C146-E350C

SHIMADZU
Excellence in Science

Microflow Liquid Chromatography Mass Spectrometry System

Nexera Mikros

Micro:
Above and Beyond Nano

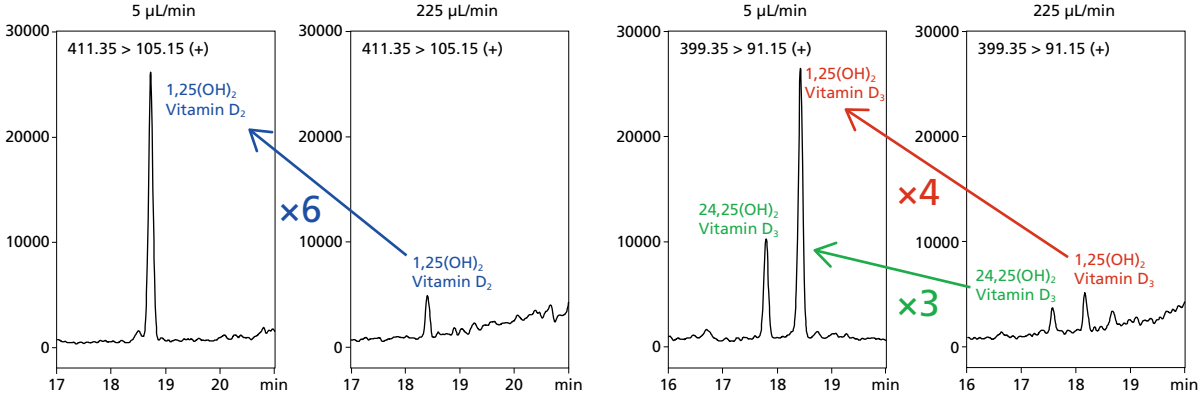
The **High Sensitivity** You Expect from a Low Flow System with the **Ruggedness of HPLC** Covering the **Complete Range** from Micro to Semi-Micro

Nexera Mikros™ Microflow LC/MS System

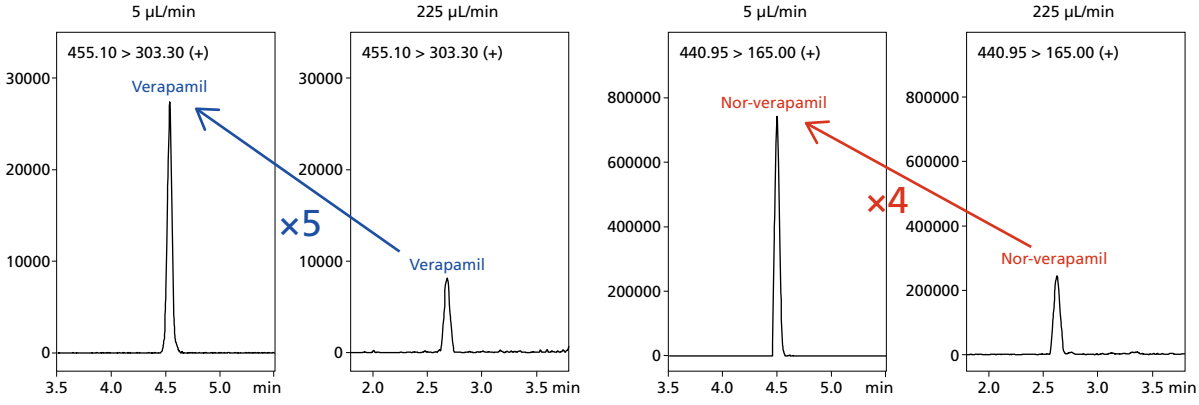
Improvement in the sensitivity of an LC/MS/MS method can generally be achieved by reducing the analytical scale of the analysis. However, reducing the analytical scale significantly to nano-flow reduces the throughput, stability, and robustness as a trade-off for greater sensitivity. Nexera Mikros, a Shimadzu microflow LC/MS system, offers a balanced solution to increased sensitivity with less compromise to throughput and robustness, using flow rates from 1 to 500 µL/min and column I.D. from 0.1 to 1 mm. It achieves stable and sensitive quantitative analysis through robust performance of microflow solvent delivery, optimized ion source design, the unique UF-Link column connection, and system variety that supports a wide range of applications.

