nSMOL Improves the Speed and Accuracy of mAb Bioanalysis

Nano-technology, limited proteolysis, and LCMS analysis

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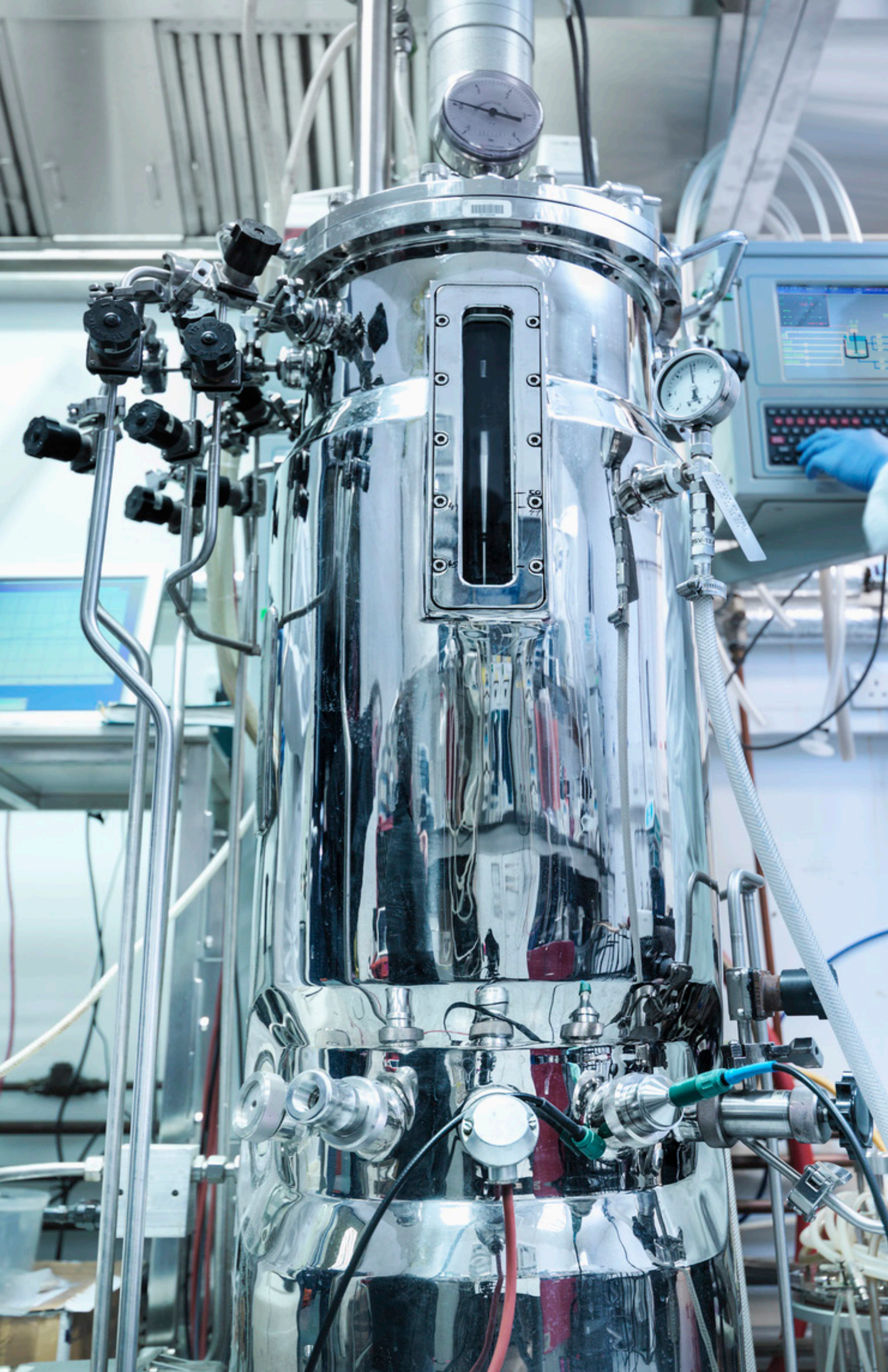


IMPROVE SPEED AND ACCURACY OF MONOCLONAL ANTIBODY BIOANALYSIS USING NANOTECHNOLOGY AND LCMS

As scientists gain an advanced understanding of diseases at the molecular level, the biopharmaceutical industry is significantly increasing research, production and manufacturing of monoclonal antibodies (mAbs). In fact, five of the top-ten best-selling drugs in the U.S. in 2016 were mAbs.

