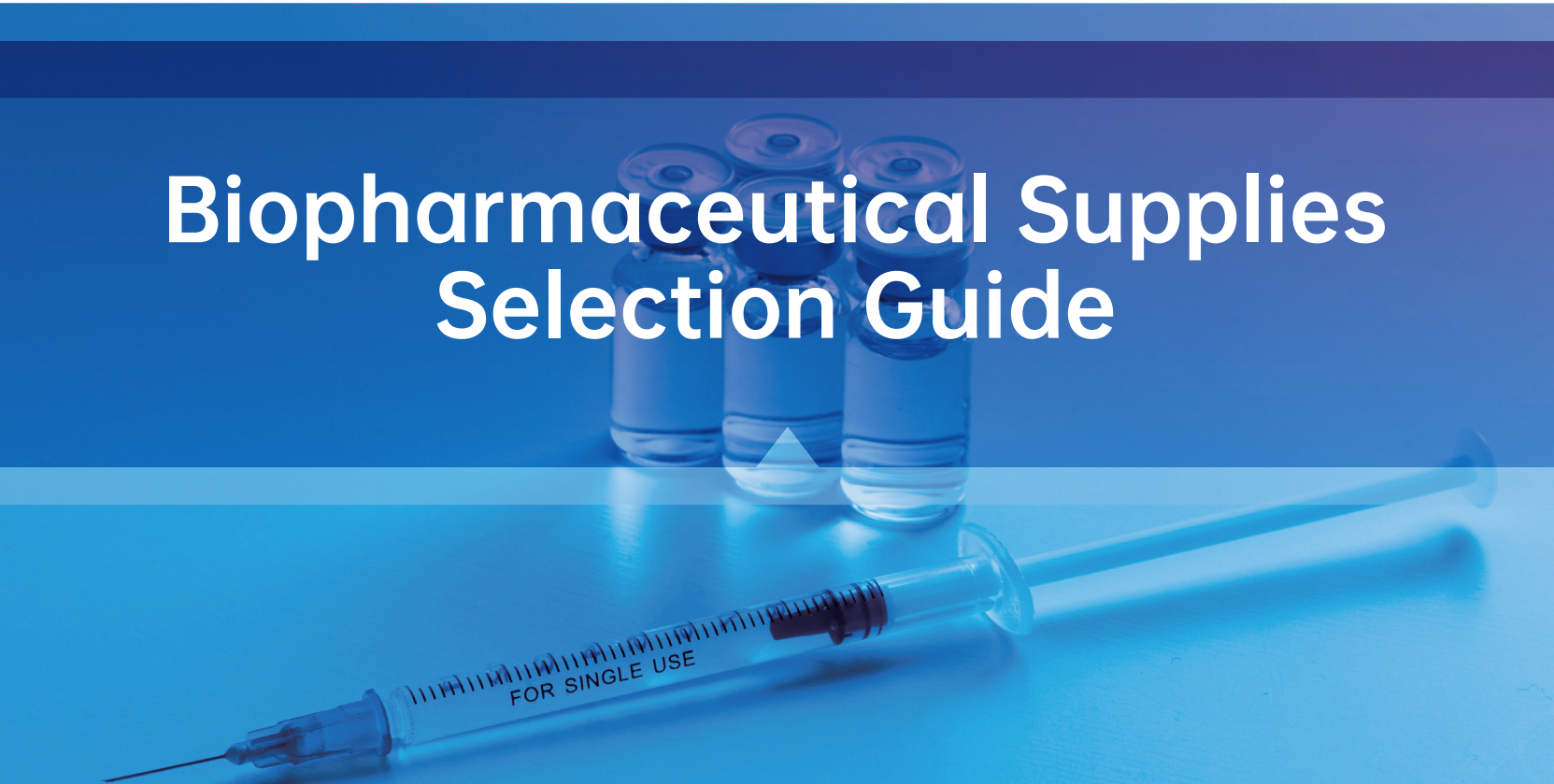
SHIMADZU
Excellence in Science

SGLC-POS-015

Biopharmaceutical Supplies Selection Guide



OUTLINE

Organizations throughout the pharmaceutical and life sciences industry are increasing their investments in biopharmaceutical development. Strategic characterization technologies provide unique insights into the development potential of large molecule therapeutics. Shimadzu (Shanghai) Laboratory Equipment Co., Ltd (SGLC), innovations in consumables are designed to address the multi-faceted analytical needs of large molecule characterization - from QA/QC standards, to clinical trials and post-marketing therapeutic drug concentration monitoring (TDM), help scientists complete their experiments faster and more efficiently.

Shimadzu Consumables family, dedicated to providing you with one-stop solutions

- Shimadzu Instrument Accessories
- Sample Preparation
- Liquid Chromatography Columns
- Standards and Reagents
- Gas Chromatography Columns
- Compact Instruments
- Low adsorption vials, Laboratory filters, Low Retention Pipette Tips

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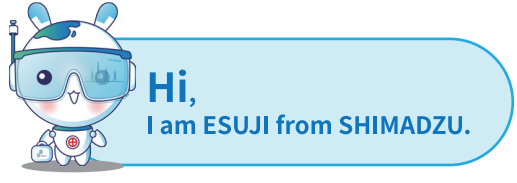


Antibody drugs using monoclonal antibodies (mAbs) poseconcerns about forming aggregates during production andstorage in terms of their impact on safety and efficacy. Therefore, monitoring these impurities by size-exclusion chromatography (SEC) is one of the most important analyses during the production of mAb. This section describes how to select **SHIMSEN Ankylo SEC columns** for the analysis of aggregates.