High Bioanalytical Sensitivity

Sensitivity remains a challenge for many vitamin D metabolites, such as 1,25$(OH)_2$ di-hydroxyvitamin D and 24,25$(OH)_2$ di-hydroxyvitamin D, because they lack easily ionizable functional groups and are in low-abundance. As shown below, microflow LC/MS/MS analysis of standard solutions of the aforementioned vitamin D analytes resulted in a three- to five-fold improvement in the signal-to-noise ratio when compared to semi-microflow analysis.



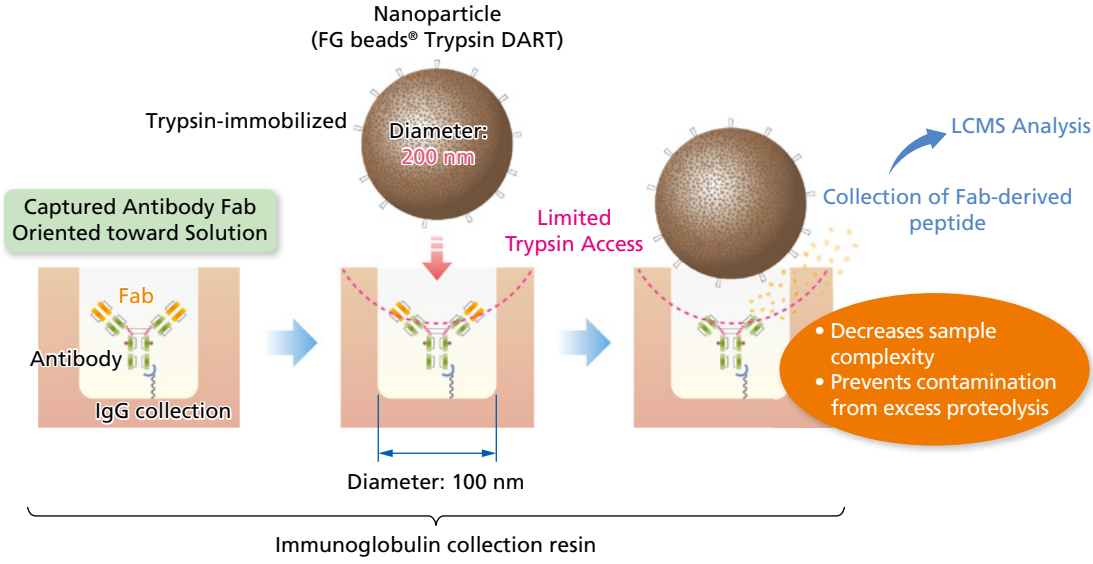
The increased sensitivity achievable by microflow LC/MS/MS, compared to semi-microflow, resulted in a five-fold increase in the signal-to-noise ratio for Verapamil and a four-fold increase in the signal-to-noise ratio of Nor-Verapamil.



Monoclonal Antibody Bioanalysis

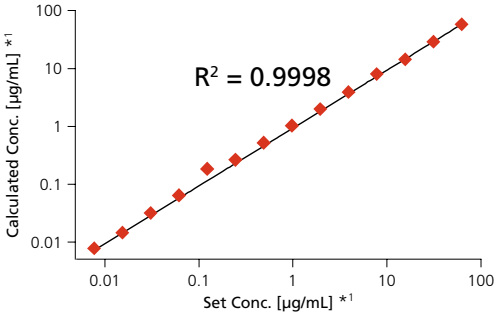
—Nexera Mikros and nSMOL™ Antibody BA Kit—

nSMOL (nano-surface and molecular orientation limited proteolysis) is a proprietary, ground-breaking process that enables selective proteolysis of the Fab region of antibodies using trypsin-immobilized nanoparticles. Fab-derived peptides are quantified via MRM measurement on a high-sensitivity mass spectrometer. The Nexera Mikros system achieved signal intensities 12 times higher than with conventional flow rates, with a corresponding lower limit of detection of 0.025 µg/mL and good linearity. The Nexera Mikros system is ideal for low-level quantitation of peptides by LCMS.

