C190-E275B

SHIMADZU
Excellence in Science

Ultra High Performance Liquid Chromatograph

# Nexera XS inert


SCL-40 SYSTEM CONTROLLER
CONTROL
ERROR
DGU-405 DEGASSING UNIT
SPD-M40 PHOTO DIODE ARRAY DETECTOR
0.3000ml MAX 105.0
79.4MPa MIN 0.0
PUMP CONC RUN PURGE
CE
del
Enter
LC-40D XSi SOLVENT DELIVERY MODULE
SET 25 ACTUAL 25.0 T.MAX 90
OVEN READY RUN
CTO-40S COLUMN OVEN
SHIMADZU

# EXPERIENCE NEWFOUND CLARITY

For many biopharmaceuticals and medium-molecule drugs, it can be difficult to acquire reliable data using a typical HPLC system, due to adsorption caused by interactions with metal ions. That tendency is particularly true for molecules that contain phosphates, carbonyl groups, or other characteristic elements or functional groups, the adsorption of which can affect analysis in various ways, such as by causing poor peak shape or inadequate sensitivity. Therefore, to increase the sensitivity and accuracy of analysis, it is important to inhibit the adsorption of compounds to the system.
Nexera XS inert UHPLC systems achieve a 105 MPa pressure capacity while inhibiting adsorption to metals by using PEEK or ceramic materials in flow channels where samples flow. They also offer excellent resistance to corrosion and a broad range of pH levels, meaning they can be operated reliably using mobile phases that contain high haloid salt concentrations or have extreme pH levels.

## Unconstrained Recovery and Sensitivity

Reduces sample loss due to adsorption to metal and achieves excellent sensitivity.

## Clear Resolution without Restrictions

Improves peak shape and achieves excellent chromatographic separation.

## Assured Reliability and Reproducibility

Reliable data for metal-adsorbing compounds with high reproducibility.


## Addition of a New Bioinert Model to the Nexera Series

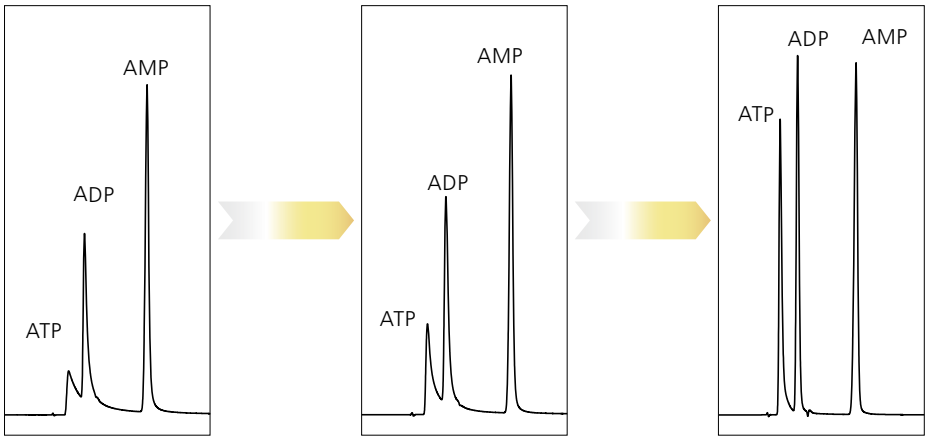
In addition to the standard Nexera models made with stainless steel for excellent pressure resistance, broad applicability, and robustness, a new bioinert model has been added to the Nexera series product line. The new Nexera XS inert model supports UHPLC analysis while also featuring PEEK and ceramic materials for flow channels exposed to sample flow. Similarly, the Nexera lite inert is an HPLC model with metal materials completely eliminated from flow channels.

| | Bioinert HPLC/UHPLC | HPLC/UHPLC (stainless steel-based) |
|---|---|---|
| UHPLC*1 |  **Nexera** XS inert |  **Nexera** X3<br>**Nexera** XS |
| UHPLC-like*2 | |  **Nexera** XR |
| HPLC*3 |  **Nexera** lite inert |  **Nexera** lite |

*1 ~130 MPa(X3), ~105 MP(XS, XS inert)
*2 ~70 MPa(XR)
*3 ~44 MPa(lite), ~20 MPa(lite inert, for aqueous mobile phase with inert kit)

## Improved Peak Shape and Separation

The Nexera XS inert uses unique proprietary technology that minimizes exposure to metal ions on internal surfaces. That inhibits adsorption of target molecules within sample flow channels, ensures good peak shape, and provides excellent separation.



