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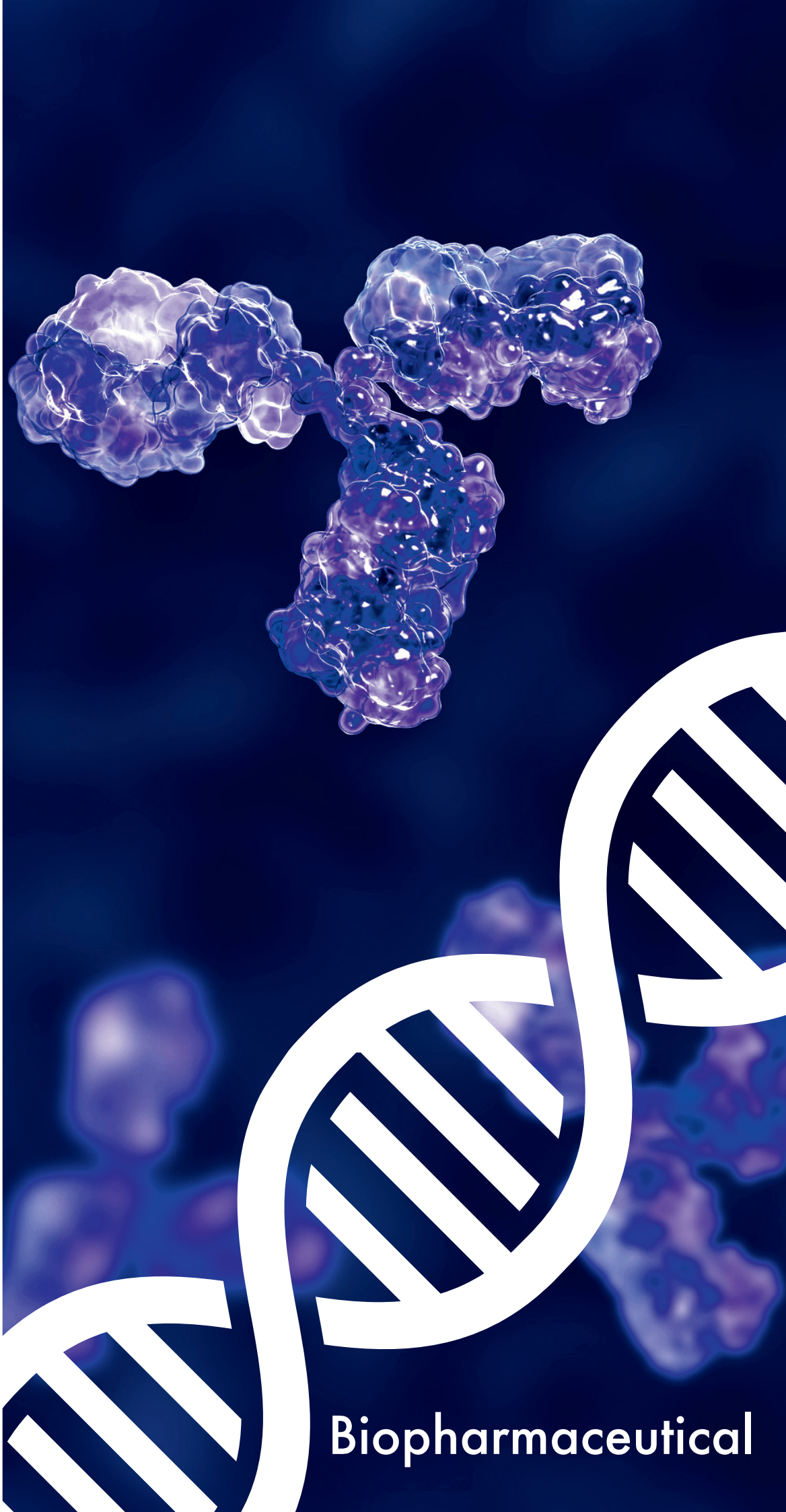
Shimadzu Journal



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Dear Readers,

Yoshiaki Mase

General Manager,
Analytical & Measurement Instruments Division

As "a company that solves challenges in society in collaboration with partners all around the world", Shimadzu Corporation aims to create and promote solutions to these challenges. Even as the impacts from the COVID-19 pandemic still continue, Shimadzu's Infectious Disease Countermeasure Projects, which were established in 2020 as important emergency challenges, entered a new stage, Phase II, in 2021. We actively engage in creating new methods, networks and systems. Fields and approaches related to analytical and measuring instruments are changing, as the movement of people and things changes worldwide, and business activities and working practices change rapidly in various countries and regions.

In the midst of such large changes across the whole of society of the world, this issue features biopharmaceuticals, which are rapidly increasing in presence at the worldwide. We introduce the researchers active on the front lines of biopharmaceutical research, as well as the applications making use of cutting-edge technology related to the biopharmaceutical field.

To begin with, we talked with Ms. Shraddha Mane, head of quality control at HiMedia Laboratories Pvt Ltd. about the development of cell culture media and related analysis systems. Next, we heard from Dr. Shivam Mukherjee of Intas Pharmaceuticals Ltd. He talked about the importance of cell culture conditions in the development of therapeutic monoclonal antibody biosimilars and about cell culture profiling. In addition, Dr. Noel Zahr of Assistance Publique–Hôpitauxde Paris (APHP) talked about the results of research related to improvements in pharmaceutical management (personalized medicine), in the context of a project to test and evaluate the pharmacokinetics of monoclonal antibodies addressed through collaborative research with Shimadzu.

In addition to these interviews, we introduce examples of the analysis of antibody drugs using our new UHPLC, Nexera XS inert, applications analyzed with HPLC, LC-MS/MS, and FTIR, and the results of research from collaborations using nSMOL(nano-surface and molecular-orientation limited proteolysis). We also introduce the latest initiatives by Shimadzu related to biopharmaceuticals.

Further social changes are likely to continue into the future. Yet Shimadzu is realizing its corporate philosophy of "Contributing to Society through Science and Technology," so as to embrace change rather than fearing it, and to create an even better society. While working cooperatively with you in the cutting-edge and innovative research and challenging activities in which you are engaged, we hope to stimulate your work through this journal.

Yours Sincerely,

Biopharmaceutical Issue2

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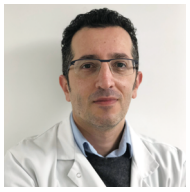

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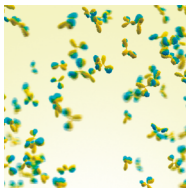

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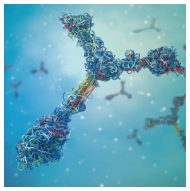

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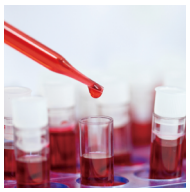

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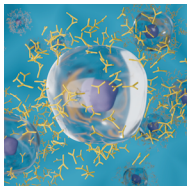

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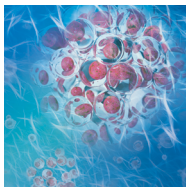

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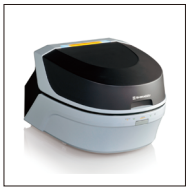

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- EDX-7200
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Interview 1

"It's a strong, reliable, and synergistic bonding."

Ms. Shraddha Mane

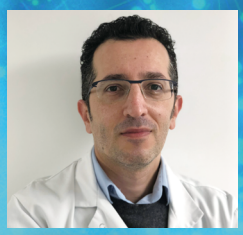
Interview 2

"It is excellent and extremely beneficial in support of our research and development process."

Dr. Shivam Mukherjee

Interview 3

"Our partnership is a great support to advance research in oncology"



Dr. Noel Zahr

Interview 1 Interview with Ms. Shraddha Mane

"It's a strong, reliable, and synergistic bonding."

We interviewed Ms. Shraddha Mane from HiMedia Laboratories Pvt. Ltd in India, a leading Bioscience company based in Mumbai manufacturing high-quality culture media for microbiology. HiMedia is amongst the top 3 brands in microbiology across the world. Additionally, they also provide advanced media and products in the fields of Animal Cell Culture, Plant Tissue Culture, Molecular Biology, Chemicals, and Lab-Aids/Equipment. HiMedia have recently progressed in the field of Higronics which is quite promising for the future of agriculture. Shimadzu have been supporting the research and development activities undergoing at HiMedia.

Hello Ms. Mane, thank you very much for spending some time for this interview. At first, could you brief us about your organization, type of research carried out, product line, etc.?

HiMedia Laboratories Private Limited is an Indian-origin bioscience company headquartered in Mumbai, with a presence across 150 countries. It has different divisions such as Microbiology, Animal cell culture, Plant Cell Culture, Higronics, Molecular Biology, and Chemicals & Lab aids.

Animal Cell Culture division has proficiency in the development & manufacture of cell culture medium for mammalian & insect cell lines, in powdered and liquid formulations. Comprehensive design of experiments, raw material qualification, and media production using automation and customization are our strengths. We supply serum-free media for CHO, Vero, MDCK, HEK, PK-15, Vero, MRC-5, BHK cell lines, and T cells, most widely used for industrial production of therapeutic recombinant proteins and vaccines. In addition, we manufacture and supply multi-compendial biochemicals and growth factors for Cell Culture.

Could you outline the research activities carried out in your department?

Our main focus is on the development of different cell culture media which is used in Biopharma for protein production and in Vaccine production to achieve a good cell count and titre.

Other research activities involve protein production, purification of the protein, spent media analysis, characterization of the protein, and media development used in vaccines where the main focus is to achieve a good count of the cells.

Congratulations to HiMedia Laboratories on being conferred with the "Innovator Award- 2020" by BIRAC. Could you share in brief more about this achievement?

Thank you so much. It was indeed a prestigious award.

HiMedia Laboratories Pvt Ltd. has been conferred with the "BIRAC Innovator Award 2020" under the category of therapeutics, vaccines, and drug delivery by the Biotechnology Industry Research Assistance Council (BIRAC- A Government of India Organization).

The award was in recognition of the significant contribution made by our team along with its collaborators, the Institute of Chemical Technology, Mumbai, towards a high level of innovation research for "Designing & commercialization of affordable chemically defined serum-free media and feed for high-value Biosimilars manufacture". This innovation will help India to

Ms. Shraddha Mane

make available world-class media & feed required for the manufacture of Biosimilar at a very affordable price as compared to imported media and feed. My team comprising of Ms.Gauri Page, Ms. Soni Shukla, and Ms. Mausami Bhattacharya worked very hard under the guidance of the directors of the cell biology division, Dr.Vishal G. Warke, and Dr.Priti V. Warke.

The award was presented by Shri M. Venkaiah Naidu, Hon'ble Vice President of India, and Dr. Harsh Vardhan, Hon'ble Union Minister for Science & Technology, Earth Sciences and Health & Family Welfare, India. The distribution of the award was done through a virtual ceremony during the Global Bio- India 2021 on 3rd March 2021.

What according to you, are the advantages of chemically defined media over conventional serum-based media?

Chemically defined media has the following advantages over serum-containing medium:

- Cost-effectiveness
- Lot to lot consistency
- Free of any adventitious agent
- Good productivity
- Easy downstream process

Could you briefly explicate how Shimadzu's Cell Culture Profiling (CCP) method package along with LC-MS is useful for developing chemically defined media?

Cell culture media analysis is an integral part of bioprocess technology. Right from the initial raw material analysis to in-process media components monitoring, it helps understand the cell growth cycle and productivity. Hence comprehensive analysis methods like the CCP method package using LC-MS/MS can help us get a better perspective towards media components selection, its impact on cell growth, and in terms of productivity. It helps for tracking the changes in chemical profile during fermentation and deciding the feed composition and time. The Ultra-Fast Mass Spectrometer (UFMS) technology of Shimadzu mass spectrometers like fast scanning speed, polarity switching time, and negligible cross talk helps in simultaneous estimation of all the media compounds in a single run.

Apart from the basic components of chemically defined media like amino acids, vitamins, etc. what are the other components that can be present in it?

Other than basic components present, media also contains trace elements, lipids, and fatty acids which are beneficial for cell proliferation. Also, during the cell culture process, there may be secretion of toxic organic elements, and identifying such elements is necessary since they may hamper cell viability and/or productivity. Overall, the analysis of such essential and trace elements helps to design the apt media and feed for commercial cell lines.

Which analytical tool you are using to determine the concentration of such trace elements and inorganic salts in media?

Shimadzu's ICPMS-2030, because of its high sensitivity, is an appropriate instrument for the determination of trace elements and inorganic salts content in the media.

What is the importance of fresh media and spent media analysis? How do you find Shimadzu LCMS-8045 and ICPMS-2030 useful for this application?

Analysis of fresh media is necessary to assess the components present in the medium before starting the cell culture. Whereas spent media analysis will help in the understanding the changes in media components, analysis of nutrient's consumption trend and its impact on cell

Ms. Shraddha Mane

viability and production. Spent media analysis will help to determine feeding intervals and replenish low media nutrients required for cells. For such applications, the usage of high-end instruments like LCMS-8045 and ICPMS-2030 will be of great assistance. Also, software like LabSolutions Insight helps to quantify vast data with easy identification using the flagging feature. The trend plots and bar graphs generated in Multi-omics Analysis software helps to keep track of the consumption and secretion of compounds throughout the cell culture process.

How are Shimadzu instruments helping you in your other biopharma research and analytics?

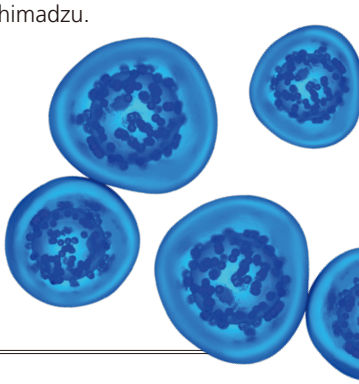
We have multiple types of equipment that are purchased from Shimadzu. HPLC (LC-2030) is being used for protein quantitation, analysis of charge variant, aggregation pattern of IgG, and study of Glycan pattern. LCMS-8045 is used for spent media analysis to design the feed media. ICPMS-2030 is mainly used for elemental analysis. So very true Shimadzu's analytical instruments are very helpful in media optimization.

What according to you, are the attributes of Shimadzu that help build your confidence in the Analytical Solutions offered by Shimadzu?

Ease in the operation of an instrument, user-friendly software and the best is the very cooperative technical & marketing team makes us more confident about analytical solutions provided by Shimadzu.

Thank you again for your time today. Finally, would you be willing to collaboratively work with Shimadzu on a project of mutual interest in the near future?

Yes, of course. the presence of high-end analytical solutions and a highly technical team make us more comfortable working closely with Shimadzu.



▸ Ms. Shraddha Mane holds a Master's degree in Microbiology from Mumbai University, and has also briefly served as Lecturer at Wilson college, Mumbai. During her tenure as the Research Assistant at Enterovirus Research Centre, Mumbai, she handled Isolation of Polio and other Enteroviruses form samples along with Serological typing of Polio Viruses by neutralization process.

At her current position as the R&D and QC head in HiMedia Laboratories Pvt. Ltd. (from 2006 onwards), she gained elaborate professional experience in development of different processes of cell culture, bioprocess and analytical lab, technical support to the customers, development of primary cell-based processes.

Interview 2 Interview with Dr. Shivam Mukherjee

"It is excellent and extremely beneficial in support of our research and development process."

We interviewed Dr. Shivam Mukherjee from Intas Biopharma Ltd. in India, a leading biosimilar product manufacturers in Asia having its own biopharmaceutical operations with a R&D facility and an EU-GMP certified bio-pharmaceutical manufacturing facility. Shimadzu have been supporting the research and development activities undergoing at Intas.

Hello Dr. Mukherjee, thank you very much for spending some time for this interview. At first, could you brief about your organization, type of research carried out, product line etc?

At Intas Biopharma unit, we focus on developing recombinant DNA and plasma derived products. We are focused on developing biosimilars of therapeutic monoclonal antibodies with the goal of 'Biosimilar for Billions'.

Could you outline the research activities carried out in your department?

The R&D department at Intas Biopharma unit consists of several functions starting from developing clones to, screening of media followed by process design and optimization of product. At all stages the process teams are supported by a strong analytical team which focusses on characterization of the developing molecule using a variety of analytical techniques.

Could you describe the importance of cultivation condition such as media, feed in mAb production and how it will impact on final mAb drug quality?

Media and feed component are important as these has direct impact on cell growth. Cell culture media provides a medium for the cell to grow and also a source for cell growth and production while feed improves the culture duration and productivity. In this context, spent media analysis from cell culture can identify the depleted components, which can then be added back to the media either through front loading in basal media or feed enhancement at different culture duration timepoints.