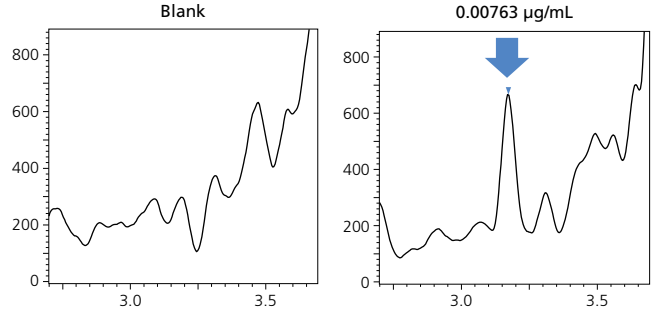


The following is an example of antibody drug analysis in plasma. The plasma spiked with trastuzumab was pretreated using nSMOL Antibody BA Kit.

In the analysis of signature peptides deriving from trastuzumab, Nexera Mikros provides a dynamic range of 0.00763 – 62.5 µg/mL, good linearity with $R^2 > 0.99$, and excellent accuracy of 101.0% (average).



Linearity (0.00763 to 62.5 µg/mL)



Chromatogram (4 µL/min)

The intra-day reproducibility of this trastuzumab-derived-peptide analysis is summarized in the table below.

These results show an excellent reproducibility, with both of accuracy and precision within 20% at LLOQ and within 15% even at other concentrations.

Concentration Setting (µg/mL)	QC set 1 *2		QC set 2 *2	
	Accuracy	Repeatability	Accuracy	Repeatability
0.00763	97.1%	5.69%	100%	11.3%
0.0229	102%	6.68%	101%	2.84%
5.86	106%	2.67%	99.4%	3.12%
50.0	94%	6.36%	91.7%	7.23%

Intra-Day Reproducibility Evaluation Results from QC Samples

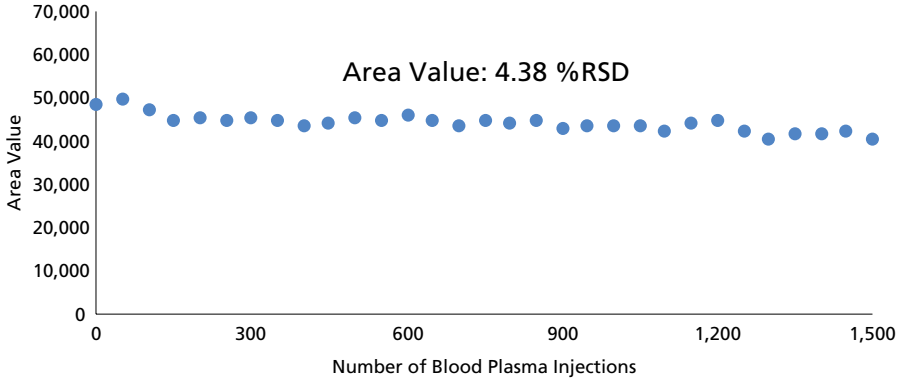
*1: This curve is drawn on logarithmic scale.

*2: QC sets 1 and 2 were analyzed over a 2-day period, with each set of concentrations analyzed five times.

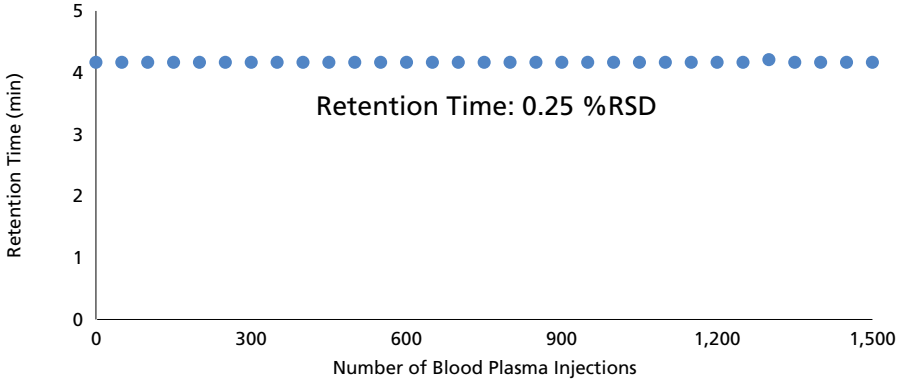
Realize the Benefits of Microflow While Enjoying the Ruggedness and Reliability of HPLC

