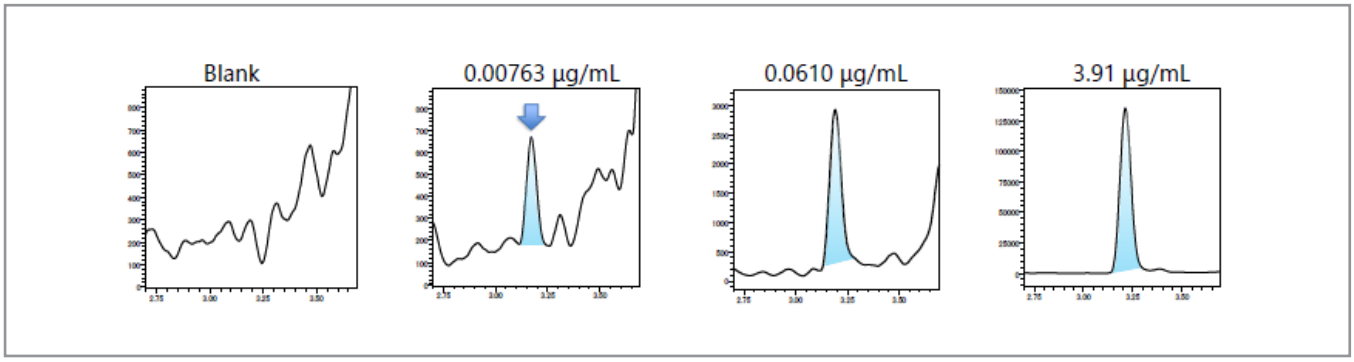
Bioanalysis Accelerating the pre-clinical/clinical phase

Bioanalytical method development is the critical step in the biopharmaceutical pipeline as it bridges the transition to pre-clinical and clinical phases. Ligand-binding assay (LBA) has been the common technique for biologics, however, LC-MS is emerging as an alternative to reduce time and cost needed for method development and to gain increased selectivity and efficiency.

To further simplify and streamline the LCMS workflow for antibody bioanalysis, Shimadzu developed an innovative nanotechnology-based nSMOL™ Antibody Bioanalysis platform for the selective proteolysis of the Fab region of antibody drugs. This increases the detection sensitivity of surrogate peptides in CDR regions, which can be accurately quantified via MRM measurements using a triple quadrupole high performance liquid chromatograph mass spectrometer.

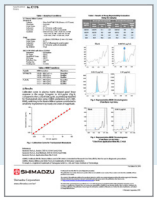
nSMOL workflow Nano-Surface and Molecular-Orientation Limited proteolysis - when nano-technology meets mAb





Representative MRM chromatograms of Trastuzumab spiked in pooled human plasma. Linearity range was 0.00762 to 62.5 µg/mL. (Download the application for details)

Application News
Microflow LC-MS/MS Analysis of Monoclonal Antibody in Human Plasma at ng/mL Level with nSMOL Proteolysis