Intas team
From L to R: Shivam Mukherjee, Sreekant Panicker, Nitin Kishor, Salma Bano, Babu Ponnusamy, Nilesh Aghera, Shrinivas Ambekar, Harshit Srivastava, Chetna Kirad.

Dr. Shivam Mukherjee

What are the major challenges in performing analysis for cell culture components of spent media?

The major challenges in identifying components in spent media are extraction methods that are used to monitor different class of analytes (amino acids, nucleic acids, vitamins) and their inherent differences in concentration.

How is Shimadzu LC-MS along with cell culture profiling method package helping you in your research?

Usage of Shimadzu cell culture package provides a number of advantages with respect to simultaneous analysis of a wide range of components including ones which might be present in less concentration. A 17-min run allows identification of 95 components using the MRM principle which distinguishes components that are difficult to monitor using conventional LC techniques. That's why we chose Shimadzu.

How would you describe the value that Shimadzu technology added to your research project?

Shimadzu CCP package combined with the ultrafast and sensitive LCMS-8060 is a very powerful and useful tool that we employ to have a quick identification of components present in media/feed and spent media. The range of compounds present in the method package and the short turnaround time in identification helps the process development teams in screening of multiple media and feed combinations, thus ensuring the best possible one is carried forward in upstream process development.

Thank you again for your time today. Finally, could you share any requests that you have with respect to analytical and measuring instrument vendors?

We have a beneficial relationship with Shimadzu instruments and related method packages. We would like to continue this and receive your updates on a regular basis which will be helpful in our research.

▸ Dr. Shivam Mukherjee did his undergraduate studies in Chemistry and then proceeded to do his doctoral studies at Stony Brook University, New York. Post completion of his thesis, he returned back to India and joined TCG Lifesciences in Kolkata. He joined Intas Biopharma Ltd. in 2019 and since then has been working in the R&D Analytical Development Laboratory where he & his team's work focusses on developing LC-MS methods used in identification and characterization of biomolecules.

Interview 3 Interview with Dr. Noel Zahr

"Our partnership is a great support to advance research in oncology"

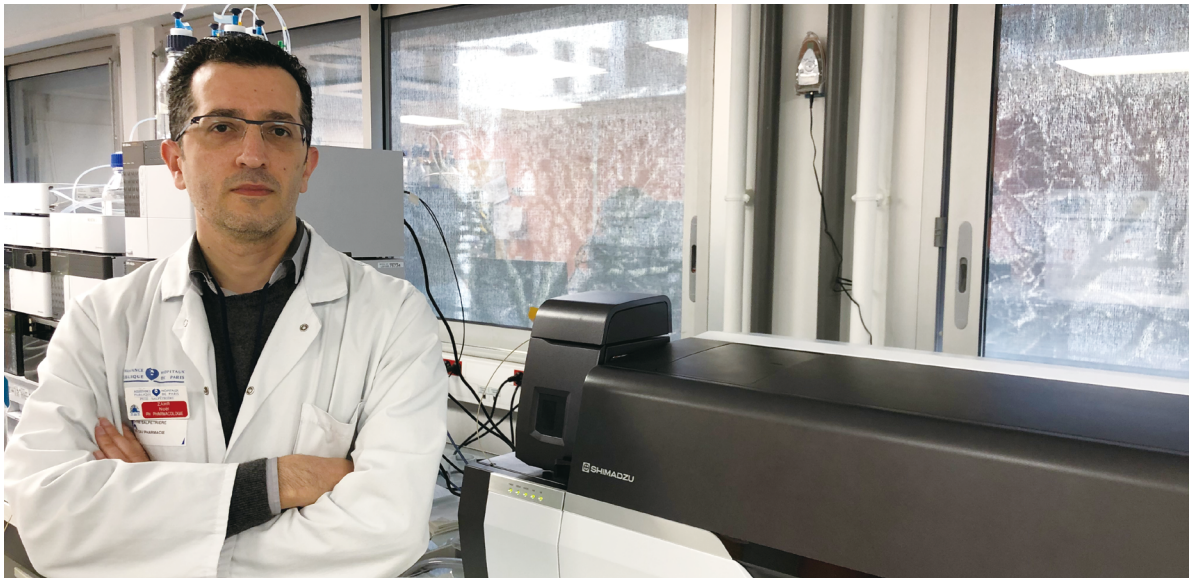
We interviewed Dr. Noel Zahr from Assistance Publique–Hôpitaux de Pari (AP-HP), Pitié Salpetrière Hospital in France, for an activity in pharmacology dedicated to improving drug management (personalized medicine) on the project of 'Monoclonal antibody pharmacokinetic assay and evaluation with the "nSMOL" kit and Shimadzu LCMS instrument' starting in 2018. APHP and Shimadzu started collaborations since the project started.

Hello Dr. Zahr, thank you very much for spending some time for this interview. At first, could you outline the research and let us know what discovery and achievement have been made so far?

We were interested by the development of monoclonal antibodies assays by mass spectrometry, but we do not have experience in this field. In the early part of this collaboration, with the help of Shimadzu and nSMOL kit, we were able to develop quickly the dosage of Belimumab by LCMS-8060 for patients with systemic lupus erythematosus*[1]. Currently, we are in the process of analyzing the results to study the pharmacokinetics and pharmacokinetics/pharmacodynamics correlation for this drug. Then, we urgently developed the dosage of Tociluzumab during Covid-19 epidemic. This method allowed us to study the rate of passage of tociluzumab into the brain, and to describe the marked clinical and biological response of a targeted treatment with Tociluzumab in a patient with a severe corticoresistant CNS toxicity of immune-checkpoint inhibitors (ICI) therapy*[2]. Finally, our main focus of development is TDM in oncology. Thanks to this collaboration, we have developed a method to quantify several simultaneously monoclonal antibodies*[3].

*1 Zahr, N., Amoura, Z., Beaulieu, Q., Llopis, B., Pha, M., Benameur, N., Mathian A. & Funck-Brentano, C. (2021) "Application and validation of LC-MS/MS bioanalysis of Belimumab in patients with systemic lupus erythematosus", Fundamental & Clinical Pharmacology, 35 (S1) , 144.
*2 article accepted in Neuroimmunology and Neuroinflammation Journal
*3 Data issues from these developments are not published yet.

Dr. Noel Zahr in front of his LCMS-8060



Dr. Noel Zahr

How are Shimadzu instruments helping you in your research?

First of all, regarding sample preparation, the nSMOL kit was very easy to use by technicians. The sample preparation time was well optimized in order to finalize all the peptide digestion steps on the same day. The HPLC system was very efficient. LCMS-8060 was very fast, sensitive and robust which allowed us to develop several multiplexing method.

Could you tell us why you chose Shimadzu as your partner when you started this project?

We have known Shimadzu Company for more than 15 years through the use of their HPLC systems. Shimadzu is a leader in the development of peptide and protein assays. Moreover, Shimadzu was the first one to offer a universal sample preparation kit for monoclonal antibodies.

What are Shimadzu's strengths compared to other vendors?

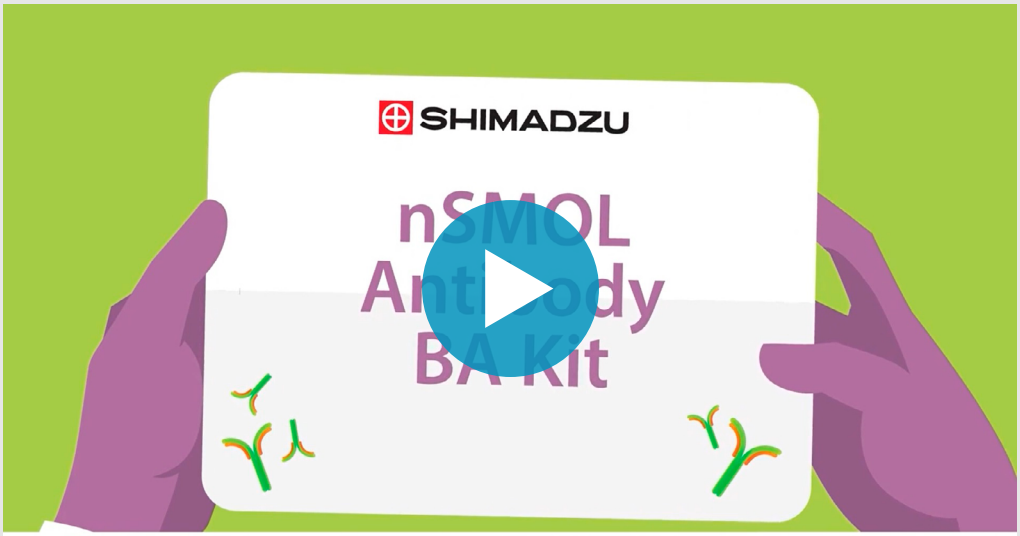
Apart from this collaboration, the Shimadzu instruments are robust and sensitive. In our laboratory, where many routine tests are carried out, it is important to have robust systems well maintained over time. Shimadzu LCMS systems are scalable, and it's very important when we cannot objectively renew it regularly. The quality to price ratio and the after-sales service are excellent.

Thank you again for your time today. Finally, could you share any requests that you have with respect to analytical and measuring instrument vendors?

What I can say to analytical and measuring instrument vendors is that they must always listen to the needs of their customers, in particular to help with the development of methods assay.

▶ Dr. Noël Zahr, pharmacologist-biologist, he is the head of the "Pharmacokinetics and Therapeutic Pharmacological Monitoring" unit and the Therapeutic Drug Monitoring Laboratory in Assistance Publique–Hôpitaux de Paris (AP-HP), Pitié Salpetrière Hospital since 2012, Dr. Zahr holds a Master's degree in research and a PhD in pharmacology. His main line of research is the evaluation of the correlation between a clinical event (toxicity, lack of efficacy) and the plasma concentrations of drugs.

Dr. Noel Zahr



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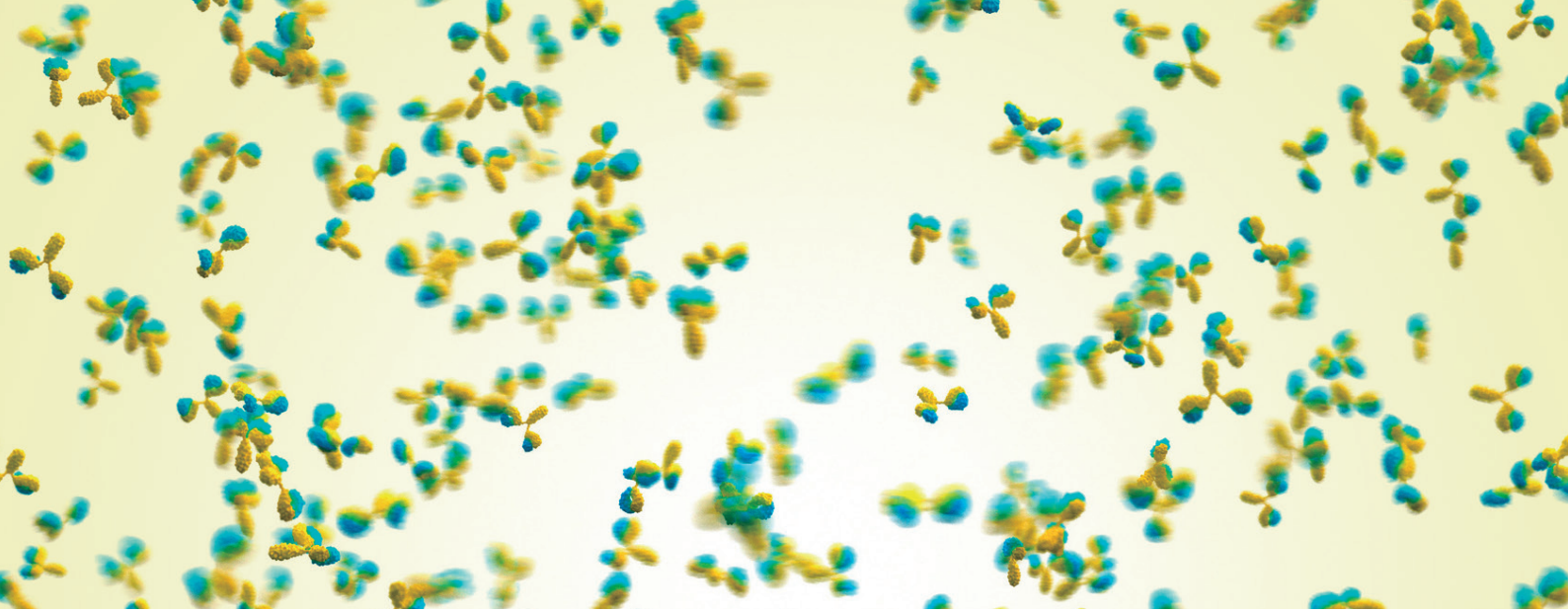
nSMOL Antibody BA Kit

nSMOL (nano-surface and molecular orientation limited proteolysis) is Shimadzu's proprietary, innovative technique that enables selective proteolysis of the Fab region of monoclonal antibodies.
By using this kit and LCMS-8050/8060, you realize high accuracy, low cost, and time reduction while simplifying the pretreatment operation, and bring a paradigm shift to the bioanalysis of antibody drugs.
>>>>Learn more



Biopharmaceutical

Method Optimization for the Analysis of Monoclonal Antibodies by Size-Exclusion Chromatography

Emiko Ando[1], Daiki Fujimura[1], Keiko Matsumoto[1]

[1]Analytical & Measuring Instruments Division, Shimadzu Corporation

Abstract Antibody drugs using monoclonal antibodies pose concerns over aggregates formed during production and storage and their impact on safety and efficacy. During monoclonal antibody production, aggregates formation is monitored, and size-exclusion chromatography is one of the most widely used techniques. However, size-exclusion chromatography is performed at relatively low flow rates and requires long analysis times. Analyses of monoclonal antibody drugs must also take into account interaction between the monoclonal antibodies and column packing materials. This Technical Report provides an example of using a column packed with small particle material to optimize an analytical method for analysis of monoclonal antibody aggregates. This article investigates the effect of mobile phase salt concentration, flow rate, and pH on chromatographic separation and peak shape. Moreover we describe an example of method optimization by using a dedicated software for improving separation, sensitivity and reducing analytical time.

Keywords *size-exclusion chromatography, antibody drug, Nexera™ XS inert, method scouting system*

Background

Pharmaceuticals have recently diversified away from low-molecular-weight compounds also to include macromolecular drugs. Biopharmaceuticals developed and produced with biotechnology have been particularly effective in treating wide range of diseases.

The most common biopharmaceuticals are antibody drugs that utilize monoclonal antibodies (mAbs). Due to their high level of specificity and affinity for target molecules, they offer the benefits of excellent therapeutic efficacy and mild side effects, and are used to treat a variety of diseases including autoimmune diseases and cancer. However, unlike pharmaceuticals that can be controlled artificially in manufacturing processes such as chemical synthesis, biopharmaceuticals are manufactured using living cells. Therefore, appropriate quality controls must be established at every production step to ensure uniformity and quality of the final product.

There are numerous steps from development to production of antibody drugs. The first step is to find an antibody effective against disease based on its affinity and specificity to target molecules. The next step introduces genetic information coding for the selected antibody into cells and determines what culture conditions ensure efficient protein expression. These culture conditions are then scaled up for mass production and the target protein is isolated from cultured cells and purified to produce the bulk drug. In order to produce high-quality antibody drugs, consistent and robust manufacturing methods are essential. The quality, efficacy, and safety of antibody drugs are assured by performing purity tests in conformance with ICH-Q6B[1], including purity analysis and structural analysis of

aggregates and isomers, and other assessments as shown in Table 1. These assessments are extremely important at every step of pharmaceutical development, from early stages to product shipment.

mAbs form dimeric or multimeric aggregates depending on production and storage conditions. Aggregates in antibody drugs not only cause a decrease in pharmacological action but also elicit an immune response, thus affecting the efficacy and safety. For this reason, ICH-Q6B requires the separation of impurities such as monomers and aggregates in antibody drugs and determines their content. This article introduces an analysis of mAb impurities and fragments by size-exclusion chromatography (SEC).

Table 1. Examples of Quality Assessment Tests for Antibody Drugs

Item Tested	Purpose	Analysis Technique
Aggregates/ Fragments	Determine levels of aggregates and fragments	Size-exclusion chromatography, Micro flow imaging etc.
Charge variants	Characterization and monitoring of charge variants	Ion-exchange chromatography, Imaged capillary isoelectric focusing etc.
Sugar chain structures	Evaluate consistency of sugar chain structures	Hydrophilic chromatography, Reversed-phase chromatography, Mass spectrometry etc.
Structure	Evaluate molecular structure and specificity characteristics of bulk drug	Reversed-phase chromatography, Mass spectrometry etc.
Antibody-drug conjugates	Calculate coupling ratio of antibody-drug conjugate	Hydrophobic chromatography, Mass spectrometry etc.
Potency	Quantify biological activity	Affinity chromatography, Enzyme-linked immunosorbent assay etc.