SEC Columns Selection Guide (What is molecular weight of you sample?)

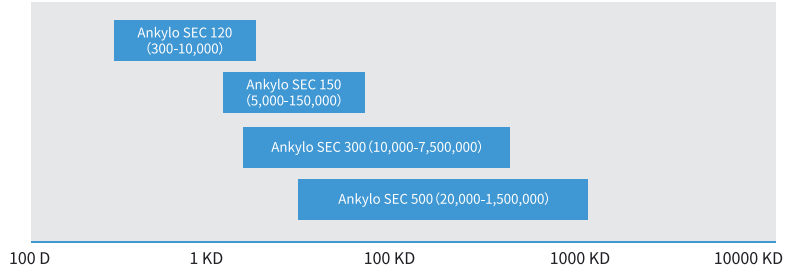
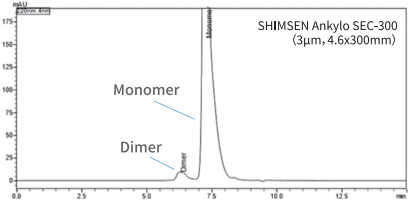
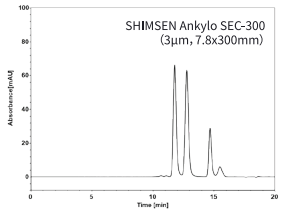


Table 1. SHIMSEN Ankylo SEC Column (Preparative column sizes can be customized)

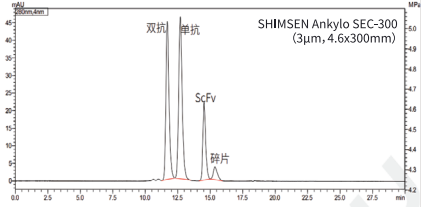
Description		Product Number	Description		Product Number
SHIMSEN Ankylo SEC-120	5µm, 7.8x300mm	380-01215-22	****SHIMSEN Ankylo SEC-300**	5µm, 7.8x300mm	380-01215-07
	3µm, 7.8x300mm	380-01215-41		3µm, 7.8x300mm	380-01215-10
SHIMSEN Ankylo SEC-150	5µm, 7.8x300mm	380-01215-01		5µm, 4.6x300mm	380-01215-08
	3µm, 7.8x300mm	380-01215-04		3µm, 4.6x300mm	380-01215-11
	5µm, 4.6x300mm	380-01215-02		3µm, 4.6x50mm	380-01215-12
	3µm, 4.6x300mm	380-01215-05		3µm, 4.6x300mm	380-01215-11
	5µm, 4.6x50mm	380-01215-03	**SHIMSEN Ankylo SEC-500**	5µm, 7.8x300mm	380-01215-65
SHIMSEN Ankylo SEC-1000	5µm, 7.8x300mm	380-01215-65		5µm, 4.6x300mm	380-01215-62
				5µm, 4.6x50mm	380-01215-63



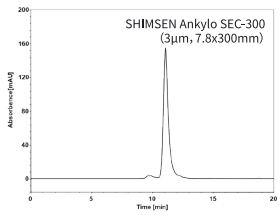
Bevacizumab



Trispecific Antibody

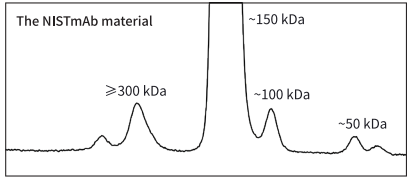


Bispecific Antibody



Recombinant Proteins

SHIMSEN Ankylo SEC columns are unique that specifically designed and QC tested with NIST-based, mAb Size Variant Standard to help ensure batch-to-batch consistency, compared to traditional SEC columns.



High resolution SEC separations of 50KDa difference in MW!

TIPS.

Attention

Care and Use Manual of your SHIMSEN Ankylo SEC Columns:

For overnight storage, SEC columns are recommended to be stored in 150 mM sodium phosphate buffer (pH 7.0). Store the column in the shipping solvent when the column will not be used within 48 hours.

Cleaning

Washing the Column:

If hydrophobic proteins or hydrophobic substances are adsorbedor retained, use an eluent with a high salt concentration (about 0.5M) for rinse.