MAbs on the market today treat debilitating and life-threatening diseases like rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, plaque psoriasis and ulcerative colitis. They are also used to fight certain colorectal, lung, glioblastoma, non-Hodgkin's lymphoma, kidney, cervical, ovarian breast, stomach and esophageal cancers.

HOW DO mAbs WORK?

To fight against foreign substances in the body, the immune system produces large numbers of antibodies. Antibodies are proteins that circulate throughout the body until they find and attach to specific foreign substances called antigens. After the antibodies attach to the antigens, they trigger the immune system to destroy the antigen-antibody complexes.

Scientists are now able to create antibodies that target a specific biomarker, like one found on cancer cells. Researchers then make mAbs, against the biomarker, in the lab. To make a mAb, researchers first have to identify the right biomarker to target. mAbs are made to act as substitute antibodies that can restore, strengthen or mimic the immune system's attack on disease-producing cells. They also are designed to bind to biomarkers that are found more often on the disease-causing cells rather than on healthy cells. This targeted therapy attacks the cancerous cells without damaging the normal cells, which can lead to fewer side effects for the person receiving the treatment.

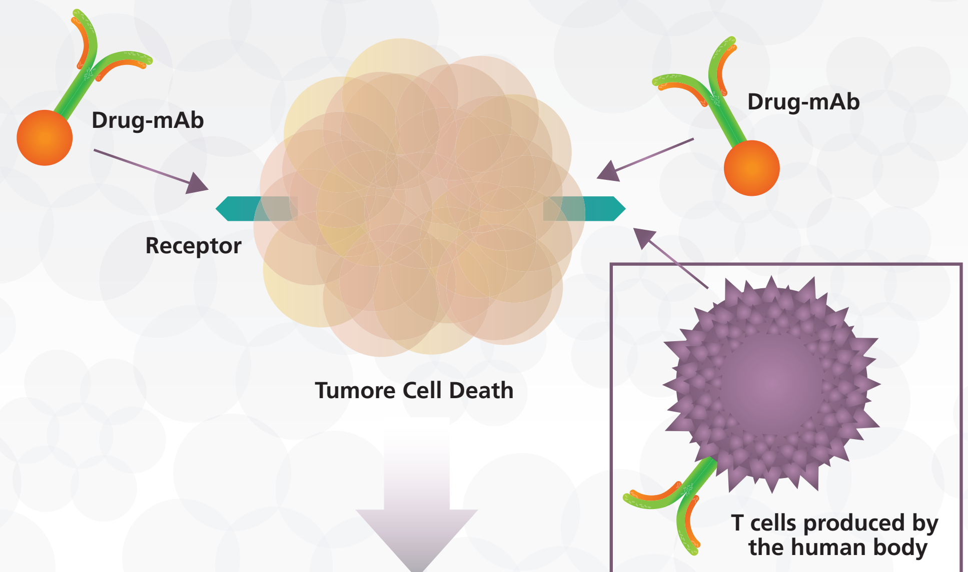
THE IMPORTANCE OF mAb BIOANALYSIS

Bioanalysis or pharmacokinetic/pharmacodynamics (PK/PD) information provides some of the most fundamental information necessary in the development of mAbs, such as drug efficacy and toxicity.

Absorption, distribution, metabolism and excretion (ADME) analysisdetermines how these four criteria influence performance and pharmacological activity, such as drug distribution in plasma and tissue. Bioanalysis also provides information to determine dosing levels.