Size-Exclusion Chromatography

Size-exclusion chromatography (SEC) is a technique that separates molecules based on their size (Fig. 1). The column packing material contains numerous pores. Smaller molecules permeate deeper into these pores and take longer to pass through the column, on the other hand, larger molecules are unable to permeate the pores. Consequently, molecules are eluted from the column in order from largest to smallest and effectively sorted according to size. Conventional SEC analysis uses long columns 300 mm in length and low flow rates with long elution times from several tens of minutes up to an hour to ensure full separation between components. Recent column development has reduced the sizes of packing materials to achieve excellent separation with shorter elution times.

However, further improvements in separation and sensitivity require not just smaller column packing materials but optimization of the analytical conditions. In an ideal SEC separation, there is no chemical interaction between the molecules, packing material surfaces, and mobile phase. But in the SEC columns containing silica-based packing materials with chemically-bound common diol groups, electrostatic interaction may arise between proteins and residual silanol groups on the packing material surface causing proteins to adsorb to the silica gel. This results in peak tailing, delayed elution times, and other phenomena. Electrostatic interactions can be suppressed by adding sodium chloride to the mobile phase to negate these negatively charged silanol groups. The strength of these interactions with column packing material varies with the type of protein; it is necessary to determine the appropriate analytical methods for each protein.

This article presents an optimization of an analytical method for mAb aggregates and fragments that investigates the effects of mobile phase salt concentration, flow rate, and pH on separation with a 150 mm long Shim-pack™ Bio Diol-300 column with 2 µm diameter packing material.

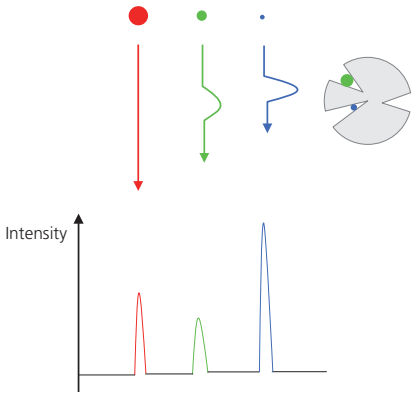


Fig. 1. Principle of Separation in SEC Analysis

Analysis

3-1. Analytical Conditions

Table 2 shows the analytical conditions that are common to all analyses. The mobile phase compositions are described in each section.

Table 2. Analytical Conditions

System	Nexera XS inert
Column	Shim-pack Bio Diol-300 *1 (150 mm×4.6 mm I.D., 2 µm)
Flow rate	0.2 mL/min (Fig. 2, Fig. 3, Fig. 9) 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4 mL/min (Fig. 5) 0.25 mL/min (Fig. 7)
Column Temp.	25 ˚C
Sample	Monoclonal Antibody Standard (Conc. 500 µg/mL)
Vial	TORAST™-H Glass Vial *2 (Shimadzu GLC Ltd.)
Injection vol.	5 µL
Detection	280 nm (SPD-M40 inert cell)

*1 P/N : 227-31010-01 *2 P/N : 370-04301-01

3-2. Impact of Mobile Phase Salt Concentration

Sequential analysis was performed to evaluate the impact of mobile phase salt concentration while automatically adjusting salt concentration by the mobile phase blending function, which is the feature of the method scouting system. Fig. 2 shows a chromatogram obtained using a 100 mmol/L phosphate buffer (pH 7.0) without sodium chloride and the same chromatogram expanded to show the area around the monomer peak in more detail. Fig. 3 also shows the same expanded area in chromatograms obtained using a 100 mmol/L phosphate buffer (pH 7.0) containing between 50 and 250 mmol/L of sodium chloride.

Fig. 4 shows the relation between sodium chloride concentration in the mobile phase and the peak symmetry factor and resolution. At 0 mmol/L sodium chloride in the mobile phase (Fig. 2), electrostatic interaction between the column packing material and mAb caused tailing of the monomer peak; but the symmetry factor of this monomer peak improved as the sodium chloride concentration was increased from 50 to 250 mmol/L. Peak resolution was also affected; it showed the best value around 50 and 100 mmol/L sodium chloride (Fig. 3, 4), which contributed to accurate peak integration. As a conclusions in this case, the better monomer peak shape and appropriate separation from aggregates were achieved with a sodium chloride concentration of 100 mmol/L. Hence a mobile phase sodium chloride concentration of 100 mmol/L was chosen for this mAb analytical method.

3-3. Impact of Flow Rate

It's known that the flow rate should be optimized based on mobile phase composition, packing material particle size, physical and chemical properties of molecules, and other factors[2]. Fig. 5 and Fig. 6 show the impact of mobile phase flow rate on separation for this analysis.

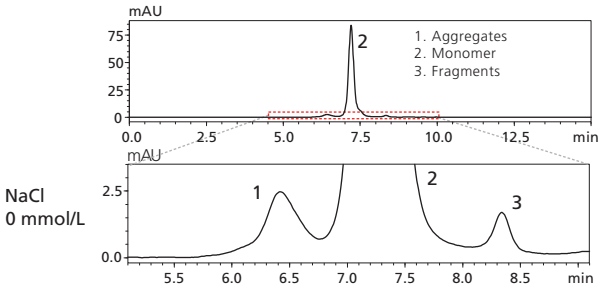


Fig. 2. Chromatograms without Sodium Chloride in Mobile Phase

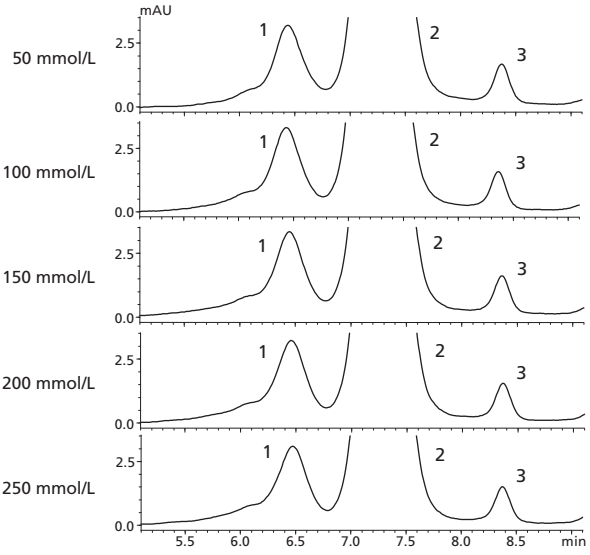


Fig. 3. Chromatograms with Sodium Chloride in Mobile Phase

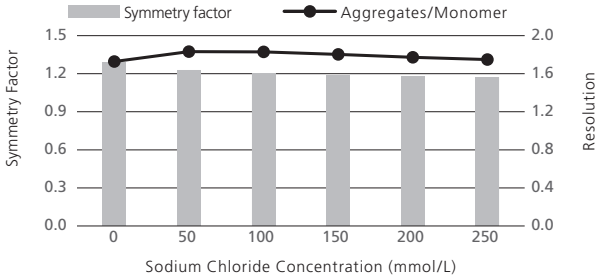


Fig. 4. Relationship between Chromatographic Performance and Sodium Chloride Concentration

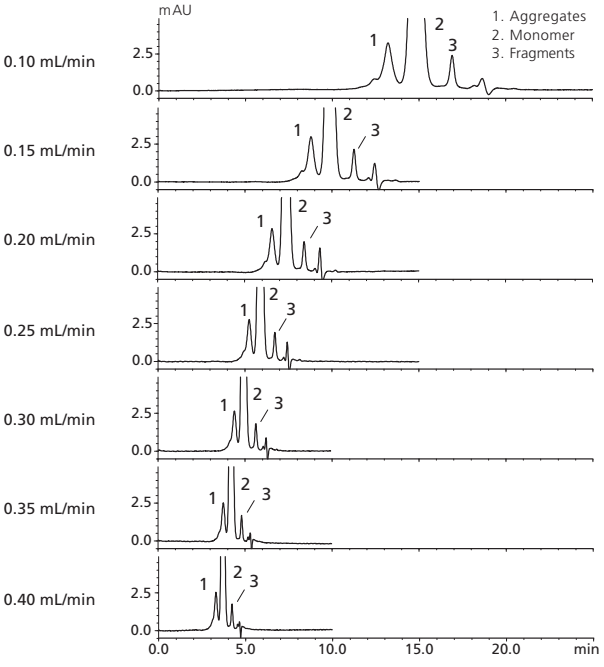


Fig. 5. Chromatograms and Flow Rate

As shown in Fig. 6, the resolution between aggregates and monomer, and between monomer and fragments improved at lower flow rate. Based on these results, a flow rate of 0.25 mL/min was chosen as it provides shorter analytical time and appropriate chromatographic separation (over 1.5 resolution).

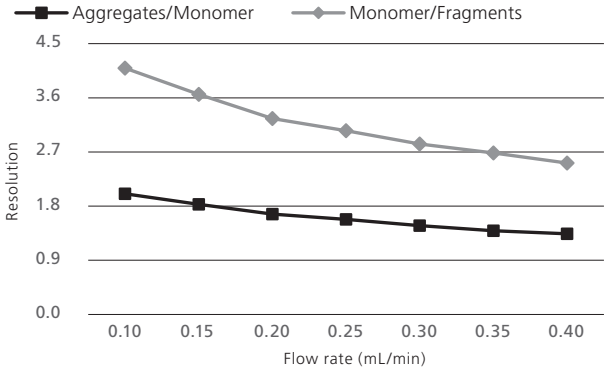


Fig. 6. Relationship between Flow Rate and Peak Resolution

3-4. Impact of Mobile Phase pH

Due to a large number of dissociable groups on protein molecule, their charge state and steric structure change depending on pH. Such changes are known to contribute to protein size and the strength of interaction with the column and thereby affect chromatogram peak shapes.

Fig. 7 shows chromatograms of mAb obtained with 100 mmol/L phosphate buffer containing 100 mmol/L sodium chloride adjusted to different pH. Each mobile phase was automatically prepared with the blending function and provided to the system. The pH 5.7 showed no aggregate and a huge unknown peak after the fragments; meanwhile, the pH 6.0 gave the smaller peaks related to aggregates and unknown components. Since the isoelectric point (pI) value of target mAb is larger than the pH level of these two mobile phases, the impact on the retention deriving from the electrical-charge interaction could be more significant in these two conditions.

As shown in Fig. 8, mobile phase pH did not significantly affected the symmetry factor at pH 6.0 and above, but the resolution between aggregates and monomer and between monomer and fragments improved with raising pH. The best resolution for the mAb in this study was obtained at pH 7.2. Proteins normally denature and recieve damages at highly acidic or alkaline solution. Hence the effect of pH should be investigated at neutral pH close to the antibody pI.

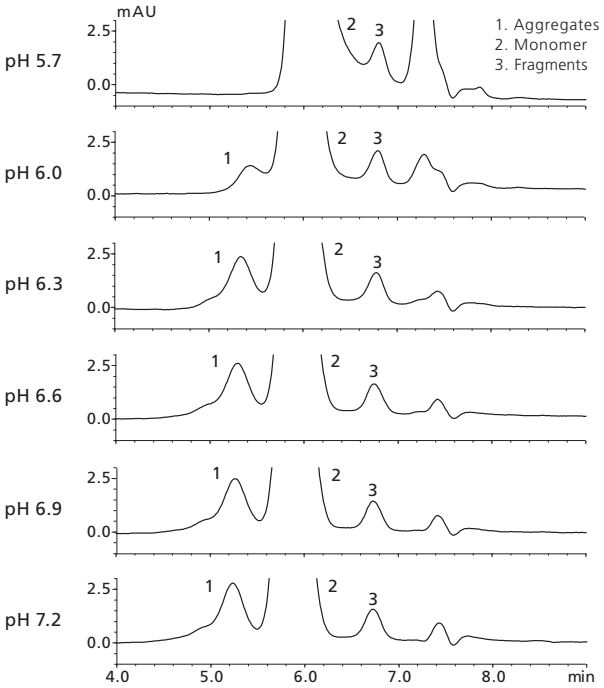


Fig. 7. Chromatograms and pH

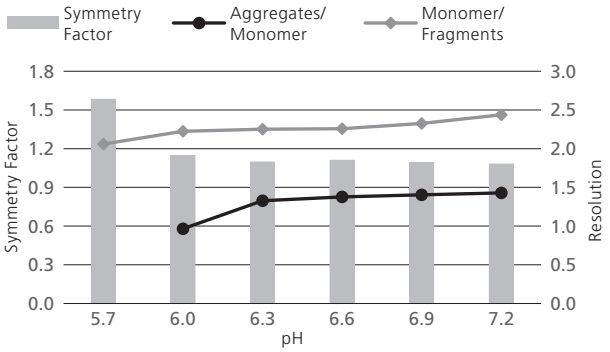


Fig. 8. Relationship between Chroamtographic Performance and pH

Column

Shim-pack Bio Diol improves the accuracy of analysis of biopharmaceuticals and medium-molecular-weight compounds. Shim-pack Bio Diol comes in four pore sizes to accommodate a wide range of molecular weights and analysis time can be shortened by the lineup of multiple particle sizes. Refer to the Shimadzu website for details[3]. Fig. 9 compares a Shim-pack Bio Diol with another commercially available SEC column installed in the same system (300 mm × 4.6 mm I.D., 3 µm, 300 Å). Better separation between monomers and fragments was achieved using Shim-pack Bio Diol-300.

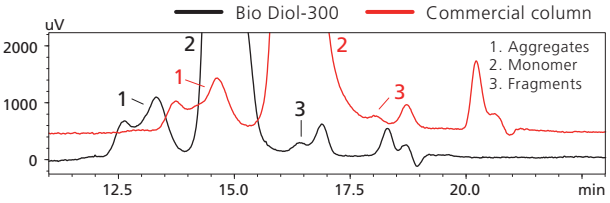


Fig. 9. Comparison of Bio Diol and Another Commercial Column (Chromatogram baselines shifted for comparison)

System Configuration

Biopharmaceutical analysis sometimes requires intense separation conditions, such as high salt concentrations and low pH, potentially harmful to HPLC systems. Additionally, molecule adsorption onto the equipment due to the interaction with the metal ion is one of the most serious issues, making it difficult to obtain highly reliable data with a standard HPLC system. Nexera XS inert (Fig. 10) is the specific UHPLC system with excellent reliability, robustness, and flexibility for such kind of analysis. It contributes to determining the characteristics of proteins, antibodies, and formulations produced with biotechnology, such as genetic engineering, cell fusion, and cell culture.

LabSolutions MD

This article indicates that screening analytical conditions with a variety of parameters is critical. On the other hand, manual operation for preparing tremendous mobile phases and methods is extremely time-consuming. The Shimadzu method development software, LabSolutions MD, allows automated creation of the combination of columns and mobile phases and the analytical sequences. The mobile phase blending function is also helpful for adjusting each mobile phase component, dramatically reducing the time to prepare numerous solvents and to optimize separation conditions. In this article, the mobile phase blending function was used to investigate salt concentration and pH.

Summary

Since aggregates of antibody drugs are known to negatively affect the pharmacological action and elicit an immune response, ICH-Q6B guides that the amounts of aggregates should be determined. This article investigated the impact of several SEC analysis parameters on mAb separation. SEC analyses are often performed at relatively low flow rates, but with Shim-pack Bio Diol-300 with 2 µm packing particle, superior separation was obtained even on a shorter column length for analysis times of faster than 15 minutes. By optimizing mobile phase salt concentration, flow rate, and pH, the chemical interaction between the mAb and packing materials was successfully controlled. Finally, aggregates, monomer, and fragments were separated with reasonable peak shapes. To correctly determine impurities in antibody drugs, it is essential to investigate these parameters and determine the optimum analytical conditions for each protein.

References

1 Specifications : Test Procedures and Acceptance Criteria for Biotechnological/Biological Products (PMSB/ELD Notification No. 571 dated May 1, 2001)

2 Niki E. and Watanabe N. (1980). Introduction to High Performance Liquid Chromatography. Journal of Japan Oil Chemists' Society, 29 (2), 127-134.