The Nexera Mikros system delivers the improvements you expect from a low-flow system with the ruggedness of HPLC. A plasma blank was protein precipitated, diluted (3×), and injected 1,500 times consecutively. During the consecutive injections, QC sample (40 ppt of nortriptyline) was injected once every 50 injections of plasma blank. Peak areas and retention times of QC sample are plotted below. With 4.38% RSD area repeatability and 0.25% RSD retention time stability, extremely stable operation was demonstrated, without loss of peak shape common in high-throughput analyses of complex matrices. Furthermore, the LCMS-8060NX with new ion guide UF-Qarray™ II and the UF-Lens™ II increases the robustness and minimizes downtime of the instrument.

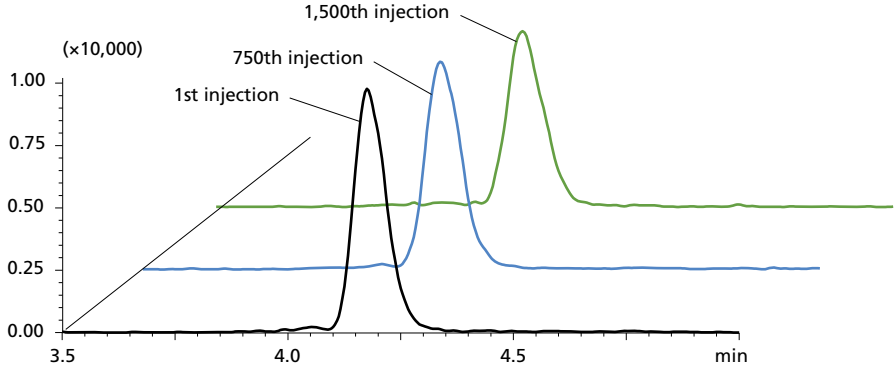
* Results obtained under our application conditions.



Area Repeatability of QC Samples



Retention Time Reproducibility of QC Samples



Chromatograms from 1st, 750th, and 1,500th Injections

Robust and Easy-to-Use Microflow ESI Source

Micro-ESI™ Sources

Shimadzu's Micro-ESI LCMS source has been designed for optimal sensitivity and ease of use. An X–Y adjustable stage and viewing camera allow for easy ESI spray needle adjustment. The column oven is mounted on the LCMS to minimize the volume between the exit of the column and the ESI needle.

The Micro-ESI probe also features an ESI spray angle optimized for microflow rates, and is available for triple quadrupole mass detection mass spectrometry and quadrupole time-of-flight mass spectrometry, respectively.

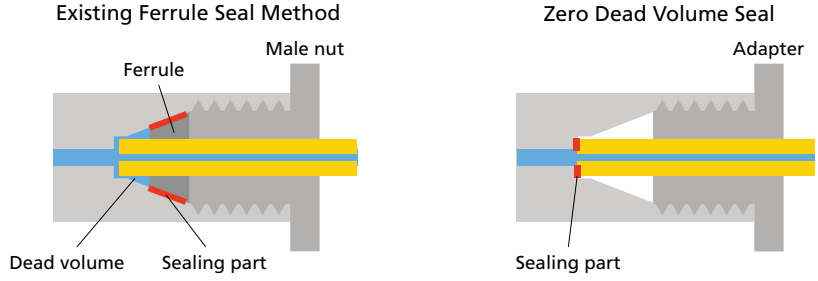
Micro-ESI 8060
(compatible with LCMS-8045/8050/8060(NX) systems)

Micro-ESI 9030

CTO-Mikros™ + UF-Link™: Easy, Dependable Column Installation

Mounted to the Micro-ESI source is the CTO-Mikros column oven. Stable column temperature, essential to retention time reproducibility, is ensured and columns up to 150 mm in length can be accommodated. To preserve the optimal source adjustment, the CTO-Mikros features an innovative snap-in column locking system, the UF-Link.