Scientists are now able to create antibodies that target a specific biomarker.

mAbs BIND TO BIOMARKERS



CURRENT METHODS FOR mAb BIOANALYSIS

Current methods for mAb bioanalysis include:

LIGAND BINDING ASSAYS AND ELISA METHOD

Ligand binding assays (LBA) involve the binding of ligand molecules to receptors, antibodies or other molecules. LBA is used to test for the presence of target molecules that will bind to the receptor. A detection method is used to determine the presence and extent of the ligand-receptor complexes. Analysts using LBA often face challenges establishing selectivity, specificity and range of quantitation. In addition, LBA's ability to obtain whole-molecule information is limited because it is insensitive to changes away from binding regions.

The enzyme-linked immunosorbent assay (ELISA) method, paired with UV spectroscopy, is often used to detect and quantify biotherapeutic drugs such as antibodies. The procedure involves immobilizing the test material on a surface and exposing it either to a complex of an enzyme linked to an antibody specific for the antigen or an enzyme linked to an antigen specific for the antibody. This is followed by a reaction of the enzyme with a substrate to yield a colored end product that correlates to the amount of analyte present in the original sample.

The ELISA method is used to measure drug concentration in blood. However, there are critical issues with the effectiveness and efficiency of ELISA, including influences from cross-reaction and inhibitory materials.

Most commonly used methods for mAb analysis – including ELISA – are far from perfect.

CONVENTIONAL LC-MS/MS

When working with large mAb molecules, LCMS overcomes the difficulties of the LBA method with selectivity and sensitivity. Using LCMS also allows for a better understanding of biotransformation and how it impacts the bioanalysis. LCMS reduces demands on LBA reagent quality because reagents are often used in separation, rather than for specificity. While LC-MS/MS reagents do not necessarily define the assay selectivity, they do enable sensitivity by reducing ion suppression and allowing low-flow ionization. LCMS also provides time- and resource-saving multiplexing advantages.

Mass spectrometry also may be able to address the issues with ELISA because of its structure-indicated analysis. Nevertheless, with mass spectrometry, direct quantitation analysis (top-down proteomics) of complex matrices, such as plasma, is not suitable for repeat analysis because the electrospray ionization (ESI) interface cannot be maintained due to the large excess of analytes.

Analysts can achieve critical measurements using LCMS instruments because of their accuracy and sensitivity. However, sample preparation can be time consuming and includes the steps of capturing, denaturing, reduction and alkylation and tryptic digestion. Analysts using LCMS face chromatographic, ionization and mass spectrometry detection challenges for intact molecules, as well as bottom-up sequence coverage challenges. In addition, regulated targeted quantitation of large molecules by intact LCMS is immature.

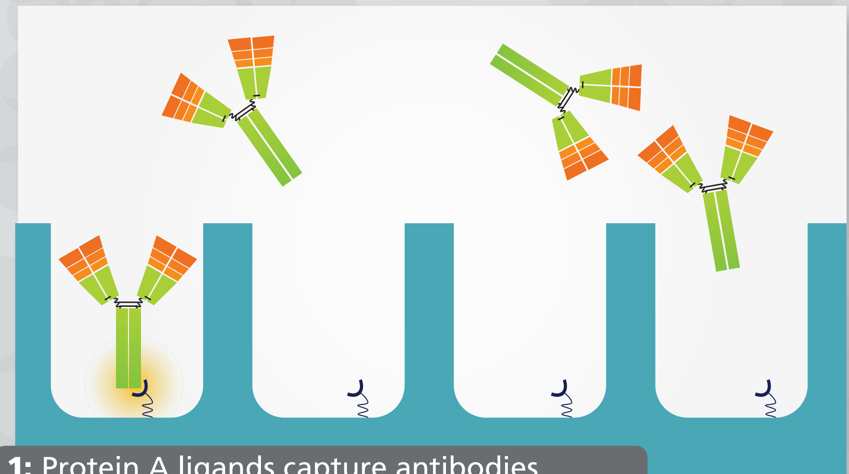
More specifically to mAb bioanalysis, the LCMS analysis of high molecular-weight proteins, such as antibodies, is normally performed after fragmentation of the protein into smaller peptides using a protease, such as trypsin or lysyl endopeptidase. However, this process also generates a large number of peptides including the signature peptides. These peptides increase the background noise and ionization suppression, and become a major challenge in the LCMS system.

