



Prominence nano

Shimadzu
Nanoflow Liquid Chromatograph



Prominence
NANOFLOW LIQUID CHROMATOGRAPH

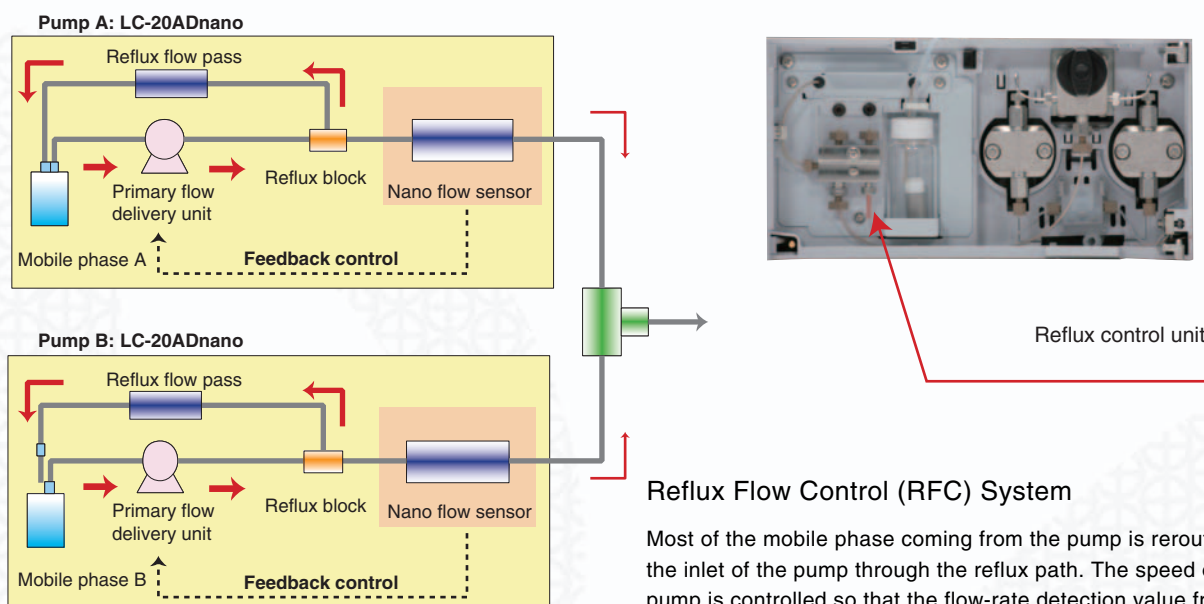
nano

Uncompromising core performance for high-precision proteome analysis

The Reflux Flow Control (RFC)* System offers superior flow-rate precision in the nano flow-rate range, making nano flow-rate gradient analysis with higher reproducibility possible. *Patent pending

Unnecessary solvent consumption is eliminated because a split drain after 2-liquid mixing is not required.

In the nano flow-rate range, stable solvent delivery and solvent consumption volume are important. The LC-20ADnano equips nano flow-rate control mechanisms for each pump independently. The flow rate is controlled by feedback from signals from the high-precision nano flow sensor, so that precise flow rates are always assured. Additionally, the RFC system offers stable solvent delivery and low solvent consumption, so that unnecessary solvent consumption is eliminated and the impact on the environment is minimized. The nano flow sensor features a precise temperature control to minimize the influence of room temperature variation on the flow rate.



Reflux Flow Control (RFC) System

Most of the mobile phase coming from the pump is rerouted to the inlet of the pump through the reflux path. The speed of the pump is controlled so that the flow-rate detection value from the flow sensor is the same as the set value.

FCV nano: a Shimadzu-developed nano valve – Superior performance and durability –

Shimadzu has developed a nano valve, which is required for trap injection and for 2-dimensional LC in nano LC. Volume between ports is as low as 25nL, and peak broadening is virtually zero in the nano flow-rate range. By combining a surface hardened stator and a PEEK rotor, low adsorption and high durability are achieved. Particles derived from the switching valve movement are minimized and clogging in nano flow paths is suppressed.

Adsorption of the analytical sample is also suppressed. The FCV nano is an optimized switching valve for bio sample analysis in the nL/min level.

PEEK is a registered trademark of Victorex plc (UK).