3 https://www.shimadzu.coam/an/products/liquid-chromatography/hplc-consumables/shim-pack-bio-diol/index.html

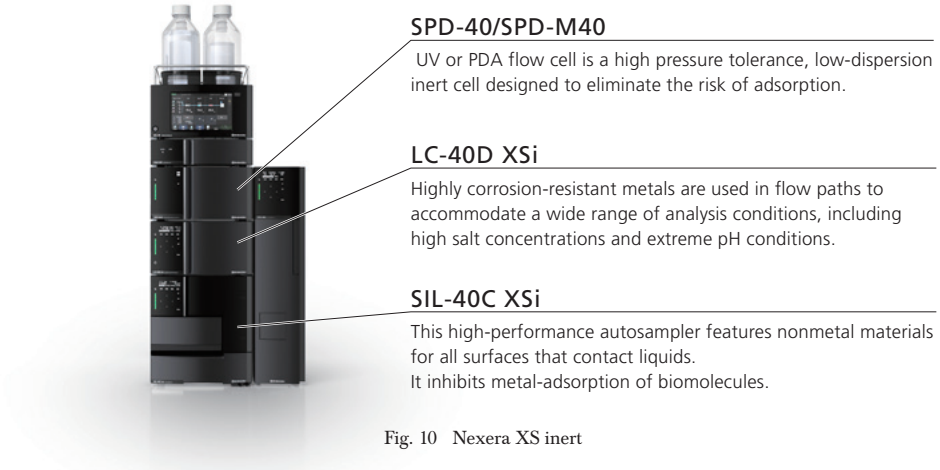


Fig. 10 Nexera XS inert

Biopharmaceutical

Liquid Chromatograph Mass Spectrometer LCMS™-9030

Characterization of Control and Stress Induced Samples of Trastuzumab Biosimilar

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[4]Shimadzu (Asia Pacific) Pte. Ltd.

User Benefits

- In-depth peptide mapping and PTM analysis methodology is described for the analysis of control and stress induced samples of mAb which can be useful in identification of sites susceptible to oxidation and deamidation modifications
- Excellent and stable mass accuracy, comprehensive fragmentation pattern and high sensitivity offered by LCMS-9030 helped obtain good sequence coverage, identification of PTMs and sites susceptible for modifications

Introduction

Monoclonal antibodies (mAbs) are a major class of biopharmaceuticals covering a large panel of diseases, from cancer to asthma, including central nervous system disorders, infectious diseases and cardiovascular diseases[1]. Throughout manufacturing, storage, transportation, and administration, mAbs are subjected to biophysical and biochemical stress from multiple sources, which may lead to their degradation via aggregation, fragmentation, and chemical modifications, such as oxidation, deamidation, or isomerization[2]. Among these undesirable degradation products, oxidation and deamidation are the most commonly observed Post-Translational Modifications (PTMs). Early identification of these prone sites enables antibody engineering to eliminate liability of leading candidates to such modifications while maintaining binding activity.

Bottom-up approach which is essentially peptide mapping helps in determination of primary amino acid sequence and identification of site–specific PTMs. In this application note, methodology for identification of sites susceptible to oxidation and deamidation is described by analyzing the control and artificial chemically stress induced (forced degradation) samples of mAb.

Experimental

Trastuzumab biosimilar sample (2 mg/mL) was incubated in water with pH 9 (adjusted using tris base solution) for 7 days at 37°C to induce deamidation. Similarly, 10 µL of 30% H_2O_2 solution was added to 2 mg/mL of trastuzumab sample and incubated for 1 hour at 37°C to induce oxidation. Oxidation reaction was quenched by adding pinch of methionine.

100 µg of trastuzumab control and stress induced samples were incubated with reduction buffer containing 8 M urea and 5 mM dithiothreitol at 37°C for 60 mins. Solutions were then alkylated with 20 mM of iodoacetamide at room temperature for 30 mins. At the end of incubation period, 50 mM Tris-HCl buffer (pH 8) was added to the solutions. Finally, trypsin was added in 1: 50 ratio and overnight digestion was carried out. Reaction was quenched with diluted formic acid and desalting was carried out using Solid Phase Extraction (SPE) cartridges. Eluent obtained at the end of SPE clean-up was evaporated using vacuum centrifugation and re-constituted in water: formic acid: acetonitrile (100:1:2 v/v) solution and analysed with LCMS-9030 (shown in Fig. 1) for peptide mapping analysis. Mobile phase consisting of 0.1% formic acid in water and 0.1% formic acid in acetonitrile was used on Shim-pack™ Arata Peptide C18 column for the chromatographic separation. Analysis was performed in Data Dependent Acquisition (DDA) mode in positive polarity using Electro Spray Ionization (ESI) interface[3]. DDA data acquisition was controlled by the LabSolutions™ LCMS software. Mass range of 200-2500 *m/z* was used for MS1 TOF survey scan. Base peak chromatogram intensity threshold of more than 1000 was used to trigger the MS/MS fragmentation with collision energy spread of 18-52 V. Use of collision energy spread allowed acquisition of comprehensive fragmentation pattern for any given precursor ion. Seven dependent (MS/MS) events were set to allow sufficient MS/MS data collection. Mass range of 100 to 2800 *m/z* was used to obtain MS/MS spectra. Ion exclusion and inclusion settings are available in the LabSolutions LCMS software to automatically exclude background ions and include ions of interest, respectively.

All data acquisition was performed with single external TOF calibration. No intermediate TOF calibration/lock masses were used during the data acquisition/processing. Details of analytical conditions are given in Table 1.

The LCMS-9030 quadrupole time-of-flight (Q-TOF) mass spectrometer is a powerful instrument that integrates the world's fastest and most sensitive quadrupole technology with TOF capabilities for accurate mass measurement. Patented technologies of LCMS-9030, UF-FlightTube™ and iRefTOF™, ensure excellent Mass Measurement Accuracy (MMA) with stability which helps in identification of different peptides and PTMs present in the sample. UFaccumulation™ and UFgrating™ offer superior sensitivity which helps in detecting low abundant PTMs present in the samples.

DDA data acquired by LCMS-9030 was processed using 'Protein Metrics' software suite[4]. Settings of precursor mass tolerance of '6 ppm' and fragment mass tolerance of '20 ppm'; maximum 2 missed cleavages; and fully specific trypsin digestion efficiency were used for peptide/PTMs identification. Carbamidomethyl modification was considered as fixed. Other PTMs like oxidation, deamidation, Gln->pyro-Glu, Glu->pyro-Glu, ammonia-loss/ succinamide formation, dioxidation, dethiomethylation were considered as variable. N-glycan 52 common biantennary database present in the software was used to obtain information about glycosylation.

Table 1. Details of analytical conditions for bottom-up approach

HPLC system	Nexera™ X2
Column	Shim-pack™ Arata Peptide C18 (100 mm × 2.0 mmI.D., 2.2 µm) (P/N: 227-32806-02)
Column oven	40°C
Mobile phases	A-0.1% formic acid in water B-0.1% formic acid in acetonitrile
Flow rate	0.3 mL/min
Gradient program (B%)	0-3 min → 1 (%); 3-45 min → 1-30 (%); 45-46 min → 30 (%); 46-46.1 min→ 30-90 (%); 46.1-50 min →90 (%); 50-50.1 min→ 1 (%); 60 min → stop
LCMS system	LCMS-9030
Interface	Heated ESI
Polarity	Positive
Acquisition mode	DDA
Mass range for TOF survey scan	200-2500 *m/z*
Mass range for precursor ion	220-2000 *m/z*
Mass range for MS/MS scan	100-2800 *m/z*
Collision energy spread	18-52 V
Temperatures	Interface: 300°C Desolvation line: 200°C Heater block: 400°C
Gas flow rates	Heating gas: 15 L/min Nebulizing gas: 3 L/min Drying gas: 15 L/min

Fig. 1. LCMS™-9030 Quadrupole Time of Flight Mass Spectrometer

Results and Discussion

Therapeutic proteins may undergo a series of modifications throughout their cellular production, upstream/downstream processing, and storage. These modifications can include the addition or replacement of functional groups, or structural changes such as folding/unfolding, cleavage, and racemization. Presence of these modifications can affect biological activity, half-life and immunogenicity[5]. Hence, it is of utmost importance to identify these modifications accurately. Moreover, it is also important to find out the sites which are prone to undergo such modifications.

Analysis of the control and artificially stress induced mAb samples can provide understanding of such susceptible sites. The bottom-up approach for mAb characterization is typically referred to as "peptide mapping,". Peptide mapping analysis not only provides information about primary sequence of mAbs but also useful in identifying sites that are susceptible to oxidation, deamidation etc.

Hence, control and stress induced samples of trastuzumab biosimilar were subjected to peptide mapping and PTMs analysis. Overlay of extracted ion chromatograms from TOF survey scan (MS1) for trastuzumab control sample is shown in Fig. 2. More than 92% of peptide sequence coverage was obtained for both heavy and light chains of trastuzumab control sample even with single enzyme digestion (shown in Fig. 3). Some of the short peptide chains (around 3 to 4 amino acid) are found to be not covered; however, use of multiple enzymes for the digestion can improve the sequence coverage.

LCMS-9030 offered excellent mass accuracies for the peptides with different chain length. Representative data demonstrating the mass accuracy (less than 2 ppm) obtained for peptides with chain length as short as 4 amino acid and as long as 63 amino acid is shown in Table 2. Obtaining such mass accuracies and stability for longer duration is of utmost important to accurately identify the PTMs.

Generally, mass shift in precursor ion m/z for the modified peptide and change in the retention time are considered to identify the presence of PTMs. Furthermore, acquiring good quality MS/MS spectra is equally important to confidently assign the location of the modification on a given peptide. Collision energy spread function of LCMS-9030 helps acquire MS/MS fragmentation pattern over a range of collision energy (18-52 V, in this case) instead of obtaining MS/MS spectra at single or few selected collision energies. Thus, comprehensive MS/MS fragmentation pattern can be obtained which in turn helps in confident site-specific PTM assignment. Examples of identified modifications like oxidation, deamidation etc. are discussed below.

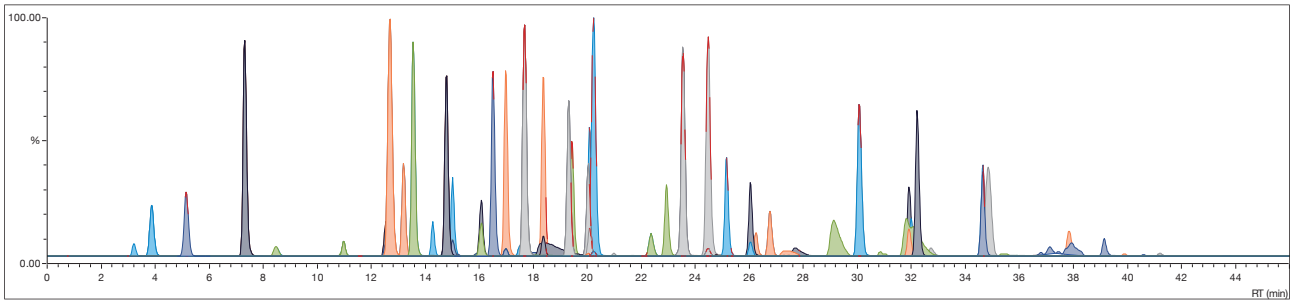

Fig. 2. Overlay of extracted ion chromatograms of TOF survey scan (MS1) for trastuzumab biosimilar control sample

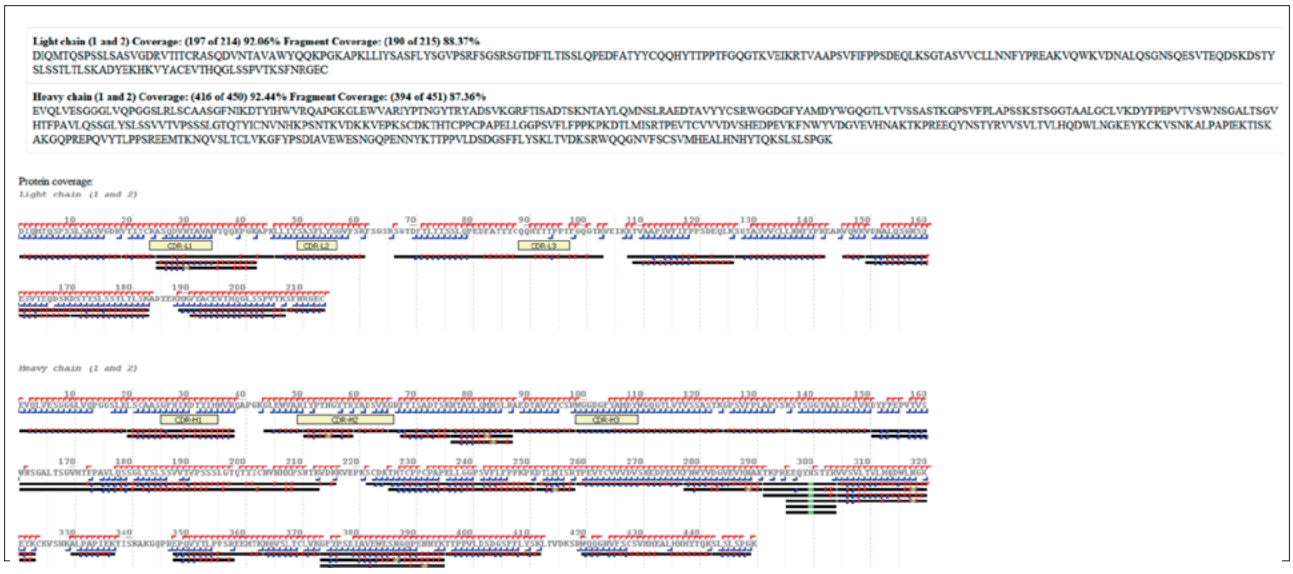

Fig. 3. Summary of peptide coverage, fragmentation coverage and PTMs for trastuzumab biosimilar control sample

Table 2. Representative results of mass accuracies for peptide sequences of different chain length for control and stress induced samples of Trastuzumab biosimilar

#	Name	m/z	TmAb_Control		TmAb_Oxidation_1		Tmab_Deamidation_pH 9	
			Found RT	Mass Error (ppm)	Found RT	Mass Error (ppm)	Found RT	Mass Error (ppm)
1	VQWK	280.6632	5.140	-1.033	5.092	-0.713	5.144	-1.710
2	YADSVK	341.6740	3.206	-0.819	3.318	-0.966	3.302	-1.727
3	DTLMISR	418.2207	14.779	0.096	14.833	-0.550	14.810	-1.124
4	DTLm (+16)ISR	426.2182	12.530	-0.493	12.572	-0.094	12.547	-1.103
5	ASQDVNTAVAWYQQKPGK	664.3376	19.423	-0.045	19.460	0.241	19.464	-0.557
6	ASQDVnTAVAWYQQKPGK	664.6656	20.432	0.135	20.454	-0.361	20.446	-0.451
7	EEQYnSTYR(NGlycan/1606.5867)	932.7044	10.889	-0.279	10.923	0.118	10.902	0.450
8	DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK	1343.4687	41.202	0.298	41.173	1.243	41.150	0.789

Oxidation of biotherapeutic proteins can alter their physical and biological properties, affecting their potency and stability characteristics. The most commonly oxidized amino acid is methionine (Met). Oxidized peptides are easily identified using MS since the addition of one oxygen atom to the Met side-chain upon conversion to Met sulfoxide increases the mass of the affected residue by +16 Da[5].

Location of oxidation modification can be easily identified from MS/MS fragmentation pattern. Identification of oxidation modification is illustrated with 'DTLMISR' peptide and shown in Fig. 4. Oxidized peptide 'DTLMISR' elutes earlier as compared to its unmodified counterpart as 'oxidation' imparts hydrophilicity to the peptide. Mass shift of +8 Da (for '+2' charge state) for oxidized peptide precursor ion m/z can be seen from MS1 spectra. MS/MS fragmentation spectra revealed that y3 fragment is same for both the peptide versions, however mass shift in fragment ions can be seen from y4 fragment onwards indicating the location of modification as y4 methionine. Met sulfoxide-containing peptides readily lose methane sulfenic acid (CH_3SOH) upon CID fragmentation and are thus easily identified by a characteristic loss of 64 Da from the fragment mass which can be seen in MS/MS spectra of modified peptide shown in Fig. 4.