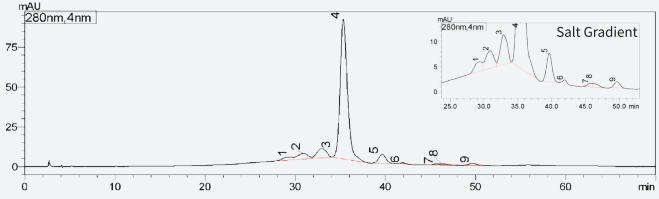
Recommend

Recommended Cleaning Solvents/Procedures:

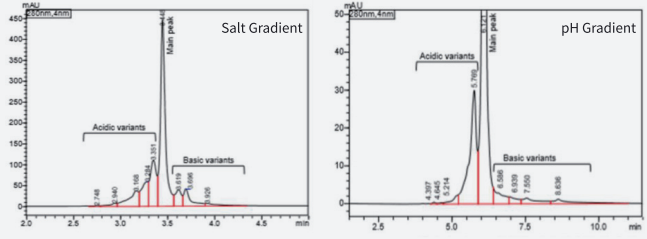
- 0.5 M sodium sulfate solution (pH 3.0): suitable for cleaning after adsorption of alkaline protein.
- 10%-20% organic solvents (ethanol, isopropanol, acetonitrile): cleaning after hydrophobic protein adsorption; before using organic solvents, please use pure water to clean the buffer salts completely.
- 4~6 M urea (pH 7.0): suitable for the cleaning of easily aggregated protein samples.
- *At this time, take care of usable pH.

Charge Variant Analysis

Charge variant levels in therapeutic proteins, including monoclonal antibodies (mAbs), are often considered to be critical quality attributes (CQAs), since they can potentially affect biological activity and safety of the biotherapeutics. One of the commonly used techniques for the purification, characterization, and routine monitoring of protein charge variants is ion-exchange chromatography (IEX). SHIMSEN Ankylo IEX and Shim-pack Bio column deliver reproducible, high-resolution, charge-based separations of mAbs and other proteins in LC and LC-MS applications using robust salt or pH gradient methods.



SHIMSEN Ankylo WCX for the Charge Variant Analysis of Pertuzumab



Shim-pack Bio IEX SP-NP for the Charge Variant Analysis of Bevacizumab

Table 2. SHIMADZU Ion Exchange Column

Product Description		Product Number
SHIMSEN Ankylo WCX	5µm, 4.6x250mm	380-01215-16
	5µm, 4.6x150mm	380-01215-15
	5µm, 4.6x50mm	380-01215-14
Shim-pack Bio IEX SP-NP	3µm, 4.6x50mm	227-31005-02
	3µm, 4.6x100mm	227-31005-03

TIPS.

Attention

IEX Care and Use Manual:

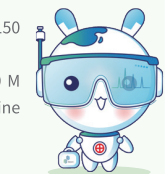
- Alcoholic reagents (e.g., methanol and ethanol) should be avoided from contacting the packing material when using IEC columns.

Cleaning & Recommend

IEX Column Cleaning:

Several different cleaning solutions may be injected to strip strongly adsorbed material or particulates from the column. Make the largest injection possible with the system configuration. With such strong cleaning solutions, it is best to disconnect the detector from the column and to direct the flow to waste.

- Anion exchange column: 75% acetonitrile solution (pH 2) containing 150 mM potassium nitrate; 0.1% TFA; 1 M HCl; 10 mM EDTA-2Na; 6 M urea;
- Cation exchange column: 50 mM phosphate buffer (pH 10) with 1.0 M NaCl; NaOH solution below 0.01 M; 10 mM EDTA-2Na; 7 M guanidine hydrochloride.

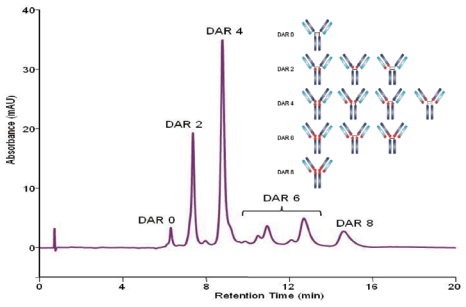


Drug-to-Antibody Ratio (DAR) Analysis

Antibody-drug conjugates (ADCs) are comprised of a cytotoxic drug attached at several locations to a monoclonal antibody (mAb) via a chemical linker. The DAR and the drug load distribution are critical quality attributes as they can change over time due to biotransformation and/or different clearance rates and should be assessed comprehensively in vivo. Analytical methods for determining DAR values include ultraviolet spectrophotometry (UV), hydrophobic interaction chromatography (HIC), liquid-mass spectrometry (LC-MS), and reversed-phase high-performance liquid chromatography (RP). Hydrophobic interaction chromatography (HIC) is the most commonly used analytical method, which can not only determine the average DAR, but also obtain the distribution of antibodies linking different small molecules of toxic substances.