Graphically assisted software operation

Instrument control through an easy-to-understand GUI

Nano-Assist control software – 2D LC operation made easy –

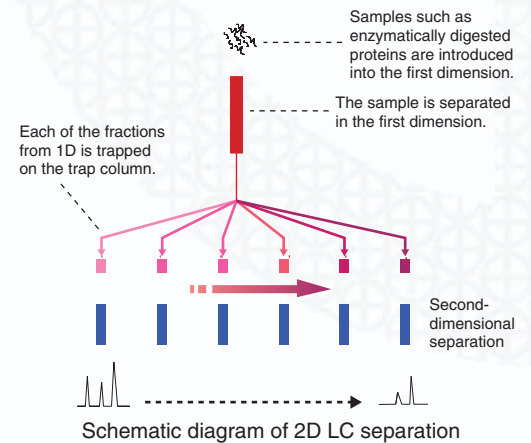
Complicated gradient programming for two-dimensional HPLC is made easy by the Nano-Assist's graphical user interface. Just input the parameters to have the method file created and downloaded to the instruments. Visual presentations of flow-line connections and gradient curves help prevent operation errors.



2D LC setting window of Nano-Assist

High peak resolution with 2D analysis

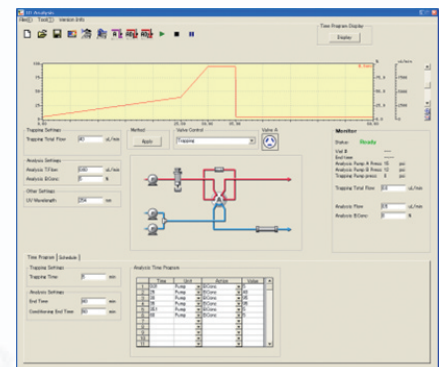
The Prominence nano's online 2D separation system combines ion exchange and reversed-phase modes as illustrated at right. Each separation mode works independently, and the combination of the two modes gives the best separation efficiency. The Prominence nano 2D system can constitute an online proteome analyzer when coupled to an LCMS with a nano ESI interface or to a MALDI spotter.



Schematic diagram of 2D LC separation

Easy-to-use 1D system

The one-dimensional nano LC system is a useful tool for identifying proteins pre-separated with SDS-PAGE. The 1D nano LC system offers simple operation, and the precise solvent delivery of the LC-20ADnano and the low dead volume of the FCV nano maximize the system's performance. Like the 2D system, the 1D system is operated using the dedicated software, Nano-Assist, which provides easy operation and prevents possible mistakes in operation settings.



1D LC setting window of Nano-Assist

Autosampler with a total-volume injection method – No waste of samples –

The combination of the proven performance of the SIL-20AC and the low dispersion of the FCV nano valves allows analysis with a minimal injection volume. The total-volume injection-type autosampler requires no extra sample volume, and the trapping column flow line with the FCV nano minimizes peak broadening, providing maximum sensitivity with the smallest sample volume. The SIL-20A also has an excellent reputation as a low-carryover autosampler, reducing adsorption of sample constituents to an absolute minimum, ensuring stable analysis with low sample carryover.

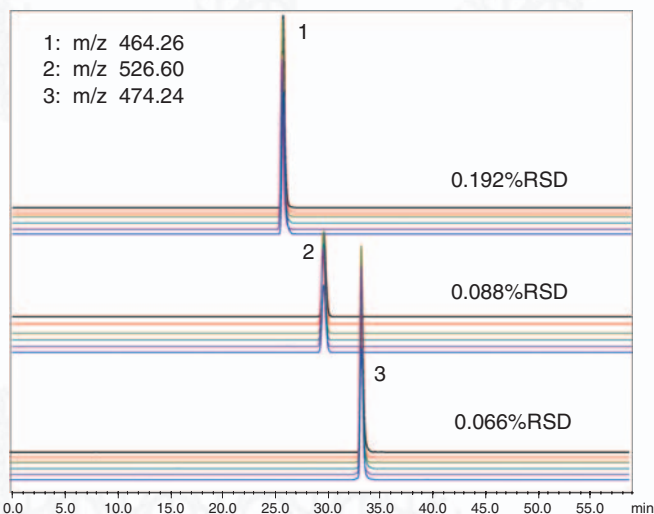


SIL-20AC autosampler demonstrates minimized sample carryover

High-precision proteome analysis by Prominence nano

Data reliability is realized by high basic performance – Exemplary retention time repeatability –

The LC-20ADnano, equipped with an RFC system, demonstrates high core performance, achieving 0.20% RSD or less (RSD% (n=6)) at a 300nL/min. flow rate. In proteome analysis, it is required to separate and identify many digestive enzyme peptides with very similar chromatographic retention characteristics. For peak comparison of samples in differential analysis, retention time reproducibility is very important. With Prominence nano, the high reproducibility to support high-precision proteome analysis is easily achieved.