nSMOL—BIOANALYSIS USING NANOTECHNOLOGY AND LCMS

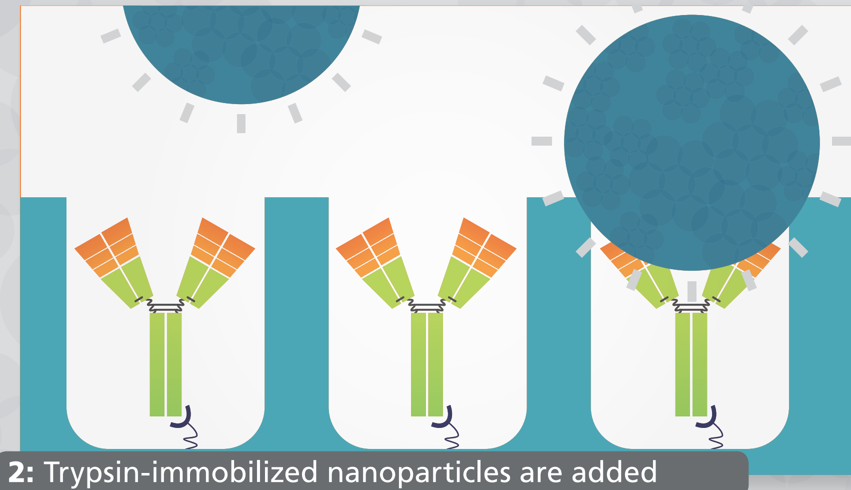
Analysts are now recognizing that mass spectrometry can be a more useful technology for mAb bioanalysis than LBA or ELISA. At Shimadzu, we have exploited a high-precision method for bioanalysis of mAbs using mass spectrometry.

The method is called nSMOL – nano-surface and molecular-orientation limited proteolysis. nSMOL dramatically improves the speed and accuracy of LCMS bioanalysis of many kinds of antibody drugs.

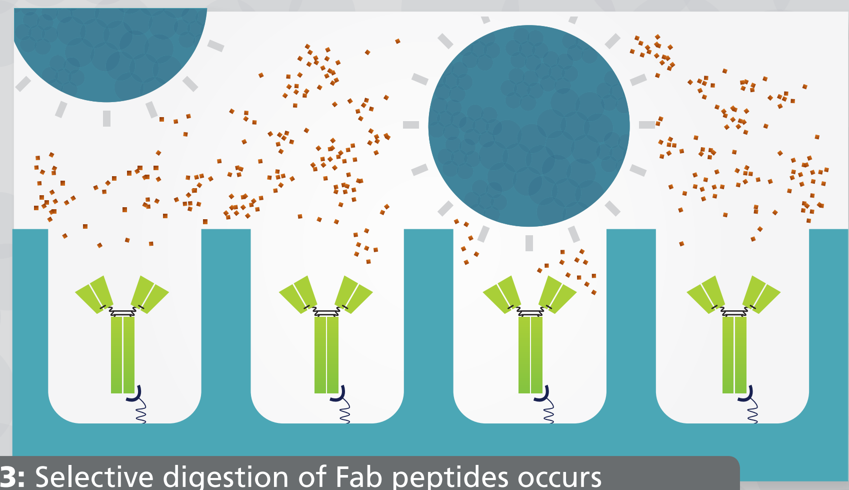
The nSMOL reagent kit is ready-to-use and optimized for capturing antibodies from blood or other biological samples using an immunoglobulin collection resin. nSMOL then enables selective proteolysis of the Fab region of these antibodies using trypsin-immobilized nanoparticles. The collection of Fab-derived peptides is easily quantified through MRM measurement on a high-performance triple quadrupole liquid chromatography mass spectrometer, such as the Shimadzu LCMS-8060.