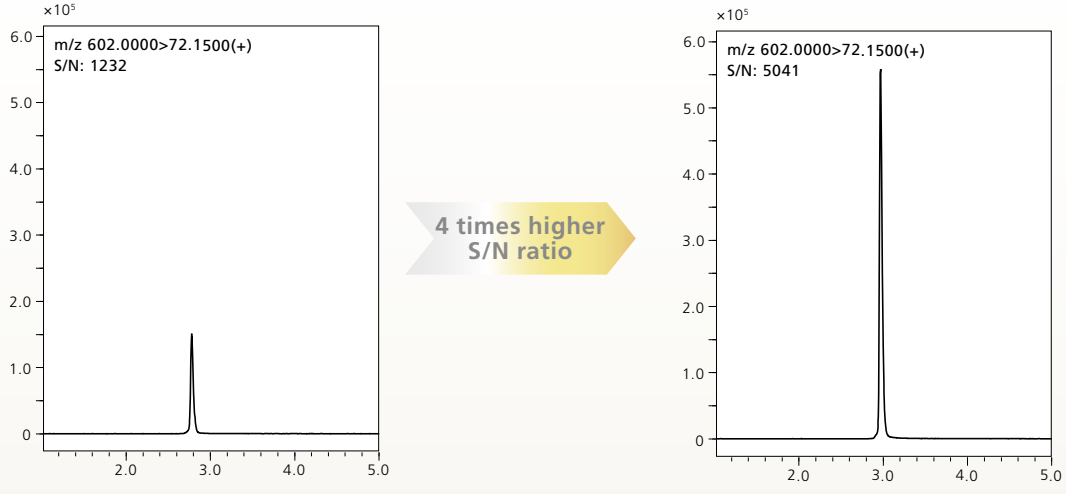

Standard UHPLC
(stainless steel-based)
+
Column with stainless steel body

Column body changed to metal-free

UHPLC system changed to
Nexera XS inert

## Adsorption Inhibited and Detection Sensitivity Improved

The Nexera XS inert prevents target component recovery rates from decreasing by inhibiting adsorption caused by interactions between metal ions and proteins, nucleic acids, or other components. As a result, the Nexera XS inert system can detect phosphorylated peptides and other molecules with a strong tendency to adsorb to metals.




Standard UHPLC
(stainless steel-based)