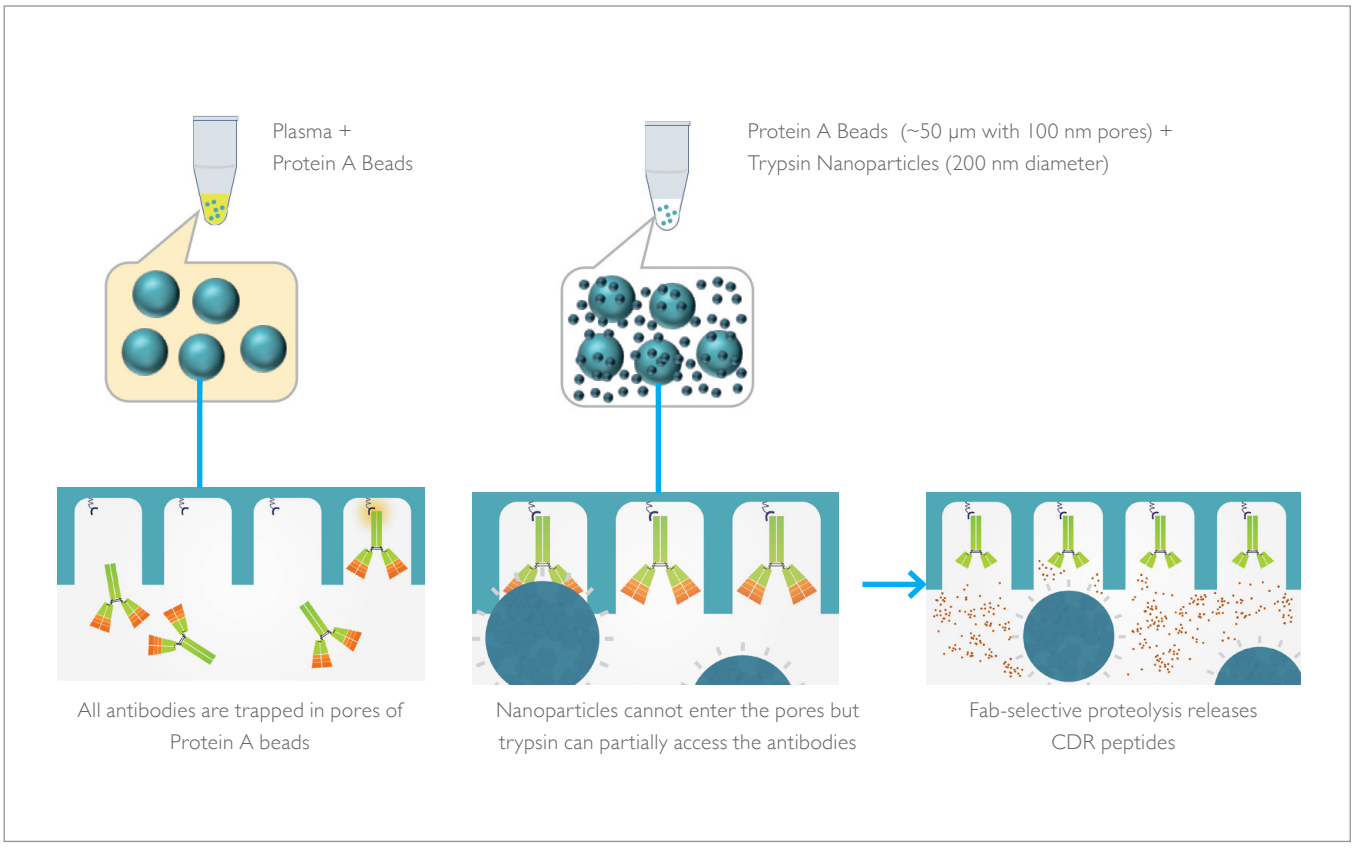


SHIMADZU
Excellence in Science

Bioanalysis Accelerating the pre-clinical/clinical phase

nSMOL™ Antibody BA Kit

Simplifying workflows and enhancing sensitivity

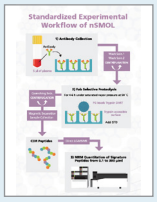


	nSMOL + LCMS	LBA
Ab for collection/detection	Not needed	6++ months to develop
Cross reactivity test	Not needed	Mandatory and tricky
Pre-validation	1-3 days	2-3 weeks
Full validation	3-4 w	3-4 w
Sample prep	3-5 h	2-4 h
Data features	Highly selective and reliable, wide dynamic range, easy to multiplex, independent of antibodies	Highly dependent on quality of detection Ab.

Development of LCMS bioanalysis in combination with nSMOL proteolysis is much faster, and can dramatically accelerate the total R&D period of biologics by alleviating the bottlenecks that typically occur when entering the preclinical and clinical phase.

Bioanalysis Accelerating the pre-clinical/clinical phase

Primer eBook:
nSMOL Improves the Speed and Accuracy of mAb BioanalysisNano-technology, limited proteolysis, and LCMS analysis.



Application News:
LCMS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL
Part 1 – Trastuzumab Analysis

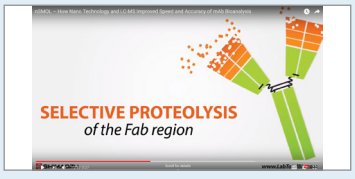
Application News:
LCMS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL
Part 2 – Bevacizumab Analysis

Application News:
LCMS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL
Part 3 – Nivolumab Analysis

Application News:
LCMS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL
Part 4 – Multiplex Analysis

Application News:
LC-MS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL - Part 5 - Instrument comparison

Video:
nSMOL – How Nano Technology and LC-MS Improved Speed and Accuracy of mAb Bioanalysis



Application News:
LCMS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL
Part 6 – For Automated Analysis

Peer-reviewed journal article:
Vialaret J, Broutin S, Paci A, Hirtz C. et al., Bioanalysis. 2018 May 1;10(10)
What sample preparation should be chosen for targeted MS monoclonal antibody quantification in human serum?

Impurities Investigation of protein aggregation and more

Biopharmaceutical products are no different from small molecule pharmaceuticals in that all impurities derived from the raw materials, manufacturing process, formulation and instability need to be fully investigated and controlled (ICH Q3). However, in addition to conventional impurities, such as elemental impurities, there are certain issues that require particular attention due to the labile nature of proteins.