When using microflow rates, even the smallest of dead volumes can cause significant diffusion and lead to loss of peak shape and sensitivity. Shimadzu's UF-Link allows any user to correctly install a microflow column without disrupting the ESI spray needle position. A sure-sealing adapter allows the column to lock into the oven and make a zero-dead volume connection with the ESI needle. Any column up to 150 mm in length with standard fittings can be accommodated.



Connection Procedure

(1) Attach the adapter to the column. Standard threads on the adapter make it compatible with a wide variety of columns.

(2) Place the column in the UF-Link mechanism inside the oven.

(3) Complete the connection by swinging over the lever.

LC-Mikros™: Innovative Microflow Pump Design

Shimadzu LC pumps are the industry standard for long life and high performance. The LC-Mikros solvent delivery pump has completely new direct-drive engineering to deliver micro to semi-micro flow rates ranging from 1 to 500 µL/min. UHPLC system pressure tolerance of 80 MPa (11,600 psi) allows the use of every column type.

The LC-Mikros pump is designed for microflow gradient elution, even at low percentages. In the example below, excellent gradient stability is shown using mobile phase compositions ranging from 1% to 90%. The LC-Mikros delivers stable retention times essential to the chromatographer.

	Average Retention Time	Standard Deviation	RSD
Gly-Tyr	5.31	0.009	0.16%
Val-Tyr-Val	7.97	0.006	0.07%
Angiotensin II	9.32	0.015	0.16%
Leu enkephalin	9.71	0.011	0.11%
Met enkephalin	10.5	0.012	0.11%

Time (min)	1	11	11.1	13	13.1	25
Concentration B	1%	40%	90%	90%	1%	1%

Flowrate: 5 µL/min
Column: Shim-pack MC C18 (0.3 mm I.D. × 150 mm)

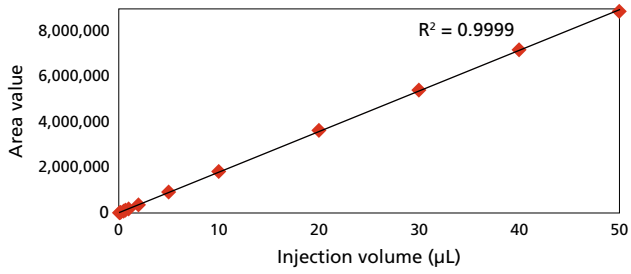
* Obtained under our specified conditions.

SIL-40C XR: Low-Volume Direct Injection Autosampler

The SIL-40C XR autosampler delivers you excellent accuracy and reproducibility as well as extremely low carry-over. Its injection volume ranges from 0.1 to 50 µL and your precious sample has never wasted due to the total injection method SIL-40C XR adapted.

It contributes to produce highly accurate results even if you had to use low injection volume (less than 1 µL). In case of micro flow rate with narrow column, optimum injection volume also gets lowered. It is also difficult to increase the injection volume, especially, if you would use a sample solvent with high elution power. In this case, sample must be diluted to weaken the sample solvents. Highly accurate micro volume injection capability of SIL-40C XR allows you to use samples with high organic solvent contents without dilution.

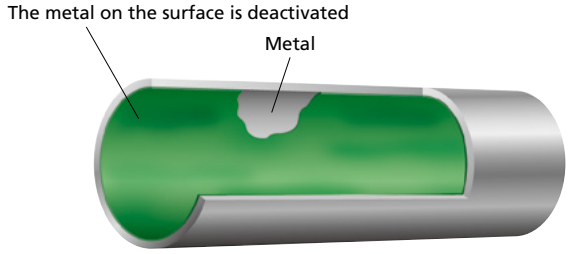
System volume and gradient delay are minimized with the newly developed "programmable loop cut-off mechanism," which automatically diverts the sample loop from the flow line using a high-pressure valve as soon as the sample in needle is drained. This is extremely important in minimizing microflow analysis run times.