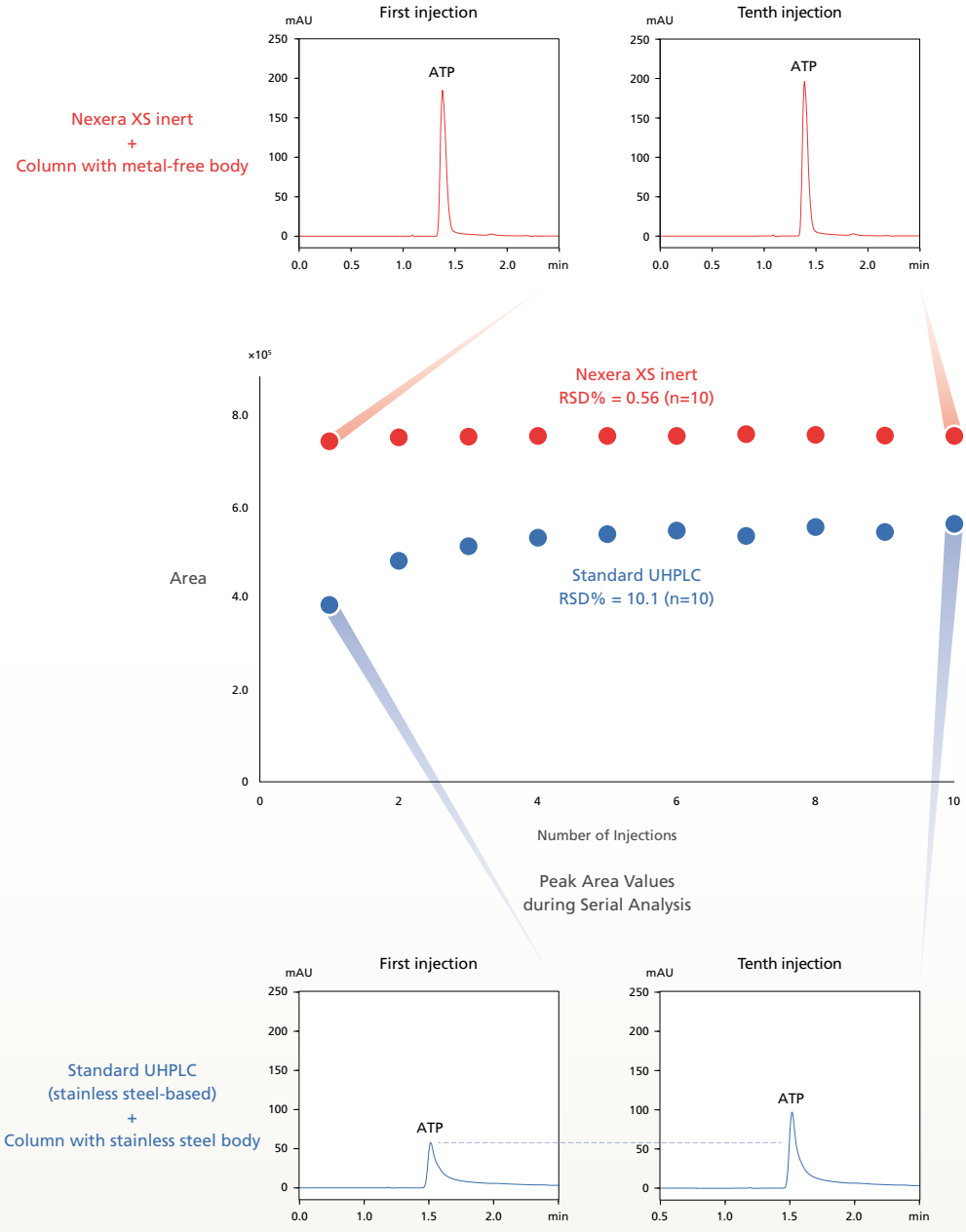
Nexera XS inert

Example of Phosphopeptide Analysis

# Assured Reliability and Reproducibility

## Consistent Analysis Reproducibility

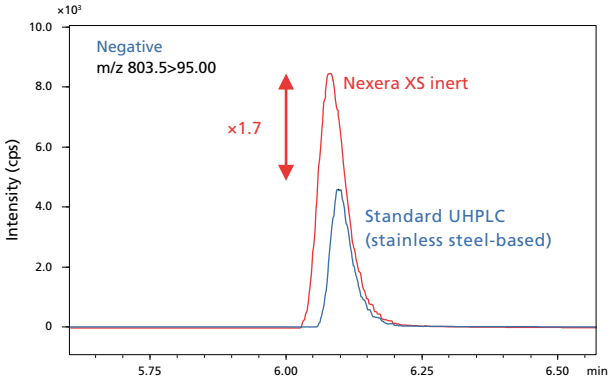
In order to reduce the effect of adsoprtion of metal-sensitive compounds, a system is often passivated by repeatedly injecting samples that contain the target compounds before starting data acquisition. However, this approach not only wastes valuable samples and time, but can make it extremely difficult to acquire reliable data due to changes in the state of passivation during continuous analysis. Nexera XS inert systems eliminate the need for the preliminary passivation and provide highly reliable data from the first injection and throughout the analytical session.




Change in Peak Intensity during Serial Analysis

## Improved Quantitative Analysis Performance

Adsorption of target compounds tends to be especially apparent in low-concentration regions, which can worsen calibration curve linearity, as well as detection and quantitation limits. Adsorption can also cause carryover. The Nexera XS inert achieves a wider dynamic range and higher quantitative accuracy in low-concentration regions by minimizing adsorption from interactions with metals.

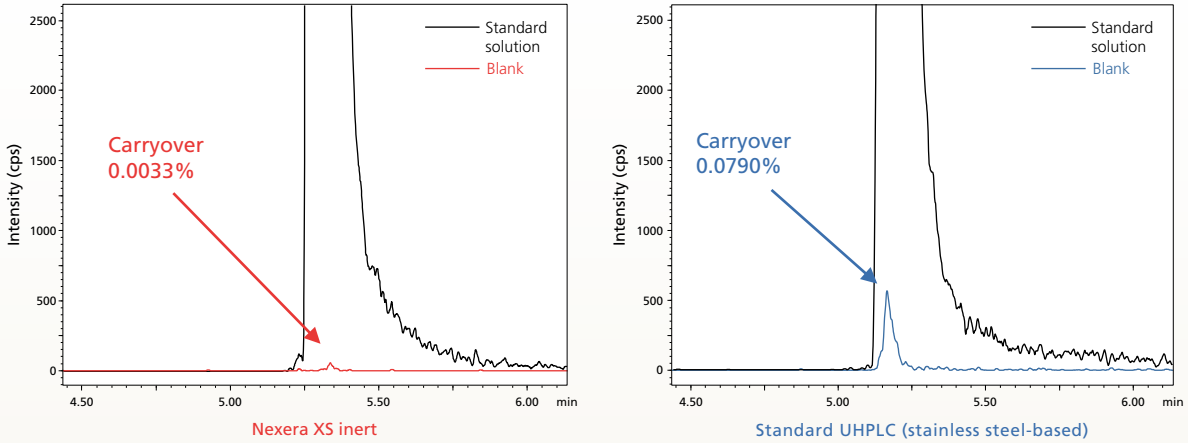



MRM Chromatogram of Oligonucleic Acid
Standard Sample (10 ng/mL)

Accuracy and Contribution Rate ($R^2$) at Each Concentration

| Spiked Conc. (ng/mL) | Nexera XS inert | | Standard UHPLC (stainless steel-based) | |
|---|---|---|---|---|
| | Measured Conc. (ng/mL) | Accuracy (%) | Measured Conc. (ng/mL) | Accuracy (%) |
| 0.5 | 0.57 | 113.5 | 2.28 | 455.7 |
| 1 | 0.93 | 93.0 | -1.04 | -104.4 |
| 5 | 5.42 | 108.0 | 2.43 | 48.5 |
| 10 | 9.13 | 91.3 | 5.62 | 56.2 |
| 50 | 50.28 | 100.6 | 26.63 | 53.3 |
| 100 | 92.77 | 92.8 | 76.39 | 76.4 |
| 500 | 497.04 | 99.4 | 588.74 | 117.7 |
| 1000 | 1010.36 | 101.0 | 965.46 | 96.5 |
| Contribution Rate ($R^2$) | 0.9996 | | 0.9721 | |

Carryover is a phenomenon where sample substances remaining inside the system or column after analysis, due to adsorption or other reasons, affect the next analysis. Therefore, carryover should be inhibited particularly for quantitative analysis of trace quantities, because of the large detrimental effects it can have on analytical results. Using a Nexera XS inert system can practically eliminate carryover by inhibiting adsorption to metal in the system.




Carryover test of oligonucleic acid

# Full-range of Bioinert UHPLC Capabilities

The Nexera XS inert system eliminates the risk of sample adsorption or surface corrosion, while still providing all the exceptional features of the Nexera series, making it the perfect solution for a wide variety of applications.

The Nexera XS inert system combines the inertness of the sample flow path with excellent resistance to corrosion. It delivers ideal chromatographic separation performance for a wide range of applications and excellent durability, even in the case of mobile phases with high salt concentrations or extreme pH values.

System Controller
**SCL-40, CBM-40/40lite**
Used to coordinate actions of the overall system, this controller offers intuitive operation that minimizes the stress involved in operating the system, from startup to shutdown.

Detector
**SPD-40/40V/M40**
Inert-type cells for UHPLC analysis eliminate the risk of adsorption on the detector. A low-diffusion cell, which has 5 mm of optical path length, can also be selected.