Table 3. SHIMADZU HIC Column

Description		Product Number	Characteristics
SHIMSEN Ankylo HIC Butyl	5μm, 4.6x250mm	380-01215-16	• Effective for separation of proteins and antibodies such as antibody-drug conjugates. • High resolution comparable to sub-3 μm with low operating pressure. • Fast analysis at high flow rates. • Usable for laboratory-scale purification.
	5μm, 4.6x100mm	380-01215-20	
	5μm, 4.6x50mm	380-01215-21	



SHIMSEN Ankylo HIC Butyl for ADC DAR Analysis

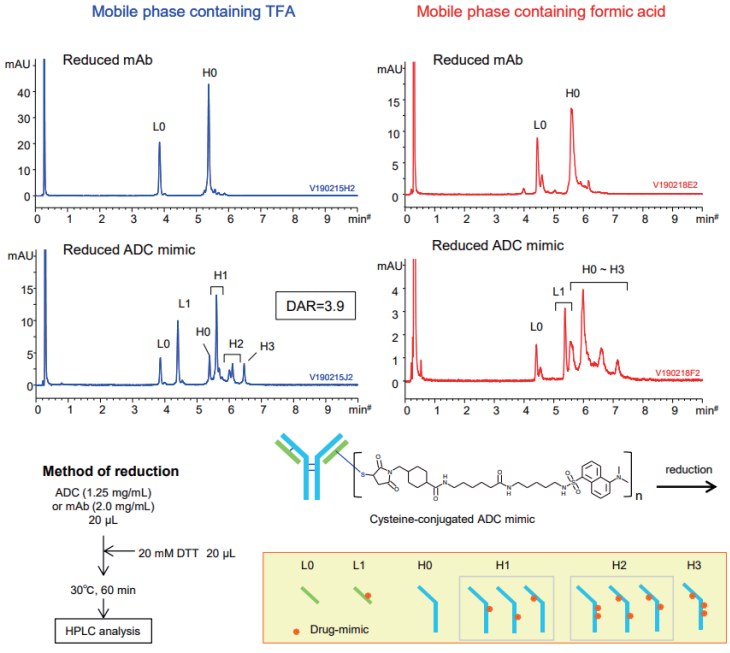
TIPS: Attention

Care and Use Manual:

- Be sure to use a filter after the injector with 0.5 μm pores to avoid plugging of the one micron pore size column frit. We also recommend a pre-injector membrane filter to prevent particles from pump seal wear to reach the column, Also, use high quality reagents, waters, and solvents for preparing buffers.
- Cleaning is not effective when the column is damaged by irreversible sample adsorption, channeling, or packing material exposure to excessive heat or shock.

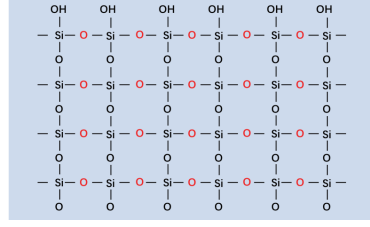
Drug-to-Antibody Ratio (DAR) Analysis

LC/MS is a popular analytical method for measuring the DAR and the drug load distribution of ADCs. It is an essential method used in the identifi cation of various drug-loaded ADCs species. In most cases, the intact ADC can be analyzed directly using LC/MS to determine the DAR value. The ADCs may be reduced before LC/MS analysis when specific DAR information on the light and heavy chains is needed.

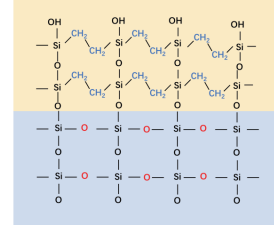


Shim-pack Scepter C4-300

common silica



organic/inorganic hybrid silica



Eluent <TFA>	: A) water/TFA (100/0.1)
	B) acetonitrile/TFA (100/0.1)
	27-42%B (0-10 min), 90%B (10-12.5 min)
Eluent <Formic acid>	: A) water/formic acid (100/0.1)
	B) acetonitrile/formic acid (100/0.1)
	22-37%B (0-10 min), 90%B (10-12.5 min)
Flow rate	: 0.4 mL/min
Temperature	: 80°C
Detection	: UV at 280 nm
Injection	: 2 µL for reduced mAb, 4 µL for reduced ADC-mimic

Peptide Mapping

Peptide mapping involves selectively cleaving the individual target antibodies using an appropriate enzyme or chemical and analyzing the peptide fragments obtained using HPLC or another suitable method. The Guidelines require identification of the peptide fragments separated by LC across as wide a range as possible, using amino acid composition analysis, N-terminal amino acid sequence analysis, or mass spectrometry.

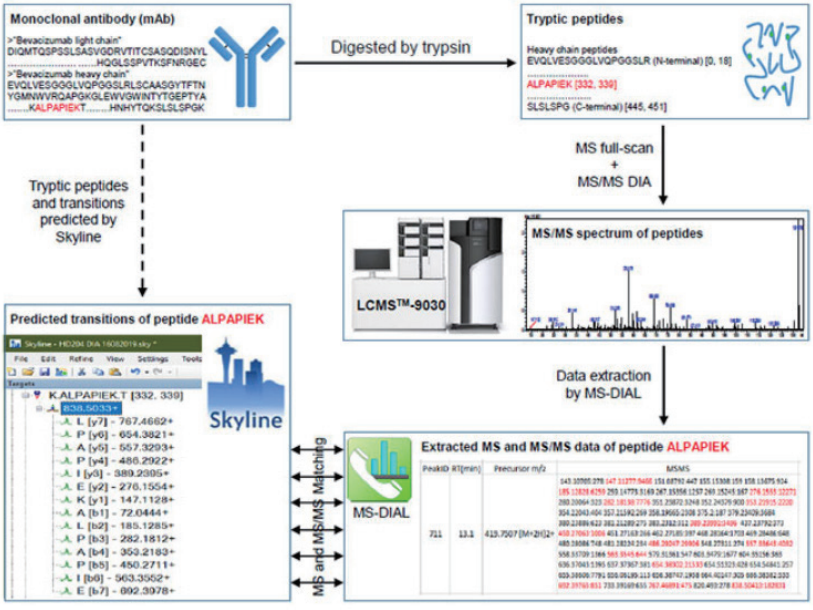


Figure 1. The *de novo* peptide sequencing approach on *LCMS-9030 (Q-TOF) for characterization* of mAb. The peptide ALPAPIEK was used as an example for illustration.