1: Protein A ligands capture antibodies



2: Trypsin-immobilized nanoparticles are added



3: Selective digestion of Fab peptides occurs

HOW nSMOL WORKS

Analysts first mix the test sample with nSMOL immunoglobulin collection resin and a binding agent.

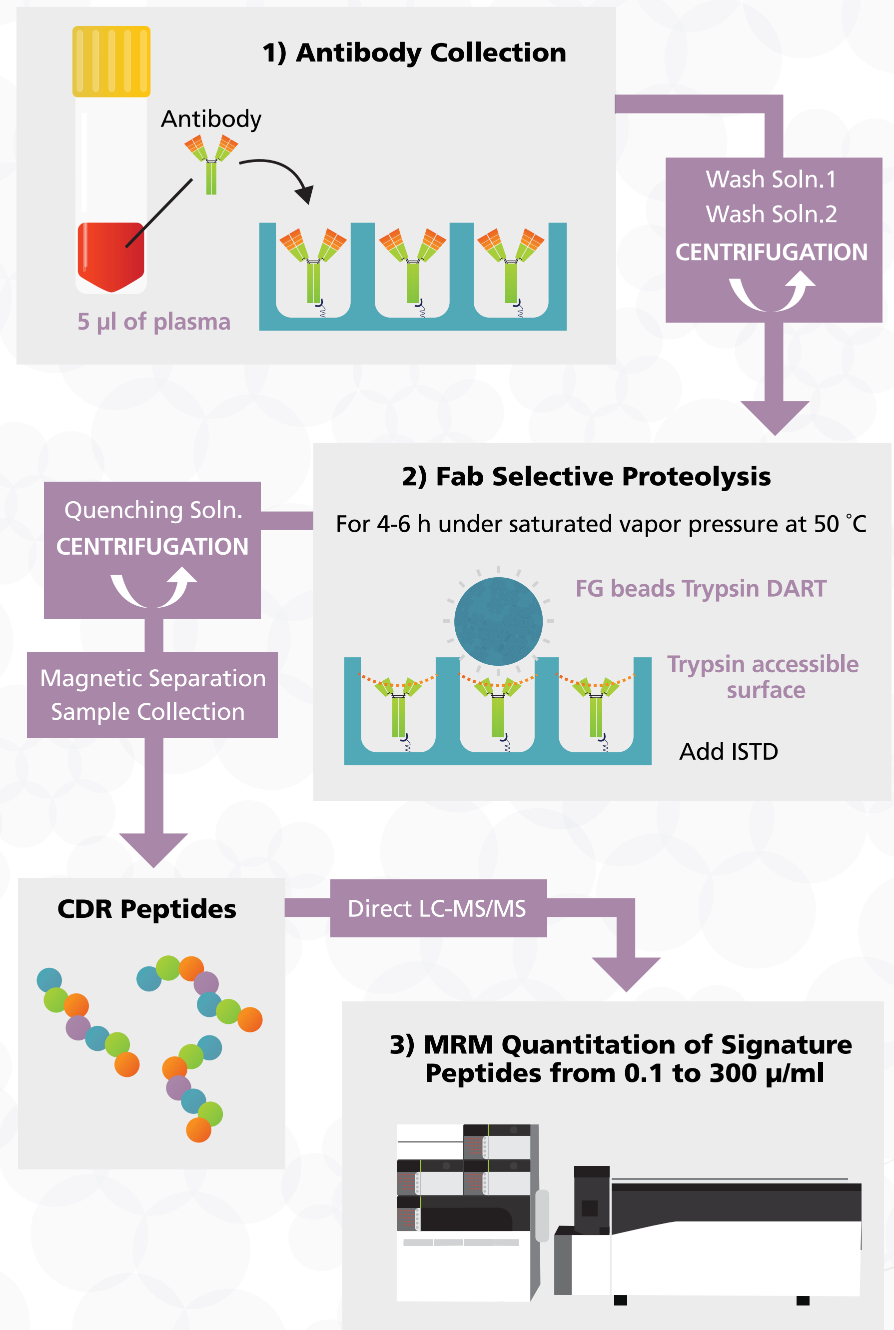
During two wash cycles, the Protein A ligands capture the antibodies and hold each one in place and in the proper orientation so that the Fab protein region is facing out from the nano-well. Matrix is removed during the wash cycles.