Solvent Delivery Unit
**LC-40D XSi**
Designed with corrosion-resistant non-stainless steel materials, it offers rugged, low-pulsation performance, advanced AI features and solvent blending capabilities. (optional).

Autosampler
**SIL-40C XSi**
This high-performance autosampler features non-metal materials for all surfaces that contact liquids. That inhibits metal-adsorption of biomolecules.

UHPLC Inert Switching Valve
**FCV-0206H2i/FCV-0607H2i**
Designed with adsorption-inhibiting materials for all wetted surfaces.

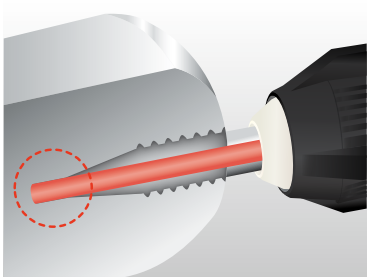
Online pH Monitor
**pHM-40 (optional)**

This monitors the pH level of mobile phases in real time and records the data in a data file together with chromatograms and instrument information.

## Inhibits Metal Adsorption and Achieves 105 MPa Pressure Capacity

To reduce the various chromatogram problems that metal adsorption can cause, the Nexera XS inert features sample flow channels made with PEEK material. Though it is generally difficult to use PEEK plastic for high-pressure conditions, the PEEK-lined tubing (developed independently by Shimadzu) used in the Nexera XS inert offers a maximum pressure capacity of 105 MPa*. Furthermore, a proprietary butt-sealed tubing configuration eliminates the need for ferrules and results in tubing joints with no dead volume. In addition, a proprietary ratcheted fitting design allows column connections to be hand-tightened even at high pressures.

* Also supports mobile phases spiked with HFIP (max. 5 %).



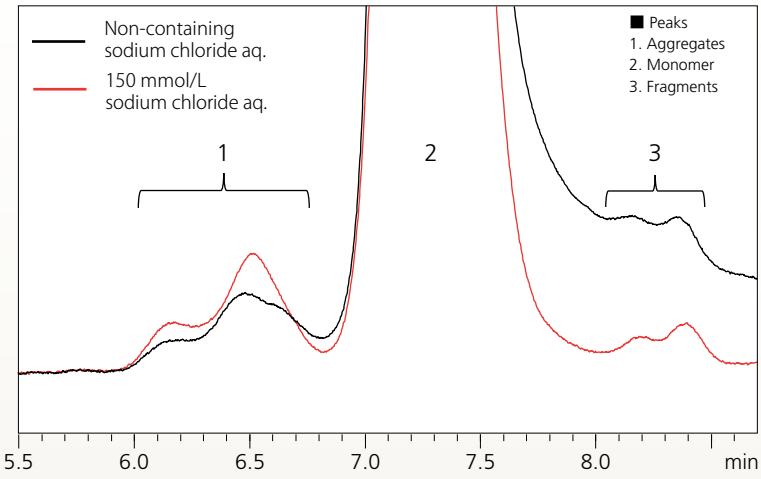
Zero dead volume with
butt-sealed tubing configuration

Columns connected easily with
ratcheted fittings

## High Corrosion Resistance and Broad pH Resistance

Nexera XS inert systems also offer superior corrosion resistance. To support mobile phases that contain high haloid salt concentrations for analyzing proteins, for example, corrosion-resistant metal is used for mobile phase contact surfaces upstream from the sample injection unit, such as in the solvent delivery pump and mixer, whereas PEEK plastic and ceramic materials are used for sample contact surfaces. In addition, the superior pH resistance of PEEK plastic ensures the system can be used to reliably acquire data for long periods even when using high-pH* mobile phases, such as for oligonucleic acid analysis.

* Supports pH levels from 1 to 14.




Example of Analyzing mAb Aggregates Using a Mobile Phase with a High Haloid Salt Concentration

Improves Efficiency of High-Sensitivity Analysis and Characterization of Oligonucleic Acid

## LabSolutions Insight™ Biologics

LabSolutions Insight Biologics is a dedicated software platform for oligonucleotide characterization using the LCMS-9030 or LCMS-9050 quadrupole time-of-flight type (Q-TOF) mass spectrometer. Main product and impurity identification includes several core editors for sequence, nucleotides, linkers, ribose and base modifications. Together with processing and integration for target modifications, Insight Biologics is a comprehensive workspace for data review, processing, and reporting. Because the Nexera XS inert system prevents the adsorption of target components to metal surfaces, combining it with LabSolutions Insight Biologics creates an ideal setup for oligonucleotide characterization.

### Complete workflow from data acquisition to analysis and reporting


Insight Biologics provides an easy method to input sequence information, configure target modifications, and set data analysis parameters. Using sequence information, Biologics comprehensively identifies chain length differences, nucleotide gaps, modifications, conversions, adducts, and other impurities. Q-TOF Data Dependent Acquisition (DDA) records MS and MS/MS spectra. MS spectra are used to identify the molecular weights of impurities, and corresponding MS/MS fragment spectra are used to confirm sequences. Visual displays of sequence coverage enhance the confidence of identifications. Seamless processing and reporting with audit trail support make Insight Biologics a comprehensive solution for oligonucleotide characterization.