With Shim-pack GISS C18 (3um, 150*3.0 mm), focusing on peptide mapping and MS/MS sequencing of C-terminal and cysteine containing peptides of a bevacizumab biosimilar on LCMS-9030, a Q-TOF system.

Description	Product Number
380-03837	**Shim-pack GISS C18 (3μm, 150*3.0 mm)**

TIPS.

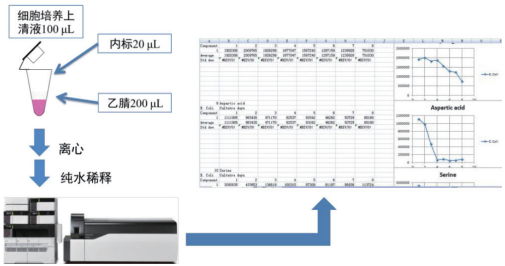
Why?

Why Shim-pack GISS C18 is the best choice for peptide mapping?

This is related to the design of the column parameters of the Shim-pack GISS C18. Most scientists applied columns with a pore size of 100 or 300 for protein identification and PTM analysis., while the Shim-pack GISS C18 is a column with a pore size of 200 to provide unique selectivities compared to 100 or 300 pore size column. In addition, the GISS C18 increases the retention of polar compounds and accelerates the retention time of less polar compounds, thus providing more information about proteins for peptide map.
This provides more protein information for peptide mapping analysis.

Cell Culture Profiling

LC/MS/MS Method Package for Cell Culture Profiling adds metabolites characteristic of CHO cells, which are frequently used in the manufacture of monoclonal antibodies. Together with existing analytes, including amino acids, vitamins, basal medium compounds, and other metabolites, this method package enables simultaneous analysis of 144 compounds in less than 20 minutes without complex method development.

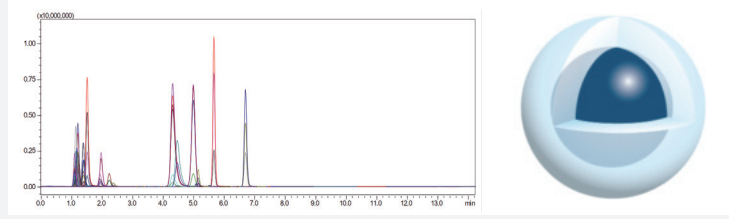


Description	Product Number
R227-32009-03	**Shim-pack Velox C18 (2.7μm, 2.1*100mm)**
R227-32019-03	**Shim-pack Velox PFPP (2.7μm, 2.1*100mm)**

The compounds are categorized into sugars, amino acids, vitamins, nucleosides, and other antibiotics, organic acids, growth factors, etc. Different groups of standards can be selected according to needs.

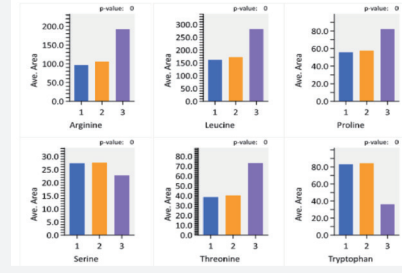
Description	Product Number
380-03837	cell culture profiling standards set (126 compounds)
380-03837-IS	Internal standard (100ppm)
380-03837-M1	38 Amino acids (100ppm)
380-03837-M2	11 nucleic acids (100ppm)
380-03837-M3	12 nucleic acids (100ppm)
380-03837-M4	7 Vitamins (100ppm)
380-03837-M6	16 organic acids

A surface porous silica gel column was used for analyzing components of cell culture, providing higher sensitivity.



Monitoring the change in culture supernatant components with culture time

The results show that the peak area ratios of each corresponding amino acid (as shown on the left), vitamin, and other compounds (not shown) in 1# and 2# media are not very different, indicating that these two media are similar in composition and proportions, whereas the 3# media samples are more different from 1# and 2#.



Glycosylation Analysis

The structure of monoclonal antibodies mainly consists of two functional regions, Fab and Fc, and there are glycosylation modifications at specific sites in the Fab and Fc segments. Antibody glycosylation testing is very important in product quality control. Glycosylation analysis mainly includes determination of sialic acid content, analysis of monosaccharide composition, determination of glycosylation sites, and determination of sugar chain structure.