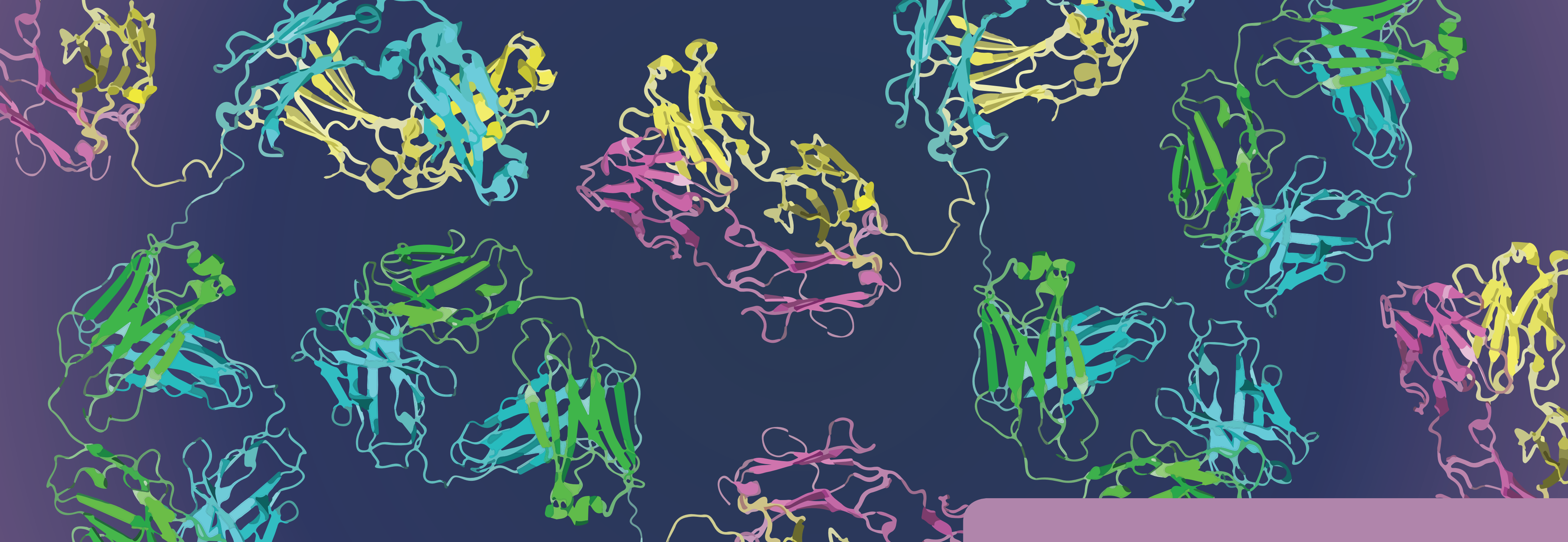
Next, a reaction solution – containing trypsin-immobilized nanoparticles – is added. The nanoparticles are perfectly sized so that only a small part fits into the nano wells on the collection resin. In this way, only the Fab region of the antibody is exposed to the trypsin.

This selective digestion of Fab peptides decreases sample complexity and limits contamination from excessive proteolysis. It's one of the things that makes nSMOL truly unique and effective. No capture antibodies or ligands are required and there is no need for solid phase extraction after reaction. Once the sample is centrifuged, the Fab peptides can be simply extracted and injected directly into the LCMS. The analysis takes only five minutes.

nSMOL is optimized to capture antibodies from blood or other biological samples.

Standardized Experimental Workflow of nSMOL





nSMOL has wide applicability regardless of the type of antibody drugs being analyzed.