# A Superior User Experience

UX 1

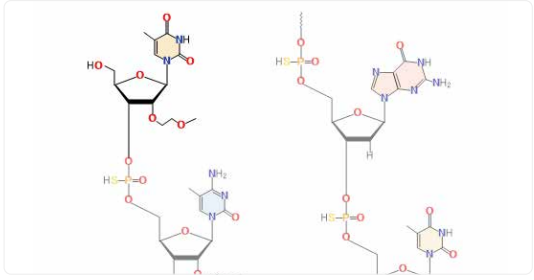
## Depiction of the Structural Formula

In the window for setting the oligonucleotide sequences, the structural formula of the sequence that was entered is displayed in real time, enabling quick and easy verification of the information. Also, the nucleobases used in the sequence, as well as the backbone linker, ribose, and base modifications, can be added and edited.

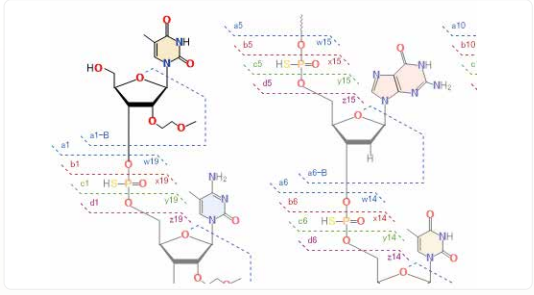
Input oligonucleotide sequences

| # | Name | Base | Base Modification | Linker | Ribose | Formula | Mono-isotopic |
|---|---|---|---|---|---|---|---|
| 1 | Cd | Cytosine | None | ---- | Deoxy | C9 H11 N3 O2 | 193.08513 |
| 2 | sTd | Thymine | None | Phosphorothioate | Deoxy | C10 H13 N2 O6 P S | 320.02319 |
| 3 | sGd | Guanine | None | Phosphorothioate | Deoxy | C10 H12 N5 O5 P S | 345.02968 |
| 4 | sCd | Cytosine | None | Phosphorothioate | Deoxy | C9 H12 N3 O5 P S | 305.02353 |
| 5 | sTd | Thymine | None | Phosphorothioate | Deoxy | C10 H13 N2 O6 P S | 320.02319 |
| 6 | sAd | Adenine | None | Phosphorothioate | Deoxy | C10 H12 N5 O4 P S | 329.03476 |
| 7 | sGd | Guanine | None | Phosphorothioate | Deoxy | C10 H12 N5 O5 P S | 345.02968 |

The structural formula of sequences is shown in real time



Base modifications can be added and edited

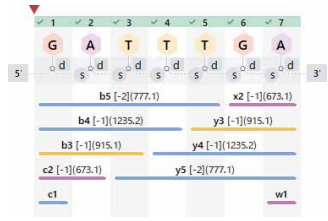


UX 2

## Display of Fragment Coverage

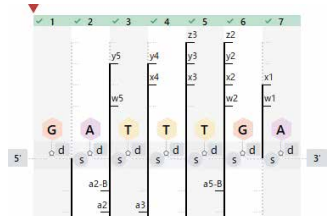
The software includes a coverage display which indicates fragment spectral assignments. The coverage display switches to match the items to be checked. Reports can also be output.

Checking ion intensity and coverage

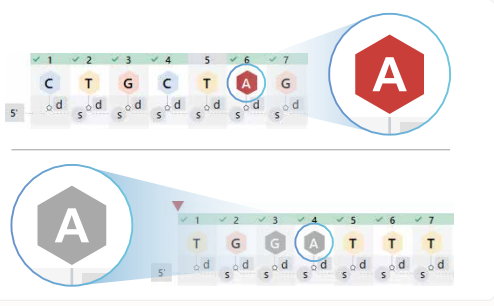



Switchable coverage display

Checking the fragment sequence



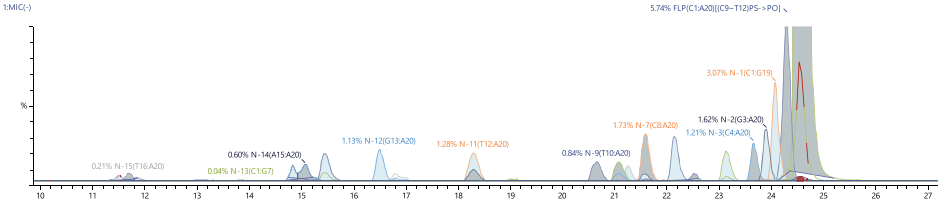

### Modification positions are also clearly identified




UX 3

## Component Chromatogram Display

The impurity peaks are displayed as a component chromatogram. The UV and MS chromatograms can be checked simultaneously.



Achieving Maximum Inhibition of Metal Adsorption in Combination with Nexera XS inert System

## Shim-pack Scepter Claris

The Shim-pack Scepter Claris is a new metal-free column with a newly developed bioinert-conditioned column body. It is packed with a Scepter series stationary phase that offers superior durability and chromatography performance.