Glycosylation Analysis Column Selection Guide

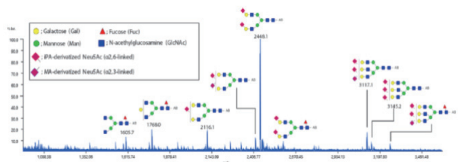
Attribute	Description	Product Number	Characteristics
sialic acid analyses	**Shim-pack Scepter PFPP (1.9μm, 2.1x150mm)**	227-31053-06	UHPLC analysis, derivatization method using a fluorescence detector
sialic acid analyses	**Shim-pack GIST Amide (5μm, 2.1x100mm)**	227-30824-04	LCMSMS analysis, negative Ion Mode
Monosaccharide Analyses	**Shim-pack Scepter C18 (1.9μm, 2.1x100mm)**	227-31012-05	LCMSMS analysis

STEP 1

The glycan modifications of the antibody were analyzed from the intact molecular weight level using Shimadzu LCMS-9030 with Q-TOF mass spectrometer.

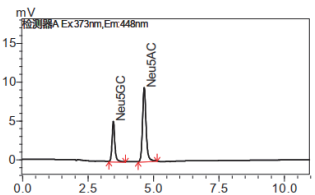
STEP 2

Usually, antibodies are cleaved using proteases to produce small peptides with molecular weights of about 0.5 to 5 kDa, which are separated by chromatography or electrophoresis and then analyzed by MALDI-MS or ESI-MS. Shimadzu MALDI-TOF and MALDI-Digital Ion Trap Mass Spectrometry can analyze related glycopeptide composition analysis.



STEP 3

The third step can be analyzed at the level of free oligosaccharides, for the analysis of free oligosaccharides there are usually three methods: (first) hydrophilic interaction chromatography, (second) capillary electrophoresis, (third) high-performance anion chromatography, for the analysis of sialic acid, Shim-pack Scepter PFPP was established for the determination of sialic acid Neu5Ac and Neu5Gc in the antibody.



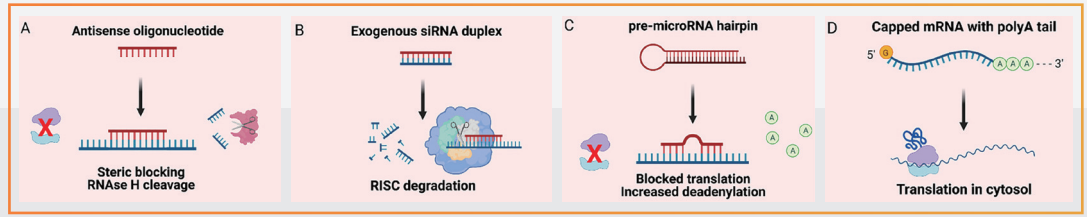
Oligonucleotide Analysis

Oligonucleotide therapeutics are nucleic acid polymers generally comprised of a few to several dozen bases (including modied bases) linked together. They are produced by chemical synthesis and act directly on organisms without being translated into proteins.

Workflow of Oligonucleotide Therapeutics

Target selection
Modification

Oligomer synthesis
Excision
Unprotected



Purification

Chromatography related:

Separation of impurities

→ Oligonucleotide therapeutics are mainly manufactured by chemicals synthesis. Separation and purification from impurities such as defect and residual protecting group formed in the synthesis process are large problems.

Characteristic analysis
Quality Control

Sequence Confirmation

The principle of quality control is “ efficacy and safety, ” and the sequence of oligonucleotide is an important factor because it is involved in the recognition of target molecules.

Confirmation of molecular weight, quantification, and concentration

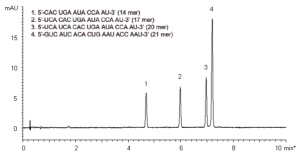
→ Confirmation of synthesized oligonucleotide product identity are examination of impurities by LC-MS precision mass spectrometry are important quality control tests.

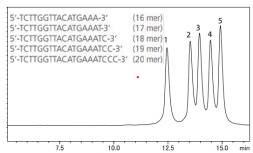
Oligonucleotide Analysis

Column selection guide

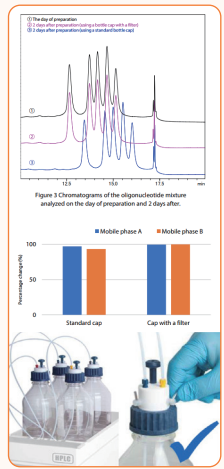
Attribute	Description	Product Number	Characteristics
CQA of SM	**Shim-pack Scepter C18 (5µm, 4.6x250mm)**	227-31020-06	Excellent peak shapes with thousands of injections
AX+HPLC	**Shim-pack Bio IEX Q-NP (5µm, 4.6x100mm)**	227-31003-03	Non-porous silica gel
RP + ion pairing	**Shim-pack Scepter C18 (3µm, 4.6x150mm)**	227-31016-05	Good separation of 60 mers length oligo
RP + ion pairing	**Shim-pack Scepter HD-C18 (1.9µm, 2.1x100mm)**	227-31026-05	Excellent separation of 20-30 mers length oligo
RP + ion pairing	**Shim-pack Scepter C4-300 (5µm, 4.6x250mm)**	227-31183-06	300 pore size for mRNA analysis
Measurements of MW	**Shim-pack Scepter C18 (1.9µm, 2.0x75mm)**	227-31011-04	High chemical stability under harsh conditions
Purity	**Shim-pack Scepter C18 [metal free] (3µm, 2.1x50mm)**	227-31073-01	Reduce NSA, minimize variability of results

Figure 1: Oligonucleotide analysis with Shim-pack Scepter C18.
Figure 2: Oligonucleotide analysis with Shim-pack Scepter C18.