Injection Volume (µL)	Repeatability (n = 6)	Injection Volume (µL)	Repeatability (n = 6)
0.1	0.80%	2	0.08%
0.2	0.46%	5	0.06%
0.5	0.32%	10	0.04%
0.7	0.21%	20	0.04%
1	0.06%	50	0.03%

Injection Repeatability (Actual Values)

Shim-pack™ MC C18



Versatility

- Shim-pack MC C18 column with 1.9 µm UHPLC particle achieves excellent separation and peak shape.
- The high pressure rating of 70 MPa allows these columns to be compatible with a range of mobile phase flow rates from 1 to dozens of µL per minute.
- The metal coordinative adsorption is suppressed by deactivating the column parts in contact with the solution.
- Both small and large molecule compounds, such as peptides, can be analyzed.
- Excellent durability can be achieved even if the biological sample analysis.

Shim-pack MC PLONAS Series

Shim-pack MC PLONAS 90Å 2.7 µm

Superior Performances with Superficially Porous Particles (SPP)

- Shim-pack MC PLONAS 2.7 µm columns reduce the back pressure yet maintain the performance of a sub 2 µm fully porous particle column. The pressure limit is 40 MPa.
- Achieve lower LODs and LOQs, better resolution, and greater reproducibility using the unique superficially porous particle found in Shim-pack MC PLONAS.
- Greater dimensional offerings allows for higher efficiency with better resolving power for challenging applications.
- An array of pore sizes designed to meet the needs of any application.
- Increased ruggedness because of larger porosity frits resulting in less clogging compared to competitor columns.
- Excellent column-to-column and lot-to-lot reproducibility.

Shim-pack MC PLONAS Series

Bonded Phase	Features and Benefits	Target Analytes
C18 (dimethyloctadecylsilane)	Suitable for a broad range of analytes	Diverse analytes ranging from polar to non polar
Aqueous-C18 (polar modified)	• Compatible with 100% aqueous mobile phases • Enhanced retention for polar molecules	Acids, bases, polar analytes
C8 (dimethyloctylsilane)	Suitable for a broad range of analytes	Diverse analytes ranging from polar to non-polar
Biphenyl (dimethylbiphenyl)	• Complementary selectivity to alkyl phases • Enhanced selectivity for aromatic compounds	Electron-poor molecules, aromatic or unsaturated compounds (ketones, nitriles, alkenes)
PFPP (pentafluoro-phenylpropylsilane)	• Complementary selectivity to alkyl phases • Enhanced selectivity for stereoisomers • Can be used in reversed phase and HILIC modes	Electron-rich compounds, aromatics, unsaturated compounds with double and/or triple bonds
HILIC (bare silica)	Can also be used in normal-phase modes	Polar and highly polar acids, bases, and neutrals, especially with log $P < 0.5$

Analytical Columns

Shim-pack MC PLONAS Series (particle size 2.7 µm)

Part Number	Bonded Phase	Diameter (mm)	Length (mm)
227-32100-01	C18	0.2	50
227-32100-11		0.3	
227-32100-02		0.2	100
227-32100-12		0.3	
227-32100-03		0.2	150
227-32100-13		0.3	
227-32102-01	AQ-C18	0.2	50
227-32102-11		0.3	
227-32102-02		0.2	100
227-32102-12		0.3	
227-32102-03		0.2	150
227-32102-13		0.3	
227-32101-01	C8	0.2	50
227-32101-11		0.3	
227-32101-02		0.2	100
227-32101-12		0.3	
227-32101-03		0.2	150
227-32101-13		0.3	

Part Number	Bonded Phase	Diameter (mm)	Length (mm)
227-32104-01	Biphenyl	0.2	50
227-32104-11		0.3	
227-32104-02		0.2	100
227-32104-12		0.3	
227-32104-03		0.2	150
227-32104-13		0.3	
227-32103-01	PFPP	0.2	50
227-32103-11		0.3	
227-32103-02		0.2	100
227-32103-12		0.3	
227-32103-03		0.2	150
227-32103-13		0.3	
227-32105-01	HILIC	0.2	50
227-32105-11		0.3	
227-32105-02		0.2	100
227-32105-12		0.3	
227-32105-03		0.2	150
227-32105-13		0.3	