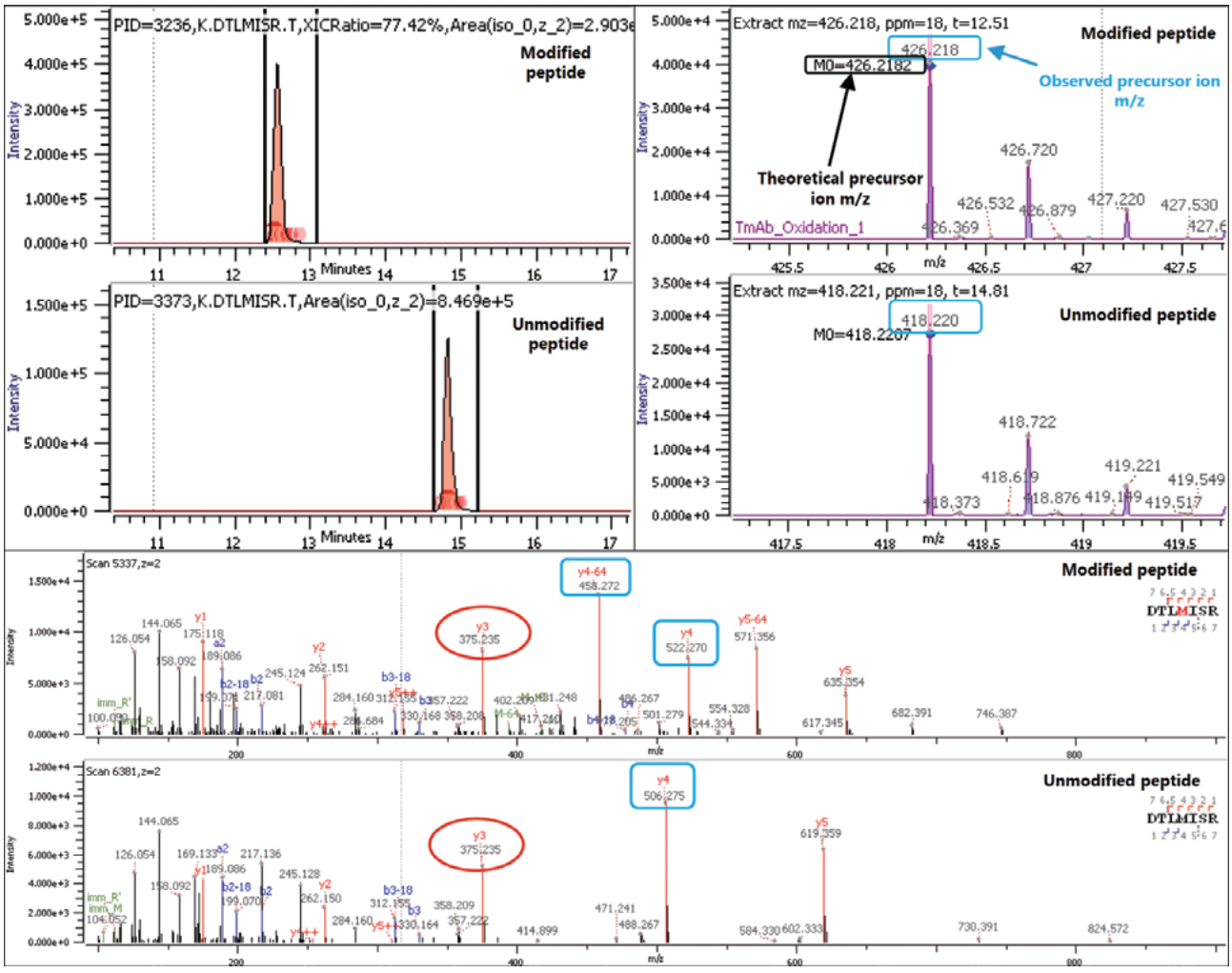

Fig. 4. Top left: Chromatograms of oxidized and unmodified 'DTLMISR'; Top right: MS1 spectra of oxidized and unmodified 'DTLMISR'; Bottom: MS/MS fragmentation spectra for oxidized and unmodified 'DTLMISR'

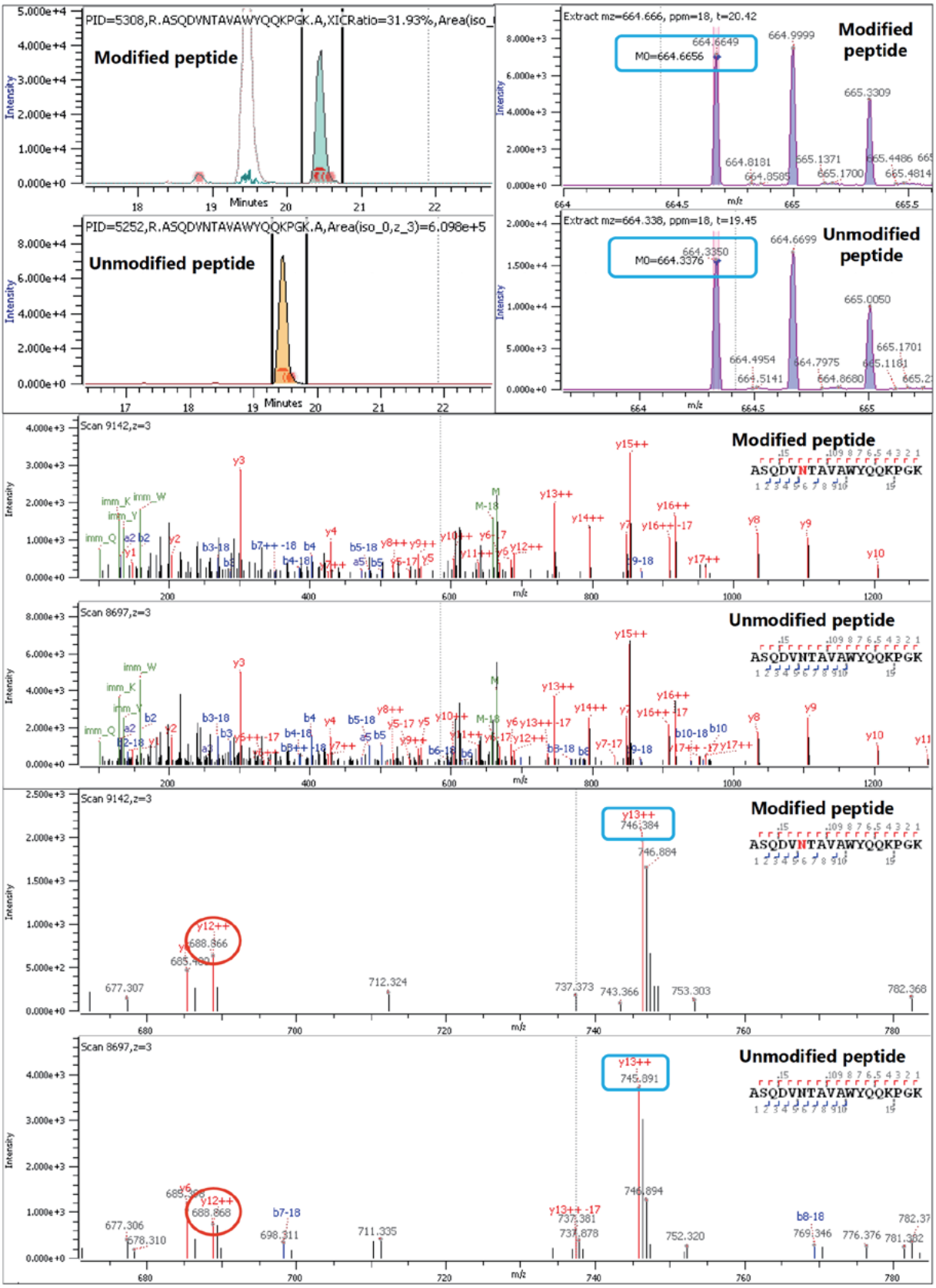

Fig. 5. Top left: Chromatograms of deamidated and unmodified 'ASQDVNTAVAWYQQKPGK' ; Top right: MS1 spectra of deamidated and unmodified 'ASQDVNTAVAWYQQKPGK' ; Middle: MS/MS fragmentation pattern for deamidated and unmodified 'ASQDVNTAVAWYQQKPGK' Bottom: Zoomed MS/MS fragmentation spectra for deamidated and unmodified 'ASQDVNTAVAWYQQKPGK'

Deamidation of asparagine (Asn) and glutamine (Gln) has an important role in regulating the heterogeneity and stability of recombinant mAbs. Deamidation is one of the most challenging PTMs to characterize using MS-based techniques. Deamidation results in conversion of $-NH_2$ to –OH (+0.984 Da), which has a very similar mass shift as the first ^{13}C isotope peak of the native peptide (+1.0034 Da)[5].

Deamidation modification example is illustrated with 'ASQDVNTAVAWYQQKPGK' peptide (refer to Fig. 5). Mass shift of '+ 0.329 Da' (for +3 charge state) is observed in MS1 scan spectra of modified peptide which is very close to first ^{13}C isotope m/z of unmodified precursor ion. Hence, careful evaluation of precursor ion mass shift, MS/MS pattern and change in the retention time is essential before assigning deamidation modification.

It is observed that deamidated peptide is eluting later than its unmodified counterpart. MS/MS fragmentation pattern revealed that product ion m/z up to $y12^{++}$ are same for modified as well as unmodified peptide. However, $y13^{++}$ fragment ion showed difference of 0.49 Da (for '+2' charge state) as seen in Fig. 5 confirming the presence of deamidation modification and its location on given peptide. Relative abundance summary of PTMs observed for control and stress induced samples of trastuzumab biosimilar is given in Table 3. It can be observed that three sites, viz., HC-361, HC-107 and HC-431 have shown methionine oxidation only in oxidative stress induced sample and are absent in control as well as deamidation stress induced samples which shows susceptibility of these sites to oxidation stress. Site HC-255 has shown methionine-oxidation in all three samples, however, relative abundance of modified peptide is higher (77.5%) in oxidative stress induced sample as compared to control and deamidation stress induced samples (6.35 and 7.29%). This site appears to be most susceptible to oxidative stress. Site HC-83 has shown minor level of methionine oxidation in all three samples with almost comparable relative abundance which indicates that this site may not be susceptible to oxidative stress.

Similarly, sites LC-30, HC-289, HC-387, HC-392, HC-55, HC-84 and HC-318 have shown deamidation. Some of these modifications could be sample preparation artefacts introduced due to elevated pH used during sample preparation. Nevertheless, sites LC-30 and HC-318 have shown elevated levels of deamidation modification in deamidation stress induced sample as compared to control and oxidative stress induced samples indicating potential susceptibility of these sites.

Table 3. Relative abundance summary of PTMs for control and stress induced samples of trastuzumab biosimilar

						MS Id ←	1	3	4
						MS Alias name ←	TmAb_Control (%)	TmAb_Oxidation_1 (%)	Tmab_Deamidation_pH 9 (%)
Sequence (unformatted) ↑	Mod. Names ↑	Protein name ↑	Mod. AAs ↑	Glycans ↑	Var. Pos. Protein ↑				
ASQDVNTAVAWYQQKPGK	Deamidated/0.9840	Light chain (1 and 2)	N		30		7.63	7.12	31.1
DTLMISR	Oxidation/15.9949	Heavy chain (1 and 2)	M		255		6.35	77.5	7.29
EEQYNSTYR	NGlycan/1241.4545	Heavy chain (1 and 2)	N	HexNAc(3)Hex(3)Fuc(1)	300		17.6	16.5	16.2
	NGlycan/1444.5339	Heavy chain (1 and 2)	N	HexNAc(4)Hex(3)Fuc(1)	300		43	43.9	44
	NGlycan/1606.5867	Heavy chain (1 and 2)	N	HexNAc(4)Hex(4)Fuc(1)	300		39.4	39.6	39.7
EPQVYTLPPSREEMTK	Oxidation/15.9949	Heavy chain (1 and 2)	M		361			59.8	
FNWYVDGVEVHNAK	Deamidated/0.9840	Heavy chain (1 and 2)	N		289		1.4	1.42	1.43
GFYPSDIAVEWESNGQPENNYK	Deamidated/0.9840	Heavy chain (1 and 2)	N		387		17.4	18.6	22.1
					392		17.4	18.6	22.1
IYPTNGYTR	Deamidated/0.9840	Heavy chain (1 and 2)	N		55		37	38.7	37.8
NTAYLQMNSLR	Deamidated/0.9840	Heavy chain (1 and 2)	N		84		1.83	2.21	1.69
	Oxidation/15.9949	Heavy chain (1 and 2)	M		83		1.96	2.08	1.43
TKPREEQYNSTYR	NGlycan/1241.4545	Heavy chain (1 and 2)	N	HexNAc(3)Hex(3)Fuc(1)	300		9.09	11.8	9.02
	NGlycan/1444.5339	Heavy chain (1 and 2)	N	HexNAc(4)Hex(3)Fuc(1)	300		33.8	46.8	33.9
	NGlycan/1606.5867	Heavy chain (1 and 2)	N	HexNAc(4)Hex(4)Fuc(1)	300		57.1	41.4	57.1
VVSVLTVLHQDWLNGK	Deamidated/0.9840	Heavy chain (1 and 2)	N		318		5.83	5.85	12.4
VVSVLTVLHQDWLNGKEYK	Deamidated/0.9840	Heavy chain (1 and 2)	N		318		11.1	12.3	49.4
WGGDGFYAMDYWGQGTLVTVSSASTK	Oxidation/15.9949	Heavy chain (1 and 2)	M		107			32.3	
WQQGNVFSCSVMHEALHNHYTQK	Oxidation/15.9949	Heavy chain (1 and 2)	M		431			35.5	

Conclusion

- Complete peptide mapping and PTMs analysis workflow for control and stress induced mAb samples is described.
- Sequence coverage of more than 92% was obtained for both heavy and light chain of the mAb even with single enzyme digestion.
- Excellent mass accuracy with good stability, comprehensive fragmentation pattern and sensitivity offered by LCMS-9030 helps in reliable and in-depth characterization of mAb and also has helped in identification of sites susceptible to oxidation and deamidation modifications. Such studies can help in deep understanding of Critical Quality Attributes (CQA) of the product and to further develop Multiple-Attribute Method (MAM).

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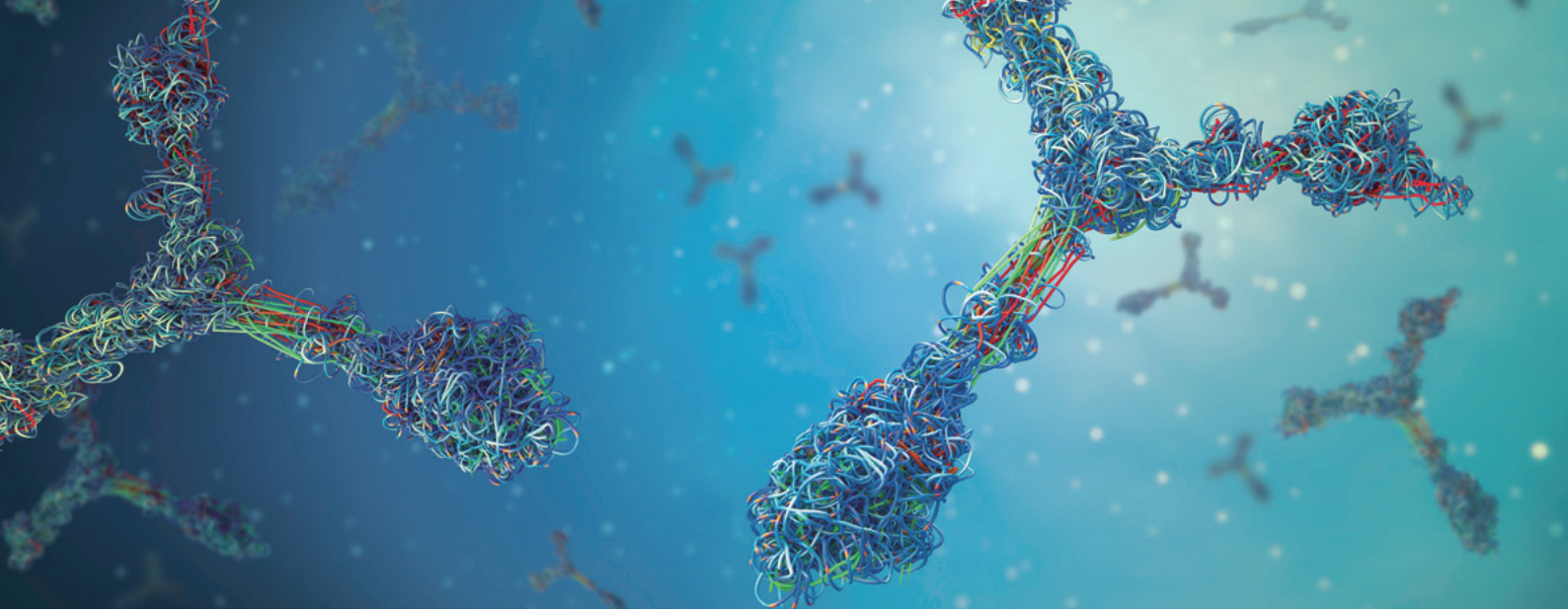

Biopharmaceutical

High Performance Liquid Chromatograph Prominence™ Inert HPLC System, LH-40

Seamless Process from Protein Purification to Evaluation

Risa Suzuki[1]

[1]Global Application Development Center, Analytical & Measuring Instruments Division, Shimadzu Corporation

User Benefits

- By choosing target peaks after fractionation, appropriate fractions can be automatically reinjected for analysis.
- Equipped column-switching valve allows automatic column switching between purification and analysis.
- It is also convenient for comparing several samples to optimize culture conditions.