ADVANTAGES OF nSMOL VS. TRADITIONAL BIOANALYSIS METHODS

The nSMOL reagent kit gives scientists several key advantages over LBA and traditional LCMS in analyzing mAbs.

nSMOL eliminates the need for capture antibodies or ligands required with LBA or conventional LCMS methods. In the case of conventional LCMS, nSMOL eliminates the steps of denaturing, reduction and alkyation normally associated with protein digestion, resulting in more efficient sample preparation and analysis. In addition, it dramatically limits background noise and ion suppression, which leads to improved response and quantitative repeatability.

nSMOL has wide applicability regardless of the type of antibody drugs being analyzed. Its selectivity of proteolysis and simple workflow attribute to its high reproducibility. The selective collection of Fab peptides limits contamination from excessive peptides or trypsin. It provides fast method development at a lower initial cost. In addition, nSMOL meets guideline standards issued by the U.S. Federal Drug Administration and Japan's Ministry of Health, Labor and Welfare.

MULTIPLEXING CAPABILITIES

Multiplex assays give scientists the opportunity to simultaneously measure multiple analytes in a single run of the assay, rather than using procedures that measure only one analyte at a time.

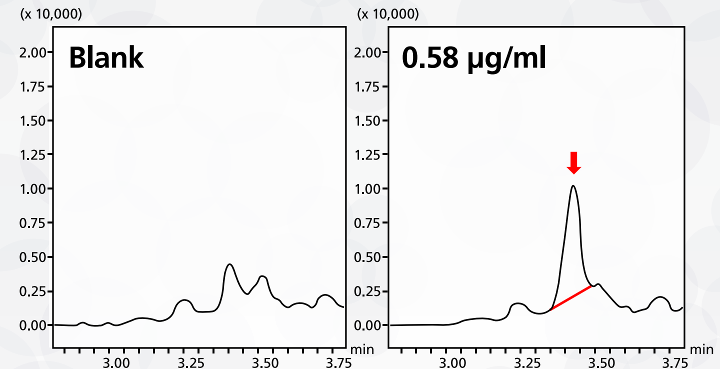
This allows researchers to obtain more information about a protein or series of proteins in less time and lets them conserve samples. nSMOL proteolysis with LCMS enables to conduct multiplex assays for mAb bioanalysis.

For example, Shimadzu researchers conducted a multiplex LCMS bioanalysis using nSMOL Fab-selective limited proteolysis of three mAb therapeutic drugs Brentuximab vedotin, Rituximab and Cetuximab combined in the same plasma samples. In this study also, researchers demonstrated the first full validation dataset for bioanalysis using nSMOL of an antibody-drug conjugate Brentuximab vedotin in human plasma using nSMOL proteolysis.

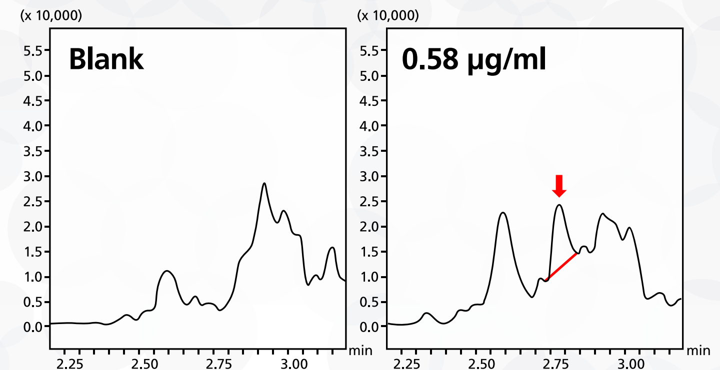
These results indicate that nSMOL is also a significant method for precise quantification of ADC in plasma, such as Brentuximab vedotin. Furthermore, nSMOL proteolysis can be applied not only to single analytes, but also to multi-analyte bioanalysis of each mAb in plasma. Therefore, nSMOL proteolysis is a feasible multiplex bioanalysis method when animals or patients are dosed with or a cocktail of antibodies or bispecific antibodies.

Obtain information about proteins in less time and conserve samples.