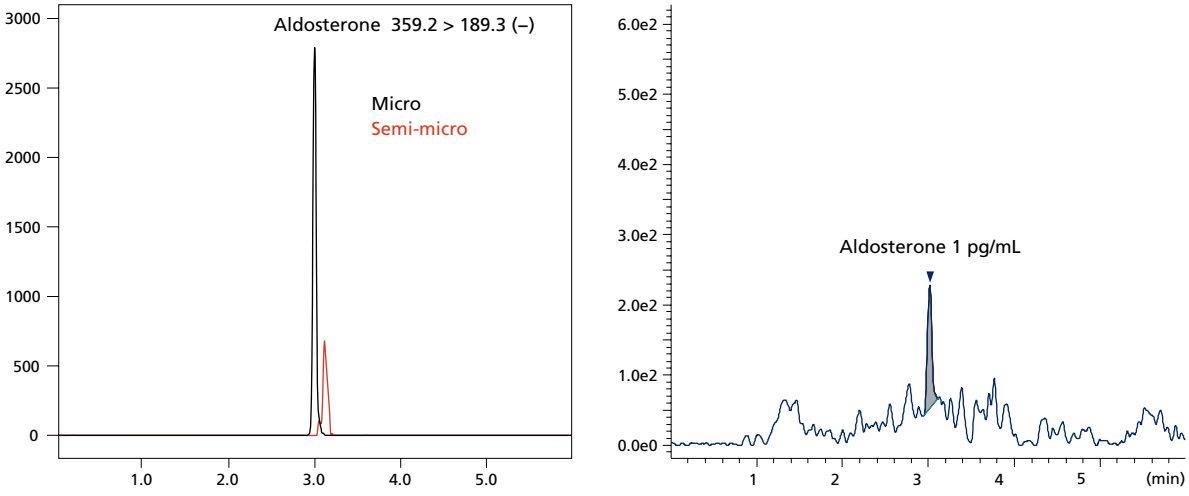
Shim-pack MC C18 (particle size 1.9 µm)

Part Number	Bonded Phase	Diameter (mm)	Length (mm)
228-59937-91	C18	0.3	50
228-59937-93		0.175	
228-59937-95		0.15	

Shim-pack MC PLONAS Series

Various molecules give an increase in the peak intensity on Nexera Mikros LC-MS/MS platform with an MC PLONAS column.

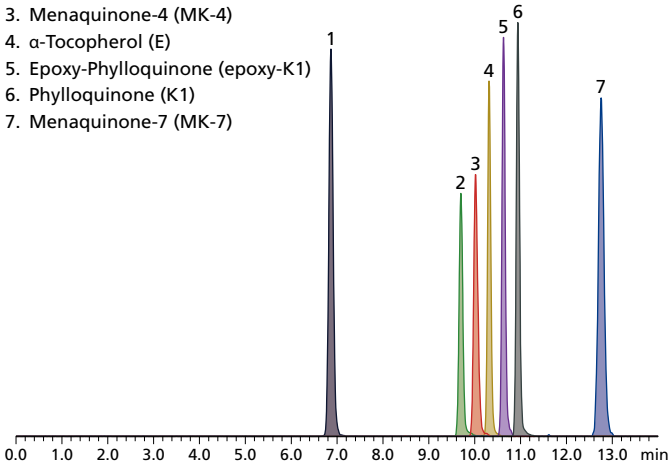
Shim-pack MC PLONAS C18



Column : Shim-pack MC PLONAS C18 (0.2 mm I.D. × 100 mm L., 2.7 µm)
* Semi-micro data acquired with the 2.1 mm I.D. column which has the same chemical profile as an MC PLONAS column.
Mobile phase : A: Water, B: Methanol, gradient elution
Flow rate : 4 µL/min
Temperature : 50 °C
Injection volume : 1 µL
MS detection : LCMS-8060, with Micro-ESI source, ESI (–)

Shim-pack MC PLONAS C8

1. All-*trans* Retinol (A)
2. Epoxy-Menaquinone-4 (epoxy-MK-4)
3. Menaquinone-4 (MK-4)
4. α-Tocopherol (E)
5. Epoxy-Phylloquinone (epoxy-K1)
6. Phylloquinone (K1)
7. Menaquinone-7 (MK-7)



20 pg on column
* each peak is in full scale intensity

Column : Shim-pack MC PLONAS C8 (0.2 mm I.D. × 100 mm L., 2.7 µm)
Mobile phase : A: Ammonium acetate aq.
B: Ammonium acetate in methanol, gradient elution
Flow rate : 4 µL/min
Temperature : 50 °C
Injection volume : 0.2 µL
MS detection : LCMS-8060, with Micro-ESI source, ESI (–)

Shim-pack Trap Columns

Trap Columns Wide Portfolio

- **Shim-pack MCT C18/C8**

  70 MPa pressure limitation and a unique modification on the column inner surface protects against absorption of the molecule.