Introduction

Size exclusion chromatography (SEC) is one technique used to separate molecules based on size, and it is one of the main methods used for verification of protein multimer formation. However, this technique can not distinguish proteins from ones with similar molecular sizes. Because smaller molecules can enter pores of materials packed in an SEC column, but larger molecules cannot. Therefore, larger molecules elute earlier than smaller molecules. In the case of target proteins in serum or culture supernatant, the samples have to be purified before SEC analysis. This article describes a seamless process of target proteins for purification, fractionation, and re-injection utilizing a liquid handler.

Seamless Analysis with Liquid Handler

The liquid handler (LH-40) serves as an autosampler as well as a fraction collector for the LC system. That means samples fractionated during the first run can be injected directly for the second run without transferring them from a fraction collector to an autosampler. For example, with this system (Fig. 1 and 2), the target protein is purified by an affinity column and fractionated as the first step, and then the fractionated protein elution is re-injected for SEC analysis as the second step. These two steps can be performed simply by specifying the method and fraction.

In this example, we evaluated IgG in human plasma using an LH-40 liquid handler installed in a Prominence inert LC system.

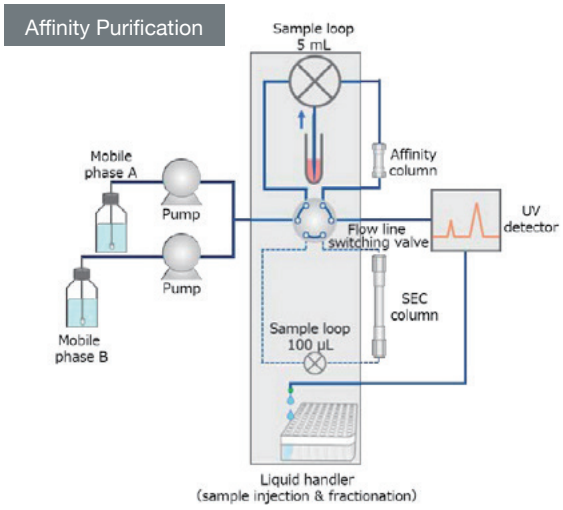

Fig. 1. Flow Path Diagram of Affinity Purification

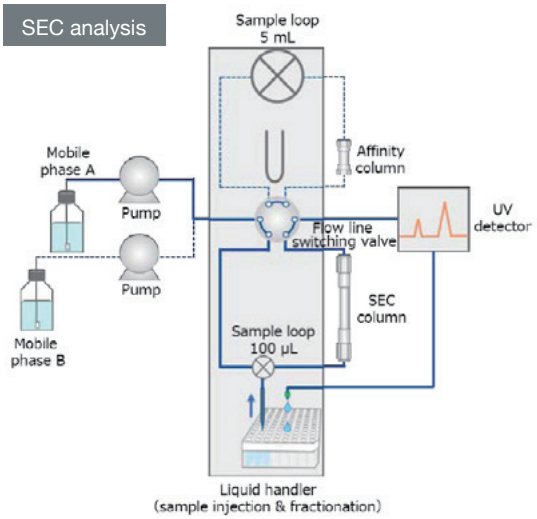

Fig. 2. Flow Path Diagram of SEC Analysis

Analytical Conditions and Samples

5 mL of commercial human plasma EDTA-2Na was placed in a 15 mL tube, diluted 5-fold using a 10 mmol/L (sodium) phosphate buffer (pH 6.9, mobile phase A), and placed in the liquid handler rack. Then the sample was purified by affinity chromatography using an IgG purification column, based on the conditions in Table 1. The fractions were collected in a 96-well deep well plate placed in the liquid handler. In this case, 100 µL of the fraction around the peak top was analyzed by SEC based on the conditions in Table 2.

Table 1. Analytical Conditions for Affinity Purification

Column	HiTrap™ rProtein A FF (1 mL, Cytiva)
Mobile Phase A	10 mmol/L (sodium) phosphate buffer (pH 6.9)
Mobile Phase B	100 mmol/L (sodium) citrate buffer (pH 4.0)
Time Program (B. Conc.)	0% (0 – 10 min) → 100% (10.01 - 20 min) → → 0% (20.01 – 35 min)
Flowrate	1.0 mL/min
Column Temp.	15˚C
Injection Volume	5 mL
Detection	280 nm (SPD-20A)
Flow Cell	Inert flow cell

Table 2. Analytical Conditions for SEC

Column	Shim-Pack™ Bio Diol-300*1 (300 mm × 4.6 mm I.D., 5 µm)
Guard Column	Shim-Pack Bio Diol-300 (G)*2 (30 mm × 8.0 mm I.D., 5 µm)
Mobile Phase A	10 mmol/L (sodium) phosphate buffer (pH 6.9)
Flowrate	0.5 mL/min
Column Temp.	15˚C
Injection Volume	100 µL
Detection	280 nm (SPD-20A)
Flow Cell	Inert flow cell

*1: P/N 227-31010-04, *2: P/N 227-31010-06

Results of Affinity Purification

5 mL of the diluted human plasma sample was injected into the IgG purification column after being equilibrated with mobile phase A. Then the non-specific adsorbates were washed out with mobile phase A (Fig. 3). Finally, IgG was eluted by mobile phase B (Fig. 4).

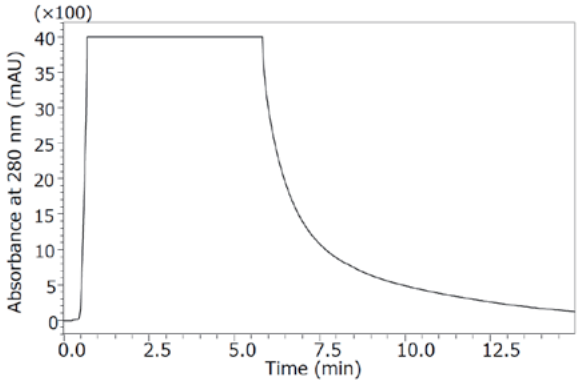

Fig. 3. Chromatogram of Binding Section during Affinity Purification

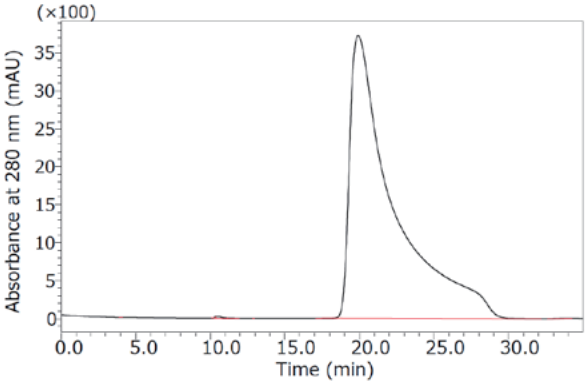

Fig. 4. Chromatogram of Elution during Affinity Purification

Results of SEC Analysis

100 µL of the fraction around the peak top eluted by the affinity purification as the first step was re-injected and evaluated by the SEC analysis as the second step. The results are shown in Fig. 5. In the SEC analysis, a main peak (Peak 2) and a broad peak (Peak 1) before Peak 2 were detected. To briefly estimate the molecular weight of the protein from the peaks obtained by SEC analysis, the mixture of thyroglobulin (669 kDa), aldolase (158 kDa), and ovalbumin (44 kDa) and the mixture of ferritin (440 kDa) and conalbumin (75 kDa) were evaluated as standard proteins by SEC (Fig. 6). The elution times of the protein standard solutions indicate that almost all the purified IgG from human plasma existed as monomers.

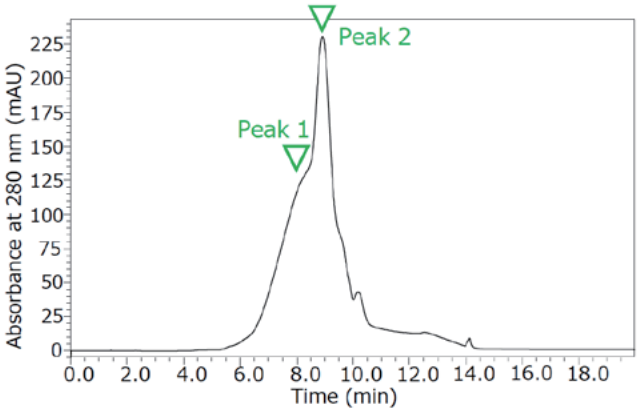

Fig. 5. Chromatogram from SEC Analysis

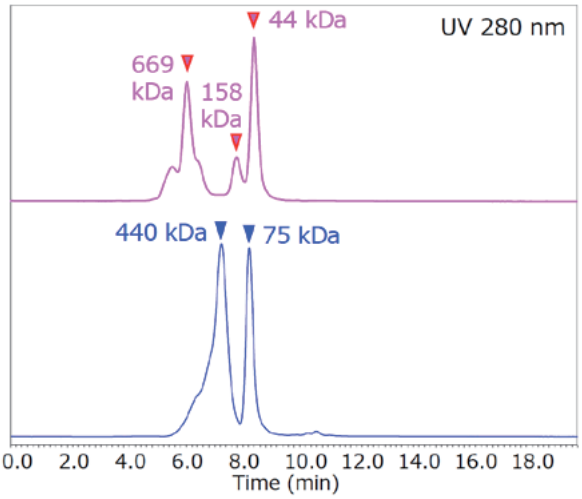

Fig. 6. SEC Chromatograms of Standard Proteins

SDS-PAGE Analysis

All fractions from affinity purification and SEC analysis were collected in a 96-well deep well plate. The peak-top fractions were evaluated by SDS-PAGE analysis (non-reducing and reducing). The peak-top fractions collected from both affinity purification and SEC analysis showed bands at the same position as standard human IgG (Fig. 7). Based on the results, both peaks 1 and 2 in SEC were IgG. Structurally similar 4 sub-classes of IgG in human blood could give broadened peak shapes due to the small differences in molecular size and/or structure.

Conclusion

The liquid handler (LH-40) installed in the LC system provided a seamless process from purification to analysis and required simply placing the sample in the liquid handler. For routine work with prespecified targets, it can be used to analyze only the target fractions. By adding one more column switching valve and increasing the number of columns, the system can also be used to screen purification parameters or purify samples in multiple steps. After fractions are collected in a 96-well plate, they can be used directly for SDS-PAGE, ELISA, or various other analytical methods.

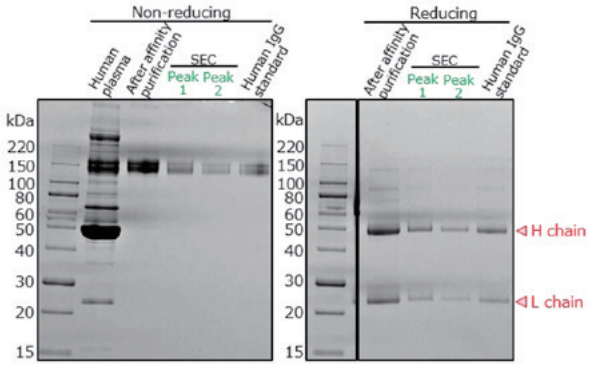

Fig. 7. SDS-PAGE Analysis

Biopharmaceutical

Uncovering the Clinical Significance of Trough Levels of Antibody Therapeutics Using nSMOL* Assay in Cancer Immunotherapy

Noriko Iwamoto[1], Takashi Shimada[1]

[1]SHIMADZU Bioscience Research Partnership (SBRP), Shimadzu Scientific Instruments

* For Research Use Only. Not for use in diagnostic procedures.

In 1986, the first monoclonal antibody (mAb) was approved by the United States Food and Drug Administration (US FDA). Supported by rapid advances in antibody engineering, the number of approved mAbs reached 100 in April 2021, and more than 900 mAbs are currently in clinical trials. With such a dramatic increase of mAbs and their use in various treatments, there is a need for assay technology that can obtain the accurate kinetics and concentration of any mAbs in serum/plasma.

Previously, we developed the universal method for mAbs quantification, nano-surface and molecular-orientation limited (nSMOL) proteolysis, as an analytical platform for LC-MS from biological samples[1]. Most of the therapeutic mAbs are immunoglobulin Gs (IgGs). nSMOL principle is based on Fab-selective proteolysis of IgGs using antibody capturing Protein A beads and trypsin-immobilized on the surface of nanoparticles (Fig. 1). IgGs captured in 100 nm of Protein A pores are oriented Fab region to the reaction

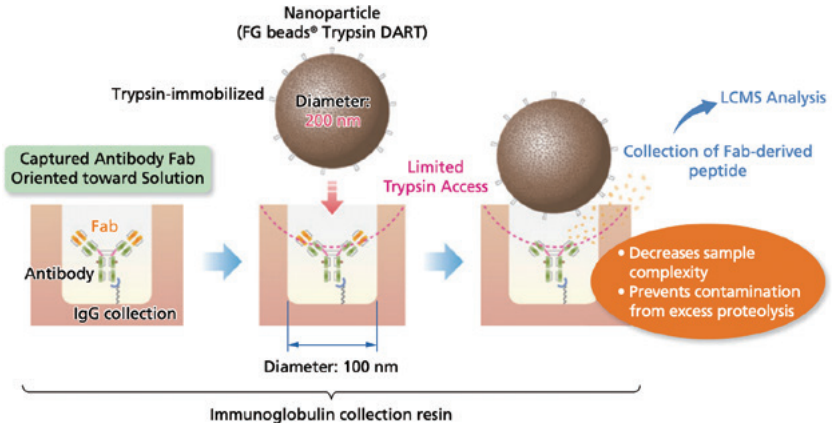

Fig. 1. Conceptual diagram of the pretreatment method nSMOL Antibody BA kit for antibody quantification using LCMS

surface and are rapidly proteolyzed by trypsin immobilized on 200 nm diameter of nanoparticles. Using the difference of two particle size, immobilized trypsin has physicochemically limited access to the Fab of IgGs on the Protein A resins. Therefore, the signature peptides that identified the specific structure of each IgG Fab can be efficiently harvested, and as a result, the peptide number to be brought to the subsequent multiple reaction monitoring (MRM) with LC-MS analysis can be reduced. Since the development of nSMOL in 2014, we have established the quantification method for more than 40 mAbs or Fc-fusion proteins[2].

In two collaborative studies with Providence Cancer Institute for the innovative cancer immunotherapy, the serum trough levels of mAb were measured using nSMOL method in patients with advanced melanoma or head and neck squamous cell carcinoma receiving pembrolizumab or ipilimumab[3, 4]. The analytical conditions of each mAb using nSMOL method were validated according to the Bioanalytical Method Validation Guidance for Industry issued by the US FDA. We have successfully established assays to quantify both these mAbs in serum, with the lower limits of quantification using conventional LC-MS of 0.5 ug/mL for pembrolizumab and 1 ug/mL for ipilimumab, respectively. The medical statistics of the relationship between clinical findings and the trough levels of mAbs in serum indicated that the therapeutic mAb monitoring can be a potential biomarker for cancer immunotherapy[2, 3].

Our findings demonstrate the importance of monitoring the trough levels of more immune checkpoint inhibitor mAbs in serum for cancer immunotherapy. In recent years, the antibody-based therapeutics for the treatment of cancer are becoming more and more diverse, supported by the active development of mAbs. Under this context, a technology such as nSMOL, which can be used for universal mAbs, can perform multiplex measurements, and ensures very sufficient reproducibility, and will be indispensable not only for therapeutic mAb monitoring but also for pharmacokinetic studies.

We envision that this technology will provide wide adaptation of mAb quantification that is easy to adopt in clinical settings by installing nSMOL with an automated system, and that will contribute to the mAb development and therapeutic optimization for many human diseases, such as cancers, autoimmune, infectious, and metabolic diseases.

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About Providence Cancer Institute

Providence Cancer Institute, a part of Providence St. Joseph Health, offers the latest in cancer services, including diagnostic, treatment, prevention, education, support and internationally renowned research. Providence Cancer Institute is home to the Earle A. Chiles Research Institute, a world-class research facility located within the Robert W. Franz Cancer Center in Portland, Oregon, and is a recognized leader in the field of cancer immunotherapy since 1993. Investigators lead more than 400 active clinical trials in key areas such as cancers of the: breast, colon/rectum, prostate, lung, esophagus, liver and pancreas, head and neck, ovary, skin and blood. Other studies are investigating treatments for COVID-19.