TIPS.

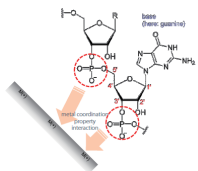


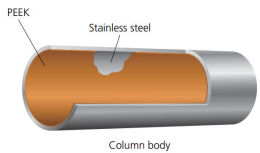
Experiments have proven that the stability of the mobile phase is significant working on oligonucleotide samples, and the left diagram compares the effect of mobile phase bottles with and without safety caps on retention time stability.

P/N	Description
107019	Safety cap
307925	Safety waster cap
610534	Exhaust Filter 1
310535	Exhaust Filter 2

Oligonucleotide Analysis

Oligonucleotide analysis comes with many unique challenges related to non-specific adsorption (NSA) as oligos may react with metal surfaces in stainless steel columns. Oligonucleotide columns with metal free technology reduce these interactions. In addition, low adsorption vial and systems should be applied.





Shim-pack Scepter [metal free]

Low adsorption vial/plate

P/N: 380-00510
300 µL

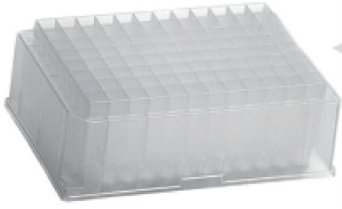
P/N: 380-00511
1.5 mL

P/N: 380-00888-50
low adsorption plate

Bioanalytical Assay of Oligonucleotides

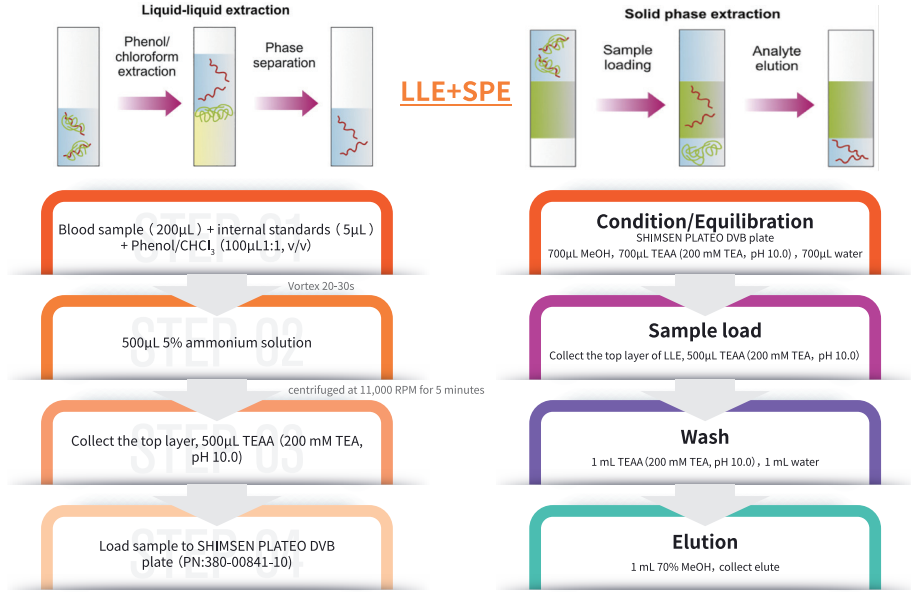
Developing robust, sensitive, and selective sample preparation and LC-MS methods for ONTs remains quite challenging due to their size, physiochemical diversity, poly-anionic nature, and known issues with protein and non-specific binding (NSB). PPT is simple and cheap but with matrix effect and low recovery. Liquid Liquid Extraction (LLE) , Solid Phase Extraction (SPE) and LLE combined SPE are the most widely used techniques for the extraction of ONTs from biofluids for LC-MS based quantification. Those methods can be easily automated to eliminate sample backlogs and provide high recovery.

Bioanalytical assay workflow of oligonucleotides



SHIMSEN Plateo

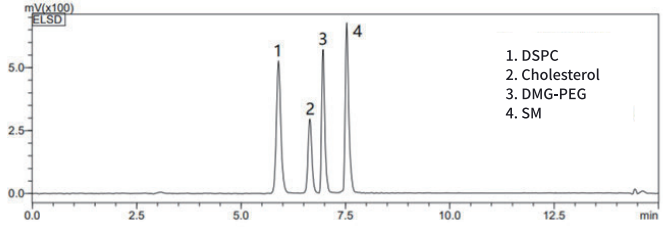
P/N	Description	分类
380-00841-10	**SHIMSEN Plateo DVB micro-SPE plate, 10mg-700μL/well**	SPE
380-00845-01	**SHIMSEN Plateo WAX SPE plate, 10mg-1mL/well**	