- 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

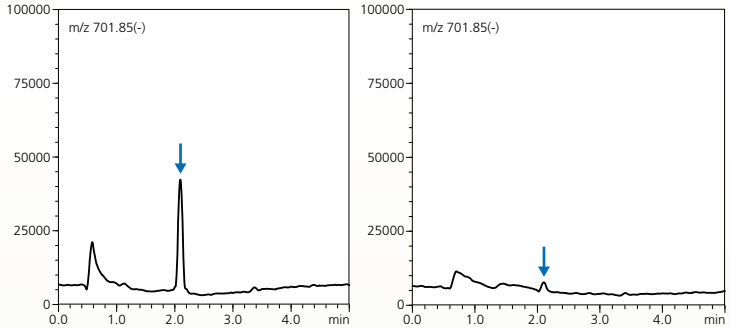
| Shim-pack Scepter | Reversed Phase | | | | |
|---|---|---|---|---|---|
| | C18-120 | C18-300 | HD-C18-80 | C8-120 | C4-300 |
| Ligand Type | Trifunctional C18<br>General Purpose | Trifunctional C18<br>General Purpose for Large Molecules | Trifunctional C18<br>High Density Type for Increased Retention | Trifunctional C8 | Trifunctional C4 |
| Particle | Organic Silica Hybrid | | | | |
| Particle Size | 1.9 µm, 3 µm, 5 µm | | | | |
| Pore Size | 12 nm (120Å) | 30 nm (300Å) | 8 nm (80Å) | 12 nm | 30 nm |
| End Capping | Proprietary | | | | |
| pH Range | 1 - 12 | | | | 1 - 10 |
| 100% Aqueous Condition | YES | YES | × | × | YES |
| USP Classification | L1 | L1 | L1 | L7 | L26 |

| Shim-pack Scepter | Reversed Phase | | HILIC |
|---|---|---|---|
| | Phenyl | PFPP | Diol-HILIC |
| Ligand Type | Trifunctional Phenylbutyl | Trifunctional Pentafluorophenylpropyl | Trifunctional Dihydroxypropyl |
| Particle | Organic Silica Hybrid | | |
| Particle Size | 1.9 µm, 3 µm, 5 µm | | |
| Pore Size | 12 nm (120Å) | | |
| End Capping | Proprietary | None | |
| pH Range | 1 - 10 | 1 - 8 | 2 - 10 |
| 100% Aqueous Condition | YES | YES | — |
| USP Classification | L11 | L43 | L20 |

## Superior Sensitivity and Separation Performance in Nucleic Acid Analysis

Shim-pack Scepter Claris C18-120 (bioinert coating) and Scepter C18-20 (stainless steel body) were compared in this analysis of Oligodeoxythymidylic acid.

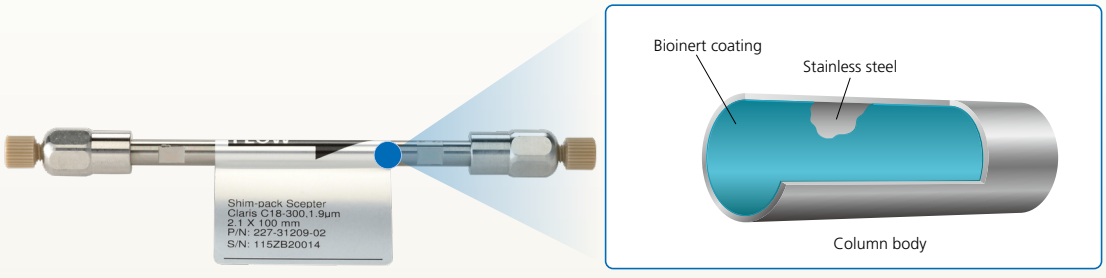
Results from Scepter C18-120 show low peak intensity, suggesting adsorption on metal surfaces. In contrast, Scepter Claris C18-120 produced a sharp, high-intensity peak.




Scepter Claris C18-120




Scepter C18-120



Ion Exchange Chromatography Columns for Protein and Nucleic Acid Analysis

## Shim-pack Bio IEX Columns

The Shim-pack Bio IEX columns are available in the following chemistries.

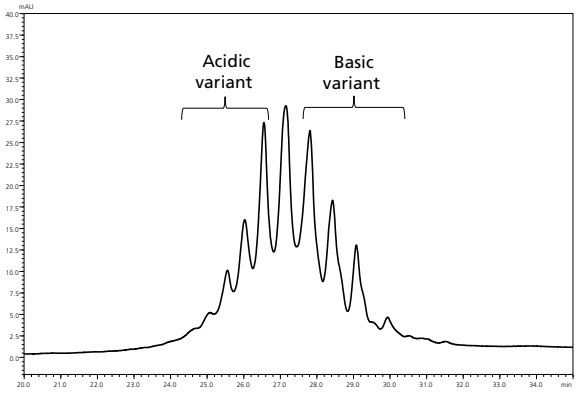
- Anion exchange quaternary ammonium (Q)
- Cation exchange sulfopropyl (SP)

The following porous and non-porous particles, both of which use hydrophilic polymer packing, are available.

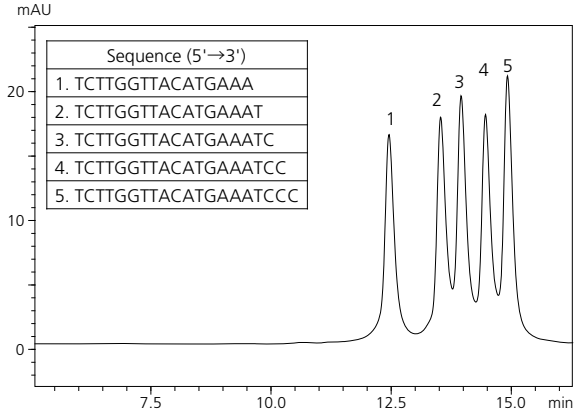
- Hydrophilic porous polymer (Q and SP columns)
- Hydrophilic non-porous polymer (Q-NP and SP-NP columns)

The porous packing materials offer highly efficient and excellent binding capacity, and the non-porous packing materials offer high recovery rates and excellent resolution.