- **Shim-pack MCT LC18/LC8**

  A small-volume cartridge column with 40 MPa pressure limit. Useful in reducing the gradient delay for a low flow rate.

Shim-pack MCT series

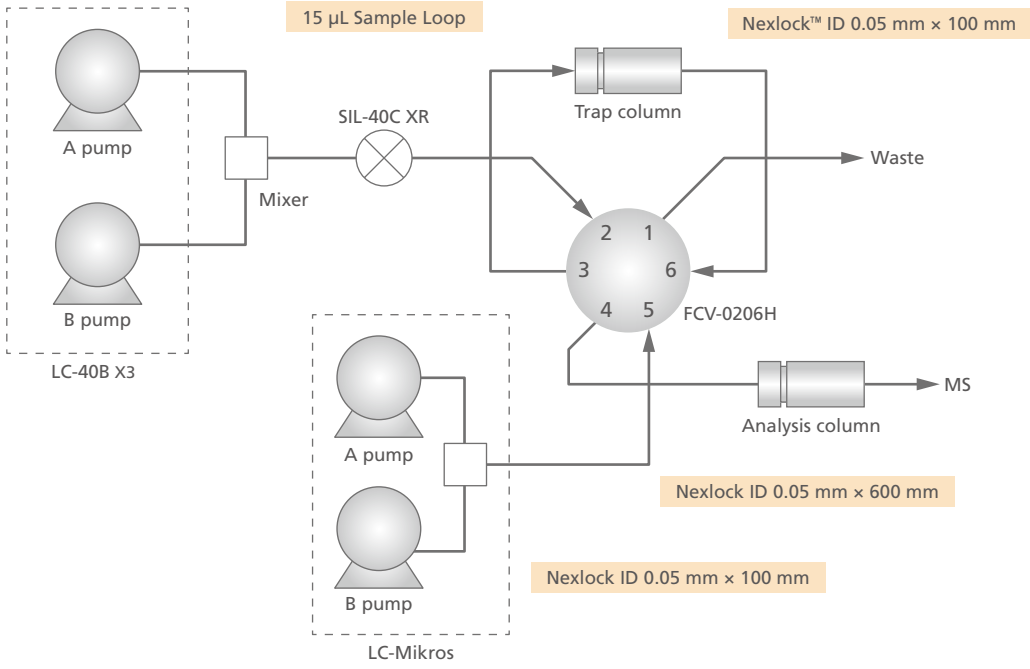
Part Number	Bonded Phase	Diameter (mm)	Length (mm)
228-59938-91	C18	0.3	35
228-59938-92		0.5	
228-59939-91	C8	0.3	35
228-59939-92		0.5	

Shim-pack MCT L series (particle size 5 µm)

Part Number	Bonded Phase	Diameter (mm)	Length (mm)
227-32701-01	Trap column holder for Shim-pack MCT L		
227-32702-01	C18	0.3	5
227-32703-01	C8	0.5	

Nexera Mikros: Trap and Elute Configuration

The trap and elute system can be constructed by using a dedicated piping kit (228-71751-42).



System Configuration Options

Direct Injection System

Ideal for sample volume-limited analyses. This system provides rapid and highly sensitive microflow LC/MS analysis without sample loss.

Trap and Elute System

Ideal for analyses with larger injection volumes or when some degree of sample cleanup is desirable.

Make-Up Flow System

The addition of a post-column make-up flow pump allows mobile phase modifiers to be added to ensure the highest ionization efficiency in the MS source.

* These systems can be constructed with both triple quadrupole mass spectrometer and quadrupole time-of-flight mass spectrometer.

Shimadzu Corporation
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