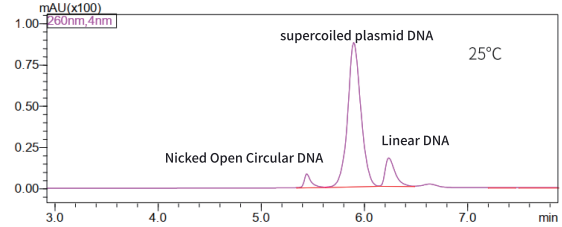
Characterization of mRNA

The first in-class therapeutic messenger RNA (mRNA) vaccines were successfully developed against SARS-CoV-2 virus. Therapeutic mRNA molecules can also be used for protein replacement therapy or vaccination approaches in cancer treatment. Since the application of mRNA technology is relatively new, regulatory guidelines and industry standards to guide non-proprietary aspects of mRNA quality during development and manufacturing are still evolving.

Attribute	Description	Product Number	Characteristics
3’ poly(A) tail length	**Shim-pack Scepter C18-300 (2.1*150mm, 1.9μm)**	227-31203-06	100mM OAA/1% HFIP/Acetonitrile 60°C/Gradient elution
5’ capping efficiency	**Shim-pack Scepter C18[metal free]**	/	Minimizes metal-mediated adsorption
Analysis of LNP Components	**SHIMSEN WP C4-300 (4.6*150mm, 5μm)**	380-01235-74	HPLC analysis, ELSD detector, 8 mins analysis time
Plasmid Analysis	**SHIMSEN Ankylo SAX-PM (4.6*150mm, 5μm)**	380-01215-64	PEEK-lined stainless steel body

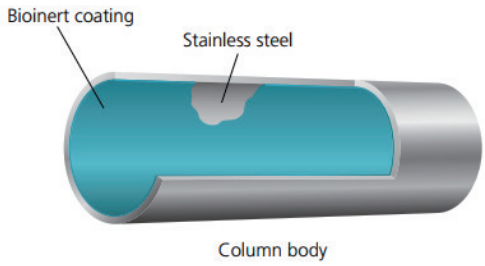


Column: SHIMSEN WP C4-300 (4.6*150mm, 5μm)
Mobile phases: A-10 mmol aqueous triethylamine solution
(The pH is adjusted to pH 7.0 by addition of acetic acid)
B-10 mmol triethylamine methanol solution
(The pH is adjusted to pH 7.0 by addition of acetic acid)
Injection volume: 10 μL
Flow rate: 1.0 mL/min
Column temperature: 55°C
Detector: ELSD-LT III



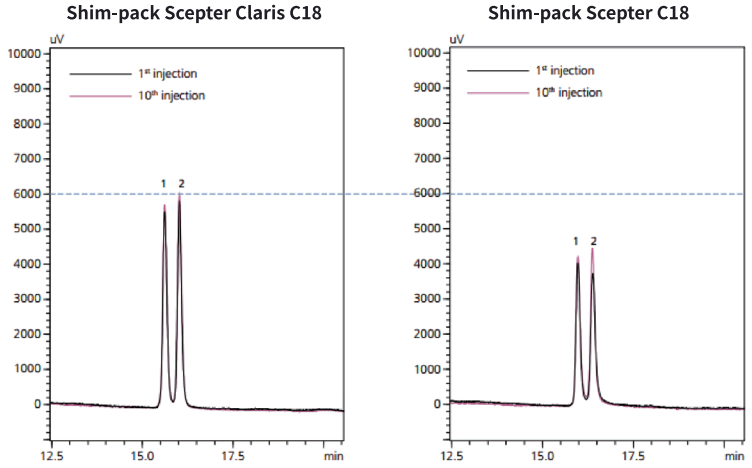
Column: SHIMSEN Ankylo SAX-PM (4.6*150mm, 5μm)
Mobile phases: A-20 mmol Tris-HCl (pH = 8.5)
B-1 M NaCl in 20 Mm Tris-HCl (pH = 8.5)
Gradient elution
Injection volume: 10 μL
Flow rate: 1.0 mL/min
Column temperature: 25°C
Detector: ELSD-LT III

Shim-pack Scepter Claris



Shim-pack Scepter Claris features a column body with a newly-developed bioinert coating packed with Scepter series stationary phases.

- Bioinert coating is applied to the column body and stainless steel frit
- Ideal for analysis of metal-coordinating and hydrophobically adsorbing compounds such as nucleic acids, proteins, and lipids
- Outstanding pH and lifetime stability due to Scepter organic silica hybrid packing



1. Synthetic oligonucleotide 20 mer (10 mg/L)
2. Synthetic oligonucleotide 21 mer (10 mg/L)

Description		Prodcut Number
Shim-pack Scepter Claris C18-120	1.9μm, 2.1*100mm	227-31210-02
Shim-pack Scepter Claris HD C18-80	1.9μm, 2.1*100mm	227-31211-02
Shim-pack Scepter Claris C18-300	1.9μm, 2.1*100mm	227-31209-02
Shim-pack Scepter Claris C4-300	1.9μm, 2.1*100mm	227-31208-02

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