Learn more
http://www.providenceoregon.org/cancer/

Drs. Fox, Tran, Koguchi (Providence Cancer Institute), Shimada, Iwamoto (Shimadzu)

Shimadzu Selection

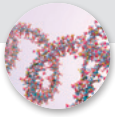
These article were selected by Shimadzu. Relating Biopharmaceutical analysis and development, they are from posters presented at ASMS 2021. They feature a variety of instruments we produce and include cutting-edge technologies. Please obtain the articles of your interest through the links on the titles.

Guide to Biopharmaceutical Solutions

This Biopharmaceutical Solutions Guide introduces the best solutions for each step of the biopharmaceutical development process, including cell line optimization, culture, purification, characterization, quality control, and pharmacokinetics.

Analysis of Protein Drugs Aggregation Using Size Exclusion Chromatography

This article describes an aggregate analysis using a Shimadzu "Shim-pack™ Bio Diol" , size exclusion chromatography column with "Nexera XS inert", an ultra high performance liquid chromatograph. This chromatograph has high salt tolerance and metal-free flow path and allows to use highly salted mobile phases and prevents sample adsorption.



Oligonucleotides analysis by Ion Exchange Chromatography and Effects of pH Changes in the Mobile Phase on Separation

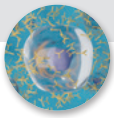
In this article, we introduce an analytical method for the separation of oligonucleotides of different length by ion-exchange chromatography, assuming that shorter length components are impurities derived from the synthesis process. In order to achieve optimal analytical performances an inert UHPLC system was used. The Nexera XS inert, which is designed to suppress the adsorption of metal-coordinating compounds containing phosphate groups. We also report the effect of changesin mobile phase pH on analytical results.

Achieving Improved Sensitivity and Reliable Analytical Performances in Nucleotides Analysis

Stainless steel is commonly used in HPLC due to its pressure proof and corrosion resistant. However, it can interact with compounds containing phosphate group(s) by metallic affinity. This is a factor that negatively affecting the shape and intensity of the peak. In order to suppress metal adsorption, cleaning flow path with phosphoric acid, addition of chelating agents to mobile phase, or repeated injections of the target compounds are often performed. Nevertheless, it is not easy to obtain highly reproducible results. This article introduces the use of the Nexera XS inert ultra-high performance liquid chromatograph, which utilizes a metal-free flow path, and a metal-free column for the accurate and reproducible analysis of nucleotides.

Charge variant analysis of mAb biotherapeutics by Nexera XS with a Shim-pack Bio IEX Column

Monoclonal antibodies (mAbs) are the most approved biopharmaceuticals used to treat severe and chronic diseases such as cancer, autoimmune, cardiovascular, respiratory, hematology, and several infections. They have an enormous therapeutic and commercial value which makes them among the top 10 best sellers of pharmaceuticals for several past years. In recent years, the use of mAbs has been expanded due to significant advances in design thus decreasing immunogenicity in humans, improving their bioavailability, and specific affinity for antigen-binding.



Determination of Protein Secondary Structures of Monoclonal Antibody using FTIR Spectroscopy

Monoclonal antibodies (mAbs) are a major class of biopharmaceuticals due to its wide application in medicine and biological science. The antibody's biological activity can be attributed to its unique structural conformation. Protein higher order structure (HOS) includes the secondary, tertiary and quaternary structures of protein, which comprise the three dimensional structures of protein. Analysis of the HOS of mAbs is essential to ensure the quality and efficacy of the protein therapeutics product. In this application news, the secondary structure of mAbs is examined by using FTIR spectroscopy and band curve-fitting data analysis.

Selective and Sensitive Method for Estimation of Liraglutide in Human Plasma using LCMS-8060

Liraglutide is a glucagon-like peptide-1 receptor agonist (GLP-1 receptor agonist) also known as incretin mimetics. Liraglutide was analyzed in human plasma using a solid phase extraction method in MRM mode on LCMS-8060. The developed method is selective and sensitive for estimation of liraglutide in human plasma. In this article, Liraglutide was analyzed in human plasma using a solid phase extraction method in MRM mode on LCMS-8060. The developed method is selective and sensitive for estimation of liraglutide in human plasma.

Simultaneous estimation of Insulin glargine and it's excipients in formulation using LC-UV-MS/MS

Simultaneous estimation plays a very important role in biopharmaceutical industry as it is very feasible and time saving. For the multi component analysis various techniques like spectrophotometric techniques (UV-VIS, IR, NMR and mass spectrometry) and chromatographic techniques (Thin Layer Chromatography, High Performance Liquid Chromatography, Ultra-High Performance Liquid Chromatography, High Pressure Thin Layer Chromatography and Gas Chromatography) is used. In this poster, an LC-UV-MS/MS method has been developed for the simultaneous determination of insulin glargine and its excipients in formulation.

Experimental Studies of a Novel Multi-turn Time-of-Flight Mass Spectrometer and Its Applications for High Mass Molecules

The needs for high resolving power and sensitivity analyses of high mass molecules has been increasing in many fields such as biopharmaceutical and material industries. Time-of-Flight mass spectrometer (TOF MS) is a suitable solution to meet these needs. A novel Multi-turn Time-of-Flight mass spectrometer has been developed for biopharma and life science. High mass resolving power of over 150k has been demonstrated for biomolecules including reduced monoclonal antibody. Here, we show the details of the instrument and the results of the performance evaluations for high mass biomolecules.

Quantitation of Nitrosamines in Metformin Active Pharmaceutical Ingredient (API) using static and dynamic headspace GCMS/MS

Health Sciences Authority (HSA) of Singapore on 4th December 2019[1], recalled 3 out of 46 locally marketed Metformin medicines after detecting presence of NDMA "above the internationally acceptable level" and this subject came into mainstream. Subsequently, both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have released regular updates into their investigations for the causes of medicine contamination. Metformin is the first-line drug control of high blood sugar levels in patients with type 2 diabetes, particularly in people who are overweight.

Got DMF? Chromatographic separation and identification of NDMA and DMF using LCMS-9030

Chromatographic separation and identification of dimethylformamide (DMF) and N-nitroso-dimethylamine (NDMA) was performed to prevent overestimation of NDMA when DMF interference is present. This method can easily be implemented for the impurity evaluation of active pharmaceutical ingredients (API), intermediates, and final drug products irrespective of triple quadrupole or high-resolution mass spectrometers.

Latest topics 1

Global Pharma Summit 2021 Report

On November 19th, 2021, Shimadzu (Asia Pacific) hosted the 8th edition of the Shimadzu Global Pharma Summit in Singapore. In accordance with the current trends and keeping in mind the needs of the customer, the summit was aptly themed - Pharma Digital Transformation or "Pharma DX". The on-demand version of the summit is available for your viewing pleasure at **www.shimadzupharmasummit.com.**

Shimadzu 8th Global Pharma Summit 2021

The Pharma Summit is a platform that Shimadzu provides to the global pharma industry to network, share and discuss strategies for the future of pharma. This year the summit was held "Phygitally" (physically + digitally). While on one had participants virtually joined the online portal showcasing a completely immersive 3D environment, on the other, key VIPs and industry leaders joined physically at multiple locations across India, Nepal and Sri Lanka. The online broadcast was done across 2 time zones (12:30pm IST & 11 am EDT) to cater to the attendees from various parts of the world like the Americas, Europe, Middle East, Africa and Asia.

This year's Phygital summit was a first-of-its kind by an analytical instrument manufacturer anywhere in the world. The milestone event witnessed participation from over 2000 global pharma leaders from over 100 global pharma organization and from18 countries, join hands in formulating strategies to transform their businesses using Pharma Digital Transformation or Pharma DX. The huge turnout of people globally at the various physical locations and over the internet, makes this the largest Shimadzu Global Pharma Summit ever till date. Majority of these participants were either CXOs, Managing Directors, Directors, Presidents, Vice Presidents or belonged to other key decision-making positions, as is the case with all previous summits over the years. The display of Shimadzu's strength at the summit in the form of its state-of-the art technology solutions and instruments or its ability to gather the key opinion leaders of the pharma industry from across the world under one virtual roof, helped build an unforgettable brand image in the minds of the customers.

Dr. Teruhisa Ueda, President and CEO, Shimadzu Corporation Japan, while delivering the Welcome Address, highlighted that, "With the advent of the pandemic, it is now more evident than ever

Dr. Teruhisa Ueda

before, how important the role of pharma is in today's world. We also recognize that society is currently undergoing major changes, especially due to the pandemic, and we are thus continuously improving ourselves to become an even stronger partner of innovation and technology. Our objective has always been to add long term value to our customers' journeys and help increase efficiency in Pharma."

A Truly immersive virtual 3D futuristic experience

This year saw the summit being showcased in a brand-new digital avatar. Leveraging on the technological advances made in CGI or Computer-Generated Imagery and Augmented Reality, Shimadzu was able to re-create and broadcast the entire experience of a physical summit over the internet on the site **www.shimadzupharmasummit.com.** The absolutely stunning graphical interfaces, combined with world-class content from global thought leaders, helped deliver an exceptionally captivating experience to the customers and other attendees, which has never before been witnessed by them in the physical world.

Speakers & Panelists

The Shimadzu 8th Global Pharma Summit 2021 witnessed participation from some of the top pharma industry leaders from across the world. Keeping in mind the extensive knowledge that these eminent individuals already possessed and the niche subject of Pharma DX, it became imperative that Shimadzu had to source the best subject matter experts from across the world, if the content of the summit was to be made valuable enough for the already enlightened audience. The wide range of speakers and panelists from across the world, who obliged to share their knowledge at the summit.

For details on the Speakers & Panelists
Speakers-and-Panellists-Shimadzu-8th-Global-Pharma-Summit-2021.pdf

The Workflow Stations

One of the most important and unique features of this year's summit were the 6 Workflow Stations, namely:

Station 1: Shimadzu's Unique Drug Discovery Workflow
Station 2: Shimadzu's Total BioPharma Workflow
Station 3: Shimadzu's Work-From-Anywhere QA/QC Workflow
Station 4: Shimadzu's Advanced Natural Products Workflow
Station 5: Shimadzu's Comprehensive Nitrosamines Workflow
Station 6: Shimadzu's Tech-celeration Solutions for Pharma

As the names indicate, each of these stations featured a comprehensive deep dive into the various workflows that the attendees could utilize in their laboratories for Pharma DX.

Launch of the World's First Living LC

The Shimadzu 8th Global Pharma Summit 2021 also saw the unveiling of the World's First Living LC. This UHPLC-ready LC, which is as dynamic and intelligent as life itself, is the first-in-line of many soon-to-come "Living Instruments". These instruments will showcase pioneering innovations and autonomous features like Self-Optimising, Self-Adapting and Self-Learning abilities and are designed keeping in mind the futuristic Smart Labs. The Living LC and other Living Instruments will play a crucial role in enabling in Pharma DX and transforming Pharma 4.0 now and in the near future as well.

Mr. Prem Anand, Executive Officer and Senior General Manager, Shimadzu (Asia Pacific) Pte Ltd, who along with delivering the opening Exordium at the event also launched the Living LC, revealed that, "Living Instruments are an emerging wave of technologies that incorporate the dynamics of human life in technology. Some examples of Living Instruments are Self-Driving Transportation and AI Surgery Robots, which are completely transforming our lives. Shimadzu has become the first-in-the-world to develop such living analytical instruments that have autonomous operation and can accomplish complex functions by themselves without human intervention. We call it Analytical Intelligence – The 6th Sense of analytical instruments."

Mr. Prem Anand

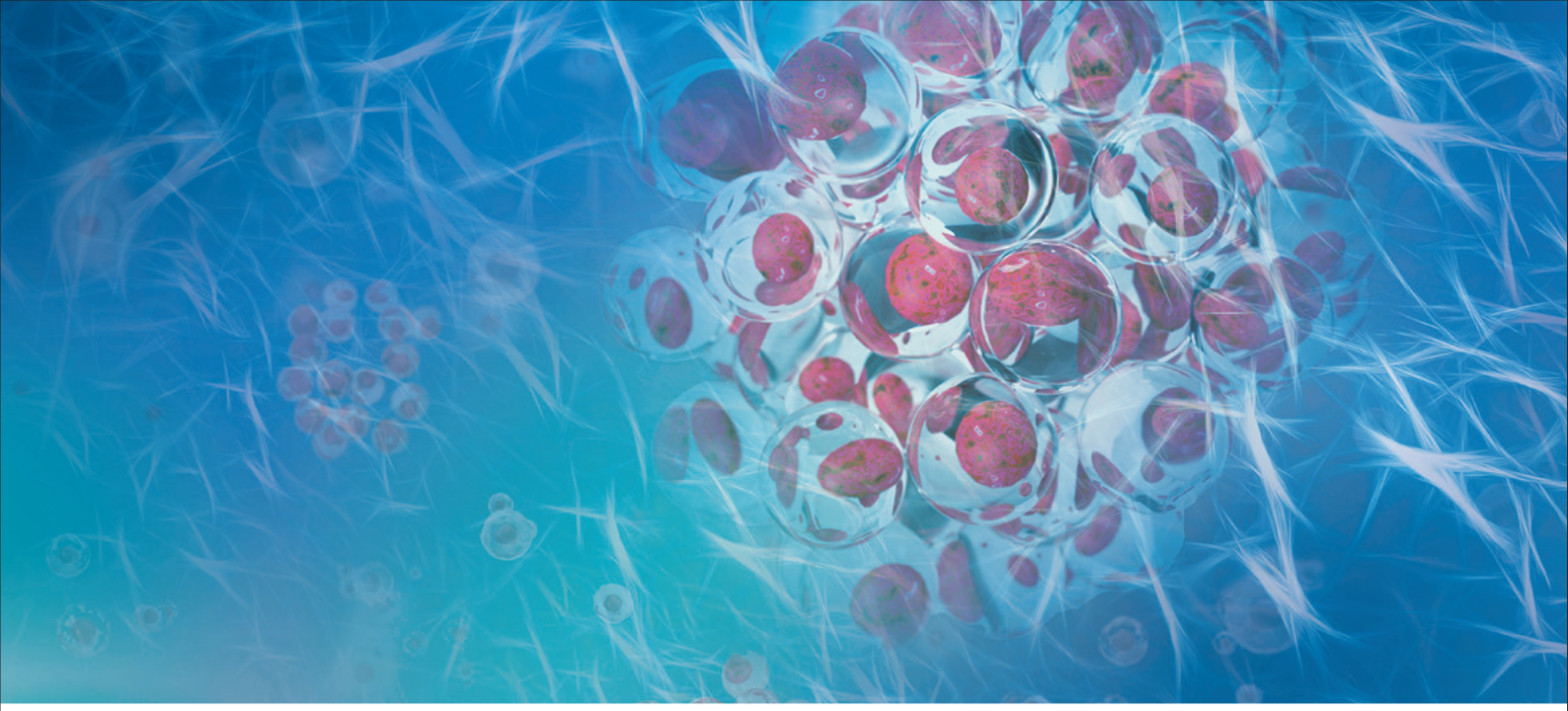
Together, Let us usher in the era of Pharma 4.0

Shimadzu's aim with the Shimadzu 8th Global Pharma Summit 2021 was to reinforce its position as a Key Leader in Pharma 4.0 and expand its outreach significantly. Looking at the testimonials received from the attending pharma industry leaders from across the world, it seems apparent that Shimadzu has been able to well achieve this objective and has further strengthened its brand presence in the hearts and minds of the customers and all the attendees alike.


Mr. Tetsuya Tanigaki

Mr. Tetsuya Tanigaki, Managing Director, Shimadzu (Asia Pacific) Pte Ltd, Singapore, who delivered the Vote of Thanks, said, "This year the theme of the summit is Pharma DX and I am very happy to see that a wide range of trending topics from AI Deep Learning to Robotics and from Continuous Manufacturing to Living Technology have been covered today. We hope that the Pharma Summit has provided you with the experience and tools to undertake Digital Transformation in your business.

See full version of the report below:
https://www.shimadzu.com.sg/an/Shimadu-8th-Global-Pharma-Summit-2021-Event-Report.html



Latest topics 2

HYDROX 3D Nanofiber Enables Three-Dimensional Cell Culturing

In cell research, drug discovery screening, regenerative medicine, and other fields, there is a need to build cell aggregates outside organisms that function similar to the given organism. HYDROX 3D nanofiber*1 (Fig. 1) is a new material developed independently by Shimadzu to assist the formation of cell aggregates by three-dimensional cell culturing. In August 2021, Shimadzu collaborated with a research group of Osaka University to show that HYDROX nanofibers can be used to efficiently derive hepatocyte-like cells from human iPS cells by applying hepatic differentiation (reference 1). That research group is headed by Professor Hiroyuki Mizuguchi and Specially Appointed Assistant Professor Yukiko Toba in the Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences at Osaka University.