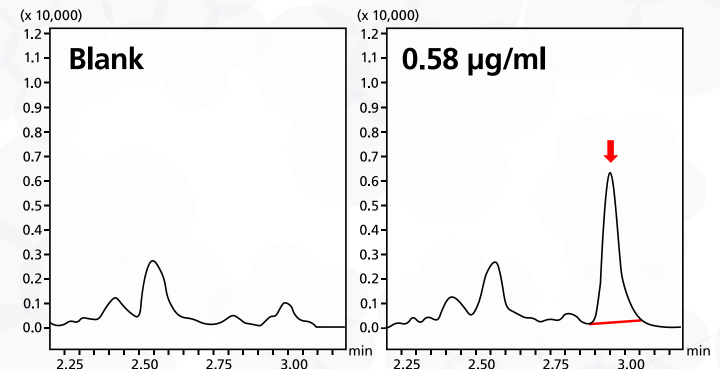
MRM Chromatograms



Brentuximab MRM Chromatograms
(in human plasma)



Rituximab MRM Chromatograms
(in human plasma)



Cetuximab MRM Chromatograms
(in human plasma)

MORE EXAMPLES OF nSMOL IN ACTION

Here are four more studies that demonstrate the accuracy and versatility of nSMOL Fab-selective proteolysis and LCMS for mAb bioanalysis.

LCMS BIOANALYSIS OF ANTIBODY DRUG TRASTUZUMAB USING FAB-SELECTIVE PROTEOLYSIS nSMOL

This study shows that nSMOL fulfills the guideline criteria for s quantitative analysis of Trastuzumab (Herceptin®) in human plasma.

Click here to read the complete application note:
http://www.shimadzu.com/an/literature/lcms/jpo117015.html

LCMS BIOANALYSIS OF ANTIBODY DRUGS BEVACIZUMAB USING FAB-SELECTIVE PROTEOLYSIS nSMOL

In this study, researchers analyzed the optimal peptide sequences for Bevacizumab (Avastin®) bioanalysis. Bevacizumab is an mAb that targets a protein called VEGF that affects tumor blood vessel growth.

Click here to read the complete application note:
http://www.shimadzu.com/an/literature/lcms/jpo117016.html

LCMS BIOANALYSIS OF ANTIBODY DRUG NIVOLUMAB USING FAB-SELECTIVE PROTEOLYSIS nSMOL

Researchers used nSMOL to perform analytical validation of Nivolumab (Opdivo®) for the pharmacokinetic monitoring into early clinical implementations. Nivolumab is a human-programmed death receptor-1 (PD-1) blocking antibody used in the treatment of metastatic melanoma.

Click here to read the complete application note:
http://www.shimadzu.com/an/literature/lcms/jpo117017.html

MULTIPLEX LCMS BIOANALYSIS OF ANTIBODY DRUGS USING FAB-SELECTIVE PROTEOLYSIS nSMOL

nSMOL supports multiplex analysis and can quantify many antibodies in a single analysis with high precision because subject molecules of nSMOL are all IgGs in plasma. This indicates that the nSMOL can be applied in antibody pharmacokinetics for combination therapy.

Click here to read the complete application note:
http://www.shimadzu.com/an/literature/lcms/jpo117018.html

CONCLUSION

As drug discovery for mAbs continues to grow around the world, it is important to develop a streamlined and universal bioanalysis method.

Shimadzu's nSMOL Antibody BA kit provides prepared reagents and protocols applicable to a wide variety of biopharmaceutical antibodies. It is optimized for capturing antibodies from blood or other biological samples using an immunoglobulin collection resin, and enables selective proteolysis of the Fab region of these antibodies using trypsin-immobilized nanoparticles.

Combining nanotechnology and LCMS analysis dramatically improves the speed and accuracy of mAb bioanalysis and supports the research and development of these important biopharmaceuticals.

For more information on the nSMOL Antibody BA kit, visit our website at www.shimadzu.eu/nsmol-antibody-ba-kit.

For more information on
Shimadzu's total solution for
biopharmaceutical labs visit
www.shimadzu.eu/nsmol-antibody-ba-kit

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