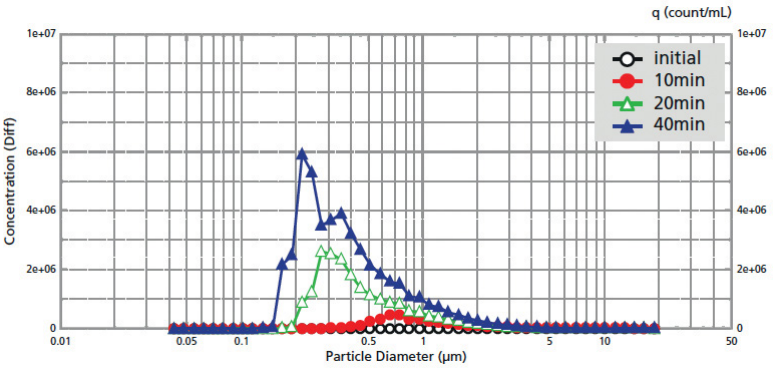
With a wide-ranging product portfolio, Shimadzu has a long history of providing solutions to address various impurities to the pharmaceutical industry.

Investigating Aggregates

Protein aggregates form at an accelerated rate if the sample is kept under improper conditions, such as temperature change, agitation, pH, surface contact and presence of other impurities. Formation of aggregates is conventionally monitored by size exclusion chromatography(Page 6).

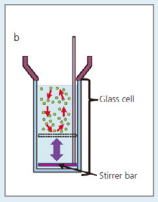
In addition, Shimadzu offers a number of different solutions for investigating the nature of the aggregates formed, in order to give researchers insight into how aggregates can be minimized.

Aggregates Sizer

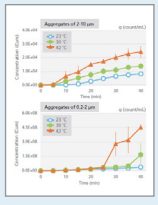


Time-course distribution of particle size of bovine IgG solutionunder accelerating conditions

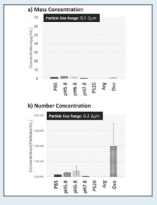
Application News:
Evaluation of Protein Aggregation Under Various Stress Conditions Using the Aggregates Sizer



Application News:
Accelerated Testing of Protein Stability Using the Aggregates Sizer TC (with Temperature Control)



Application News:
Aggregates Sizer Enables Evaluation of Biopharmaceutical Additives to Inhibit Protein Aggregation



Peer-reviewed journal article:
Yoneda S, Uchiyama S et al., J Pharm Sci. 2019 Jan;108(1) Quantitative Laser Diffraction for Quantification of Protein Aggregates: Comparison With Resonant Mass Measurement, Nanoparticle Tracking Analysis, Flow Imaging, and Light Obscuration

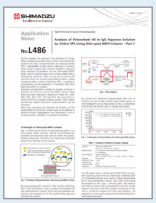
Impurities Investigation of protein aggregation and more

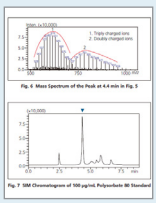
Analysis of Polysorbate 80

Polysorbate 80 (polyoxyethylene sorbitan monooleate) is commonly used as an additive to stabilize recombinant protein in the final formulation. As reagent, it is a polymeric mixture of a range of carbon numbers and is prone to degradation. Thus, the composition and quantity of polysorbate 80 must be analysed both as raw material and in the final formulation. Shimadzu offers both GC and LCMS solutions to address this need.

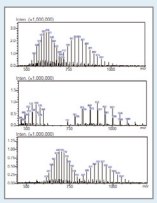
Application News:
Purity Testing of Polysorbate 80 by GC

Application News:
Analysis of Polysorbate 80 in IgG Aqueous Solution by Online SPE Using Shim-pack MAYI Column – Quantitative Analysis





Application News:
Analysis of Polysorbate 80 in IgG Aqueous Solution by Online SPE Using Shim-pack MAYI Column – Quality Analysis



Elemental Impurities

Application News:
Analysis of ICH Q3D Guideline for Elemental Impurities in Drug Products Using ICPMS-2030

Extractables and Leachables

Application News:
Analysis of Styrene Leached from Polystyrene Cups Using GCMS coupled with Headspace (HS) Sampler

Application News:
Determination of Leachables in Orally Inhaled and Nasal Drug Products (OINDP) by GC-MS/MS