| | Q-NP | SP-NP | Q | SP |
|---|---|---|---|---|
| Particle Material | Hydrophilic non-porous polymer | | Hydrophilic porous polymer | |
| Particle Size | 3 µm, 5 µm | | 5 µm | |
| Ion Exchange Group | - $CH_2N^+(CH_3)_3$ | - $(CH_2)_3SO_3^-$ | - $CH_2N^+(CH_3)_3$ | - $(CH_2)_3SO_3^-$ |
| Operating pH Range | 2 - 12 | | | |
| Operating Temp. Range | 4-60°C | | | |
| Column Material | PEEK | | | |




Charge Variant Analysis of Antibodies




Ion Exchange Chromatography of Oligonucleotides

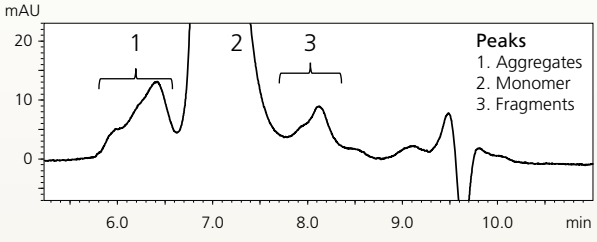
Application

Size Exclusion Chromatography Columns for Mid- and Large-Size Molecules

## Shim-pack Bio Diol Columns

Shim-pack Bio Diol columns are size exclusion chromatography columns with packing particles of various pore sizes for analyzing aggregates and fragments of antibodies, oligonucleotides, carbohydrates, and other analytes. The lineup covers a wide range of use, from traditional analysis to laboratory-scale purification. In addition, the 2 µm-particle columns for high-speed analysis enable rapid characterization.

| | Diol-60 | Diol-120 | Diol-200 | Diol-300 |
|---|---|---|---|---|
| Particle Material | Silica gel | | | |
| Functional Group | Dihydroxypropyl (Diol) | | | |
| Particle Size | 3 µm, 5 µm | | 2 µm, 3 µm, 5 µm | |
| Pore Size | 6 nm (60Å) | 12 nm (120Å) | 20 nm (200Å) | 30 nm (300Å) |
| Operating pH Range | 5.0 - 7.5 | | | |
| Molecular Weight Range | 10,000 or less | 1,000 - 100,000 | 5,000 - 300,000 | 20,000 - 1,000,000 |
| Maximum pressure | 2 µm : 45 MPa (Usually at 30 MPa or less) | | 3, 5 µm : 20 MPa | |




Analysis of Antibody and Antibody-Drug Conjugate Aggregates

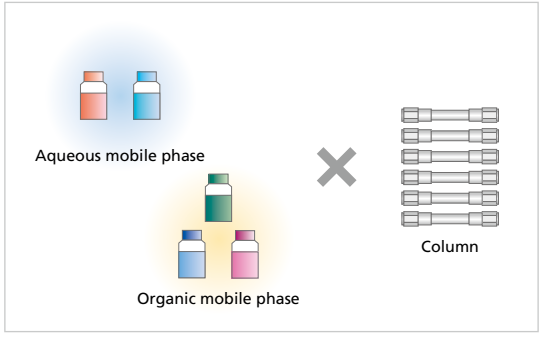
Application

Improving the Efficiency of Process Steps in Analytical Method Development

## LabSolutions™ MD

When evaluating manufacturing processes or testing the stability of pharmaceuticals, separating principal components and impurities in chromatograms is very important for characterization analysis and quality control, but establishing the appropriate separation parameters requires a significant amount of time and effort. LabSolutions MD enables efficient development of analysis methods using an Analytical Quality by Design (AQbD) approach based on scientific evidence and risk. All steps involved in the screening, optimization, and validation phases of the method development process can be completed using LabSolutions MD, from analyzing samples by automatically switching between respective mobile phases and columns to using the analytical results to build a design space, deciding the optimal analytical condition settings, and then evaluating robustness (validation).

### Screening Phase — Initial Screening of Analysis Methods

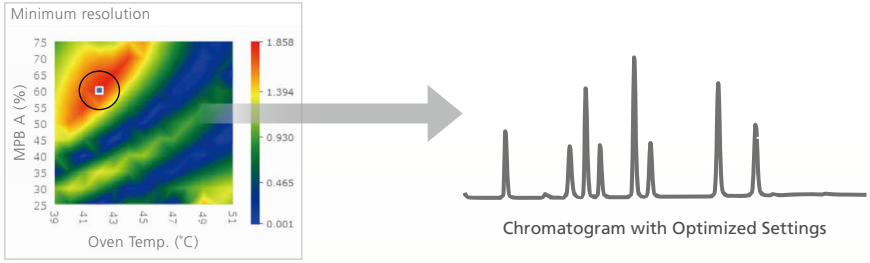



| | | | Response |
|---|---|---|---|
| Column Nick Name | MPA pH | MPB A (%) | Evaluation Value |
| Scepter-Phenyl-120 | 6.8 | 50 | 546.000 |
| Scepter-C8-120 | 6.8 | 0 | 469.894 |
| GIST-C18-AQ | 2.7 | 0 | 465.124 |
| GIST-C18-AQ | 6.8 | 50 | 443.580 |
| Scepter-C8-120 | 6.8 | 50 | 436.241 |
| Scepter-Phenyl-120 | 2.7 | 50 | 419.659 |
| Scepter-C18 | 2.7 | 0 | 419.338 |

Automatic switching between mobile phases and columns improves analysis efficiency. It automatically searches for optimal combinations from the massive amounts of data generated.