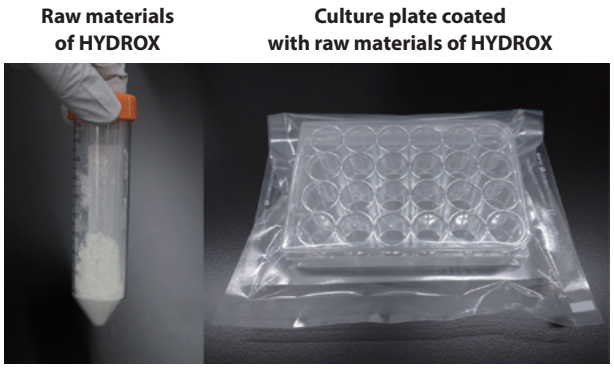


Fig. 1 Appearance of samples
Raw materials of HYDROX (the left picture) and culture plate coated with dried gel derived of raw materials of HYDROX (the right picture)

Conventional cell culturing is typically performed by two-dimensional "plate culturing," but the majority of human cells have three-dimensional structures. Consequently, three-dimensional culturing has gained significant interest in recent years. However, the animal-based components used as a scaffold material*3 for three-dimensional culturing involve difficulties with reproducibility, safety, handling, and manufacturing.

In 2016, Shimadzu Corporation developed HYDROX 3D nanofiber from Shimadzu's own chemically synthesized polymer (HYDROX raw material polymer) *4 (Fig. 2 and reference 2) and determined that HYDROX can be used to enable easy three-dimensional culturing. The actual culturing steps are illustrated in Fig. 3.

(1) Powdered HYDROX raw material polymer dissolved in solution is dripped onto a culture plate and dried to form a dried gel coating at the bottom of the plate. Then a cell suspension is added.
(2) The dried gel absorbs the water content to form HYDROX 3D nanofibers.
(3) Cultured cells trapped in the nanofibers assemble to form three-dimensional cell aggregates.
(4) The HYDROX nanofiber structure is broken down by adding an excessive quantity of water to easily recover only the cell aggregates.

Since all the culturing steps are easy and safe, HYDROX offers an excellent alternative material to animal-based ingredients, so Shimadzu intends to commercialize it as a product from 2023. As part of the commercialization process, Shimadzu Corporation partnered with Osaka University in joint research using HYDROX nanofibers to efficiently obtain hepatocyte-like cells from human iPS cells by applying hepatic differentiation or to maintain hepatic-specific functions of primary-cultured hepatocyte cells. That research demonstrated that HYDROX can be used to form cell aggregates with enhanced functional characteristics and can be used to improve the various liver functions of differentiated cells (reference 2) .

In addition to using the HYDROX 3D nanofiber to enable easy three-dimensional culturing, Shimadzu also intends to promote its widespread use for basic cell research, drug discovery screening, regenerative medicine research, and other applications.

*1 HYDROX is a registered trademark of Shimadzu Corporation. Nanofibers are fibrous substances that range from 1 nm to 100 nm in diameter and have a length that is at least 100 times greater than the diameter.
*2 iPS cells are artificially cultured pluripotent stem cells. The world's first iPS cells were created by Professor Shinya Yamanaka and others at Kyoto University.
*3 This material mimics the functions of an extracellular matrix that maintains the cell structure.
*4 Amphiphilic polymer formed from poly(l-lactic acid) and poly(sarcosine).

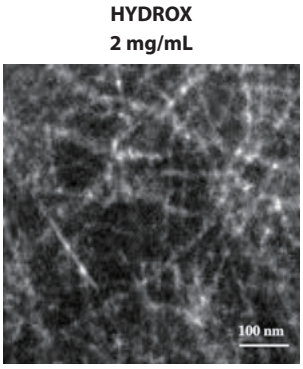


Fig. 2 Observation of HYDROX by transmission electron microscopy
The image shows a result using 2 mg/mL HYDROX by transmission electron microscopy.

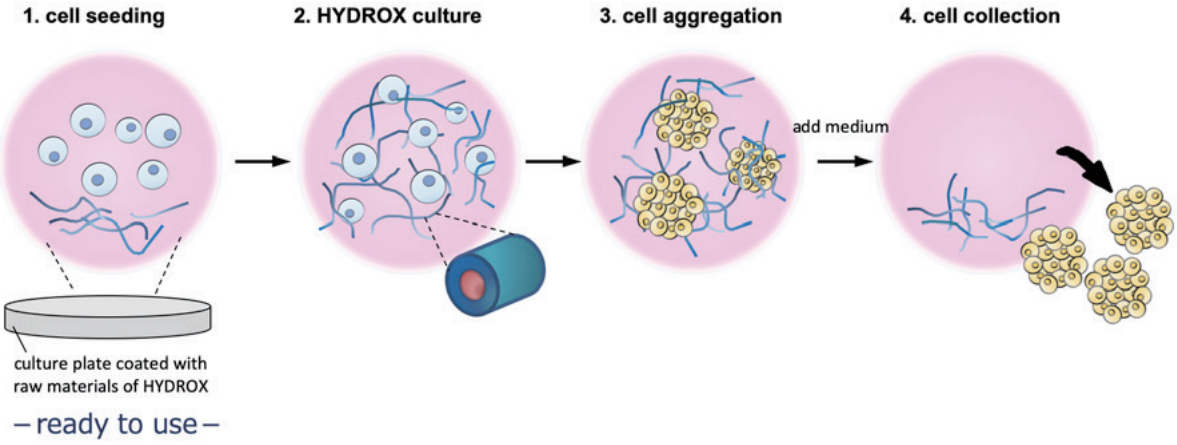


Fig. 3 Schematic of the preparation of HYDROX, the 3D cell culture and the collection of the cells
The raw material solution of HYDROX was coated onto a culture plate and dehydrated to form a dried gel (1). Then a cell suspension was added to the dried gels, leading to the formation of 3D nanofibers (HYDROX) (2). The cells gradually assembled and formed 3D aggregates (3). For the separation of the cultured cells from HYDROX for the subsequent analyses, additional culture medium was poured into the HYDROX-containing plates, leading to conformational collapse of nanofibers. Then the cell medium was collected in tube and, by only centrifugation, the cells could be easily separated from HYDROX without any additional reagents (4).

References

1. Enomoto, J.; Toba, Y.; Yamazaki, H.; Kanai, M.; Mizuguchi, H; Matsui, H. Development of a 3D cell culture system using amphiphilic polydepsipeptides and its application to hepatic differentiation. ACS Applied Bio Materials. 2021, 4, 9, 7290–7299.
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Latest topics 3

Celebrating 50 Years of Total Organic Carbon (TOC) Analysis Innovation

2022 marks the 50th anniversary of the first TOC analyzers (TOC-100 and TOC-10 models) released by Shimadzu Corporation. To commemorate the occasion, Shimadzu is planning a variety of activities throughout the year.

TOC 50th Anniversary Website :
https://www.shimadzu.com/an/products/total-organic-carbon-analysis/en/

A total organic carbon (TOC) analyzer is an analytical instrument that measures the total amount of organic carbon contained in water. It is used for quality control of public drinking water, control and evaluation of pharmaceutical manufacturing processes, and a wide variety of other applications.

A History of Cutting-Edge Innovation

The history of Shimadzu TOC analyzers began in 1972 when Shimadzu developed and released the TOC-100 automatic water quality monitoring system and TOC-10 laboratory total organic carbon analyzer. In 1983, Shimadzu released the TOC-500, which was the world's first TOC analyzer with 680°C combustion catalytic oxidation capability. That significantly extended the life of combustion tubes and catalysts and improved ease of maintenance. In 1989, the TOC-5000 featured an automatic sample injection mechanism that expanded the measurement range from previous ppm levels to ppb levels. That also expanded the applicability of TOC measurements to include purified water, making Shimadzu the global leader in TOC analyzers. The TOC-V series released in 2000 featured additional functionality for measuring solid samples, volatile organic carbon (POC), and total nitrogen (TN), which expanded the applicability of TOC analyzers. Today, the TOC-L series released in 2011 continues to serve at the forefront of TOC analysis.

Shimadzu TOC-10A Total Organic Carbon Analyzer

Initially, TOC analyzers were mainly used for ensuring the water quality of environmental waters, industrial effluents, and so on, but later, as TOC performance improved, the scope of applications expanded. Now they are also used for quality control of purified and ultrapure water, for ensuring compliance with public drinking water quality standards, for controlling/evaluating pharmaceutical manufacturing processes, and for researching carbon-neutrality. Thus they serve a vital role in various fields important for people and the Earth.

Applications for Biopharmaceuticals

Takahide Hiramatsu
Pharmaceutical
Application Specialist

At pharmaceutical manufacturing sites, ingredients and rinse water must be controlled based on pharmacopoeia requirements in respective countries. The following describes an application of using TOC analysis for biopharmaceutical manufacturing.

■ **Culture tank cleaning validation:** Using an online TOC analyzer to validate the culture tank cleaning process can shorten the validation time and reduce human errors.

Biopharmaceutical production lines include a large number of culture tanks. Inadequate cleaning of culture tanks can prevent the culturing process from progressing as expected, which can sometimes waste an entire tank of ingredients. However, using an offline TOC analyzer to check the cleaning status of culture tanks requires people to sample the rinse water and measure the samples in a laboratory, which is time-consuming and expensive. In contrast, using an online TOC analyzer can reduce waiting times by periodically measuring automatically sampled rinse water and successively sending that data to a host computer.

Cleaning Validation System for Biopharmaceutical Cell Culture Tanks Using On-Line TOC Analyzer

■ **Extractables and leachables from plastic materials:** A TOC analyzer compliant with standards for substances extracted from plastic materials in pharmaceutical packaging (USP 661) can be used to easily check for extracted substances with high sensitivity.

Pharmaceutical packing materials interact with pharmaceuticals. Substances that can be transferred from packaging materials to pharmaceuticals under normal storage conditions are referred to as "leachables," whereas components that form under more severe storage conditions are referred to as "extractables." Due to the increase in biopharmaceuticals, there has been growing interest in extractables and leachables in recent years. Because most biopharmaceuticals are injectable drugs, there is a high risk of biopharmaceuticals interacting with packaging materials. Furthermore, to reduce biopharmaceutical manufacturing costs, momentum is also increasing for using continuous production methods. The use of plastic materials in single-use production equipment also adds the risk of leachables being introduced from such equipment. Shimadzu TOC analyzers can be used as a physicochemical testing parameter for extractables.

TOC – Determination according to USP 661.1 Testing of Plastic Packaging Systems and their Materials of Construction

TOC – Determination according to USP 661.2 Testing of Plastic Packaging Systems and their Materials of Construction

Looking forward to the future of TOC

Ever since Shimadzu released its first TOC analyzer in 1972, Shimadzu has been constantly improving TOC analyzer accuracy, sensitivity, functionality, the product line, and options. Today, as the leading TOC analyzer company, Shimadzu now has the largest global market share. In the future as well, Shimadzu remains committed to "Realizing Our Wishes for the Well-being of Mankind and the Earth" by continuing to develop additional systems.

Image: Environmental TOC Development Team

Latest topics 4

Release of the Nexera XS inert High-Performance Liquid Chromatograph

Shimadzu Corporation announces the release of the Nexera XS inert high-performance liquid chromatograph globally. The Nexera XS inert incorporates rust-resistant materials and nonmetallic materials that limit the adsorption of molecules to provide stable measurements of biopharmaceuticals and medium molecule pharmaceuticals*1. Through this product, Shimadzu aims to expand its business performance in the market for biopharmaceuticals such as antibody drugs and medium molecule pharmaceuticals such as nucleic acid drugs.

In recent years, in the pharmaceutical industry, the development of biopharmaceuticals, such as antibody drugs, as well as medium molecule pharmaceuticals utilizing peptides and oligonucleotides has accelerated. These new modalities*2 have chemical characteristics that differ from low molecular compounds, which are currently the mainstream in pharmaceuticals. Accordingly, new techniques will be required in situations such as research and development related to drug efficacy and toxicity as well as in manufacturing and quality control. High-performance liquid chromatographs (LC) and liquid chromatograph mass spectrometers (LC-MS) may also pose problems. For example, molecules prone to adsorption by metals become adsorbed within the instrument during analysis, leading to data inaccuracies and reducing sensitivity. In addition, depending on the test method, the LC system might rust, and performance might deteriorate because of long-term use or insufficient cleaning after use.

Nexera XS inert, based on the outcome of cooperative research with Professor Yasushi Ishihama of the Graduate School of Pharmaceutical Sciences, Kyoto University, was designed and developed in order to resolve such problems. Nonmetallic materials are used for the flow lines through which the samples pass, preventing adsorption of molecules prone to metallic adsorption by the instrument. As a result, sensitivity in LC and LC/MS tests is not prone to decrease, and the accuracy of data is maintained even in repeated analyses. Additionally, rust-resistant materials are used in the design, so that the instrument provides stable operation and highly reliable data, even after long-term use of saline and other solutions for corrosive to metals.

As an LC manufacturer, Shimadzu has the top share of the Japanese market, and our flagship Nexera series LC system is used by a wide range of customers thanks to its high basic performance level and excellent usability. The Nexera XS inert is intended to expand our global share by strengthening our share of the pharma/biopharma market. By continuing to heighten data reliability, Shimadzu is promoting more streamlined analysis and testing, thereby contributing to the rapid development of bio- and medium molecule pharmaceuticals.

*1 Medium molecule pharmaceuticals: Pharmaceuticals that use molecules with a molecular weight between 500 and 2000
*2 Modality: The type and form of pharmaceuticals

For more details, visit Nexera XS inert

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New Products

EDX-7200

Energy Dispersive X-ray Fluorescence Spectrometer

A flagship model of the EDX series in pursuit of high sensitivity, high speed and high precision. This model supports new regulations and directives for consumer and environmental compliance, such as RoHS/ELV, REACH, and TSCA with full exclusive screening analysis kits.

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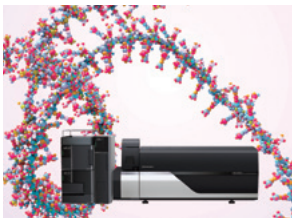
LC/MS/MS Method Package for Modified Nucleosides

For LabSolutions™ LCMS

This Method Package provides optimized analytical conditions, including chromatographic separation and MS parameters, for the determination of two types of modified nucleosides and factors for normalization.



Learn more

SialoCapper™-ID Kit

Sialic Acid Stabilizing Kit for Linkage Isomer Discrimination

The SialoCapper-ID Kit improves sialoglycan sensitivity while stabilizing sialic acids and reducing their degradation. This kit enables highly reliable measurement data acquisition and labor-saving experiments.

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LabSolutions MD

ANALYTICAL INTELLIGENCE

Solution for Method Development and Analytical Quality by Design

The LabSolutions MD uses an Analytical Quality by Design (AQbD) approach for achieving efficient method development. It streamlines all workflow steps from experimental design to design space construction, enabling even non-experts to develop optimal analytical methods.

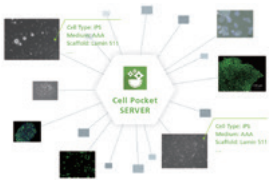
Learn more

Cell Pocket Ver. 2

Web Application Supporting Cellular Observations

This system can perform image analysis using AI technology (deep learning) on cell images taken with a microscope and aggregate and manage experimental data including cell images through a Web browser.

*This product is only available in Southeast Asia, China and Oceania.



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AP W-AD Series

Analytical Balances

New automatic doors and touchless sensors enable the entire range of weighing operations to be performed without touching the operating panel on the main unit, which helps prevent infections and reduces the risk of foreign matter contaminating analytical samples.

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Nexera XS inert

ANALYTICAL INTELLIGENCE

Ultra High Performance Liquid Chromatograph

The Nexera XS inert incorporates a non-metallic material in the flow path where the injected sample comes into contact, reducing the adsorption of metal-adsorbing molecules to the LC system and provides corrosion resistance to mobile phases containing high concentrations of salt.

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MALDI-8030

Dual-Polarity Benchtop Linear MALDI-TOF Mass Spectrometer

The MALDI-8030 is the class-leading instrument – versatile, compact and affordable. It is specified for dual-polarity to cater for compounds best suited for analysis in negative ion mode. Its outstanding performance is a result of its high mass accuracy and sensitivity.

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