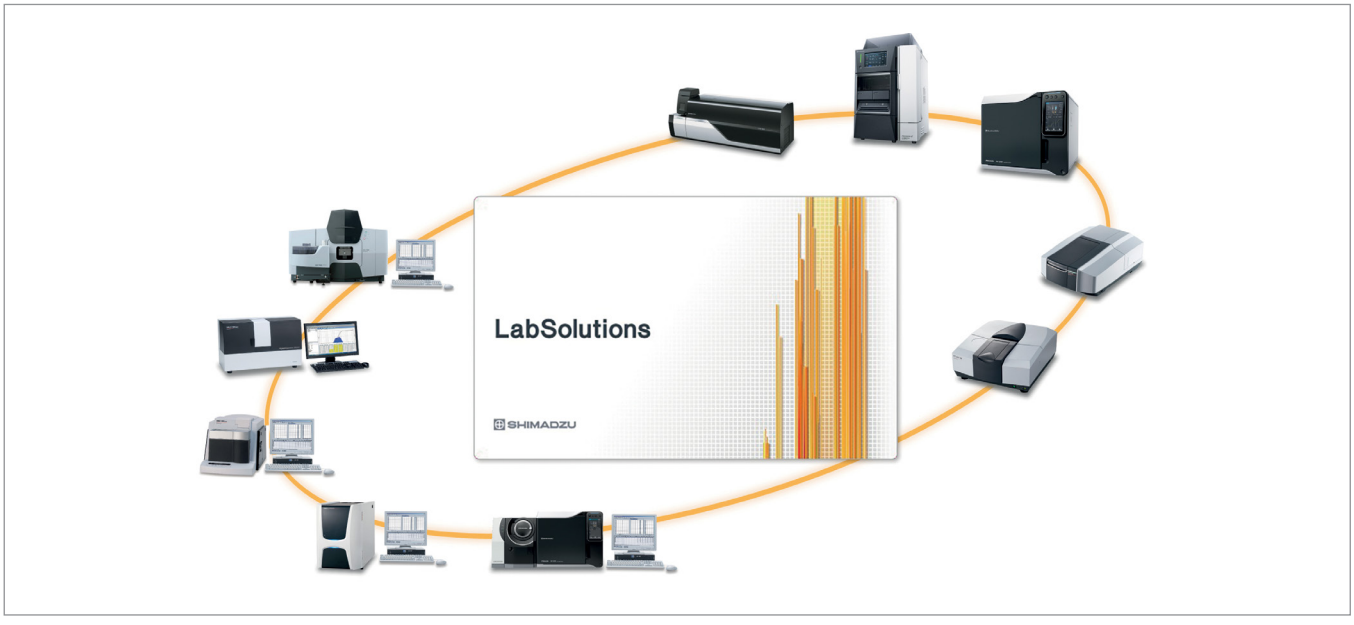
SHIMADZU
Excellence in Science

Total Solution for Regulatory Compliance

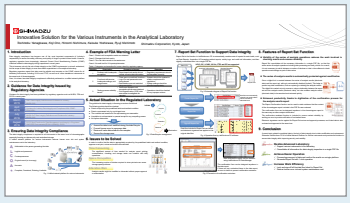
Data integrity compliance has become one of the most important components of the pharmaceutical industry's responsibility to ensure the safety, efficiency and quality of drugs to protect human health. However, regulatory agencies have observed an increasing number of Current Good Manufacturing Practice (CGMP) violations related to data integrity in recent years.

Regulatory agencies expect data to be reliable and accurate on the CGMP criteria for all laboratory instruments, including UV-Vis and FT-IR, as well as all other standalone instruments.

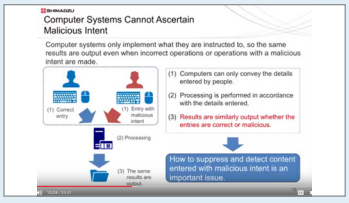
Shimadzu offers the LabSolutions CS platform, the unified network platform that is able to control all Shimadzu instruments via the network with administrative access control and logging, complete audit trail and reporting functionality to ensure data integrity compliance.



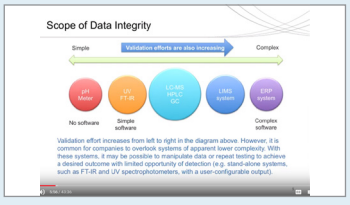
Poster Presentation (Pittcon 2018):
Innovative Solution for the Various Instruments in the Analytical Laboratory



Video:
"Supporting Data Integrity"



Video:
"Solutions Supporting the Reliability of Test Data"



Find us on

Find us on

Find us on

Contact Us

https://www.shimadzu.com/contact/

SHIMADZU
Excellence in Science

Shimadzu Corporation
https://www.shimadzu.com/an/