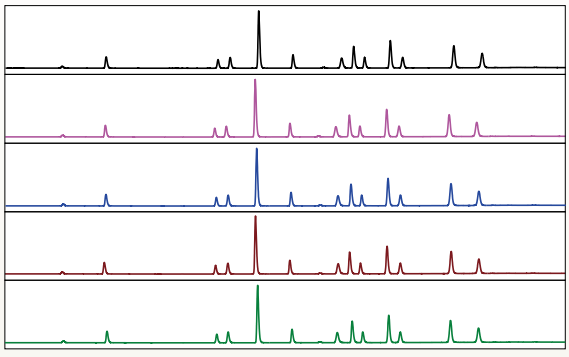
### Optimization Phase — Analysis Method Optimization

ANALYTICAL INTELLIGENCE




It automatically searches for robust chromatograms with good peak separation by using a design space to visualize separation.

### Validation Phase — Validation



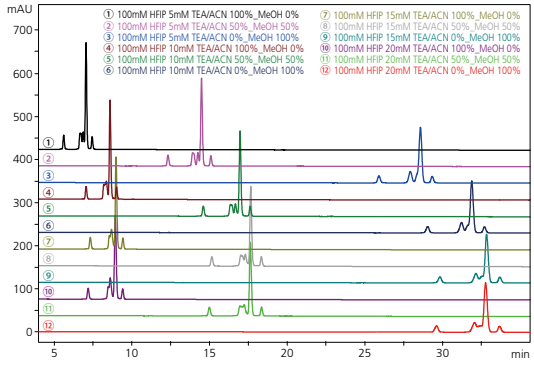
It automatically generates validation analysis schedule.

Brochure

Example 1:

## Nexera XS inert with LabSolutions MD Provides Optimized Separation Parameters

When developing methods for testing substances related to oligonucleic acids, separation patterns can differ depending on the chain length and base composition, the presence/absence of modifications, and other factors. Therefore, separation must be optimized for each target sequence. Good peak shape and separation can be achieved by using a Nexera XS inert system with a Shim-pack Scepter Claris column to inhibit oligonucleic acid adsorption to metals. Using LabSolutions MD to search for separation parameters enabled a comprehensive evaluation of a variety of LC parameter settings, such as the mobile phase, oven temperature, and gradient program.




Chromatogram Obtained by Screening Mobile Phases

| MPA Nick Name | MPB Nick Name | Evaluation Value |
|---|---|---|
| 100mM HFIP 10mM TEA | ACN 50%_MeOH 50% | 54.074 |
| 100mM HFIP 15mM TEA | ACN 50%_MeOH 50% | 53.77 |
| 100mM HFIP 5mM TEA | ACN 50%_MeOH 50% | 52.477 |
| 100mM HFIP 20mM TEA | ACN 50%_MeOH 50% | 51.919 |
| 200mM HFIP 20mM TEA | ACN 50%_MeOH 50% | 47.016 |
| 200mM HFIP 15mM TEA | ACN 50%_MeOH 50% | 46.926 |
| 200mM HFIP 10mM TEA | ACN 50%_MeOH 50% | 46.836 |
| 200mM HFIP 5mM TEA | ACN 50%_MeOH 50% | 45.719 |
| 100mM HFIP 10mM TEA | ACN 100%_MeOH 0% | 38.822 |
| 200mM HFIP 10mM TEA | ACN 100%_MeOH 0% | 37.732 |

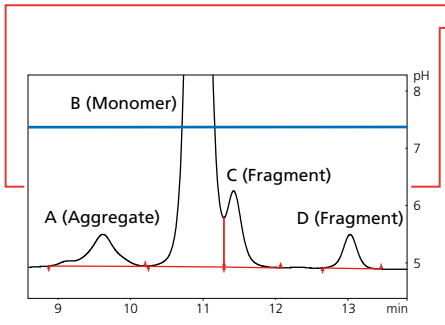
Parameter Settings Ranked by Evaluation Score
(Top 10 settings listed in descending order of evaluation scores)

Application

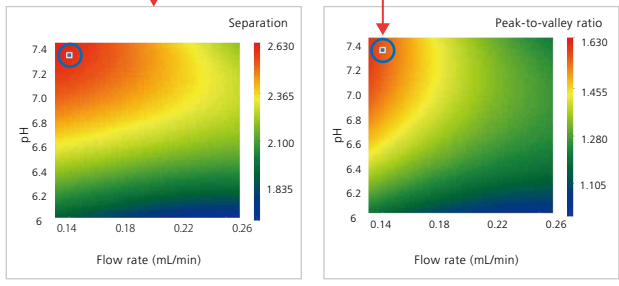
Example 2:

## Corrosion-Resistant System Assists Method Development for Analyzing Antibody Drugs

Antibody drugs made with monoclonal antibodies (mAb) can form dimers, multimers, or other aggregates depending on the manufacturing processes or storage conditions used. Therefore, it is important to monitor the aggregates by size exclusion chromatography during manufacturing processes. Using a corrosion-resistant Nexera XS inert system for that analysis enables reliable data acquisition even for mobile phases with high salt concentrations. The visualization of the design space with LabSolutions MD streamlines the process of identifying the analytical conditions, resulting in ideal peak separation of aggregates / monomers / fragments.




Chromatogram of mAb
(Blue line: pH value monitored using pHM-40 unit)




Design Space
(Left: Separation between A and B, Right: Peak-to-valley ratio of B and C)

Application

Shimadzu Corporation

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