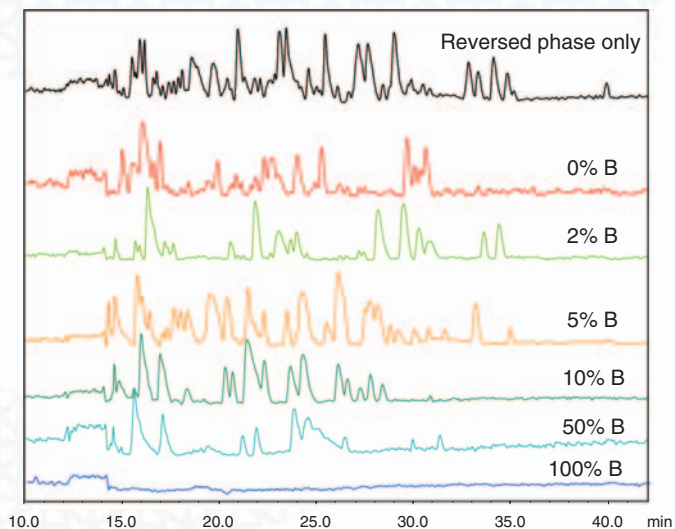
| | Analytical conditions |
|-----------------|---|
| Column | PicoFrit (100 mm L. x 75 µm I.D.) |
| Mobile phase | A) Water / Acetonitrile / Formic acid = 98/2/0.1 (v/v) B) Water / Acetonitrile / Formic acid = 5/95/0.1 (v/v) gradient elution |
| Flow rate | 300 nL/min |
| Temperature | Ambient temperature |
| Trapping column | L-column Micro (5 mm L. x 300 µm I.D.) |
| Detection | LCMS-IT-TOF |

Chromatograms illustrated in the left figure are MS chromatograms of each targeted m/z.

Reproducibility for digested BSA sample (Retention time reproducibility of major peaks)

Higher separation by 2-dimensional LC – Higher peak capacity –

With complex samples, such as an enzyme digestion protein mixture, 1-dimensional separation using a reversed-phase mode does not offer sufficient peak capacity. Therefore, 2-dimensional separation using a combination of separation modes, which are independent of each other, should be used because 2-dimensional separation provides a larger peak capacity and powerfully separates complex samples. Using the Prominence nano system, it is possible to construct a 2-dimensional LC with a combination of cation exchange and reversed-phase modes* that achieves sufficient performance to quality as the principal separation instrument for proteome analysis. The figure below shows an analysis of 200fmol of protein from yeast. Overlapped peaks with 1-dimensional LC are separated into different fractions by 2-dimensional separation.



Separation by 2-dimensional LC for enzymatically digested yeast proteins

| [1 st dimension] | Analytical conditions |
|-----------------------------|---|
| Column | Polysulfoethyl A (50 mm L. x 1 mm I.D.) |
| Mobile phase | Formate buffer / Salt step gradient |
| Flow rate | 40 µL/min |
| Trapping column | L-column Micro (5 mm L. x 300 µm I.D.) |
| Trapping period | 5 minutes |
| Desalting solvent | Water / Formic acid = 100 / 0.1 |
| Flow rate for desalting | 40 µL/min |
| Desalting period | 5 minutes |
| [2 nd dimension] | Analytical conditions |
| Column | PicoFrit (100 mm L. x 75 µm I.D.) |
| Mobile phase | A) Water / Acetonitrile / Formic acid = 95 / 5 / 0.1 (v/v) B) Water / Acetonitrile / Formic acid = 5 / 95 / 0.1 (v/v) gradient elution |
| Flow rate | 600 nL/min |
| Detection | LCMS-IT-TOF |
| Sample | Digested yeast proteins mixture (200 fmol as proteins) |

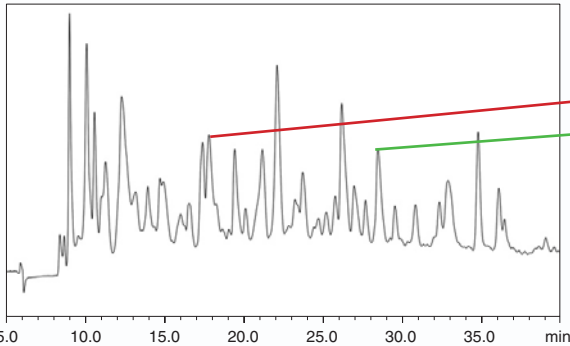
* Other combinations of separation modes can be adapted for 2-dimensional analysis using different types of columns than those shown here. In such cases, there may be some limitations in the separation or detection method due to the influences between each separation mode or the types of mobile phase used.

Prominence nano provides ultra flexibility

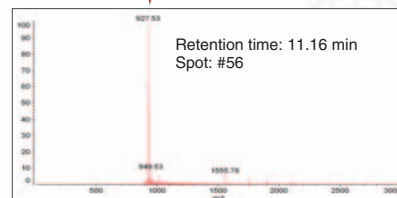
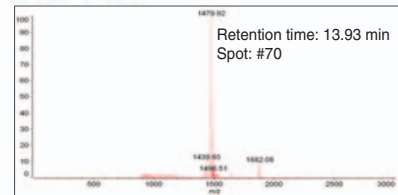
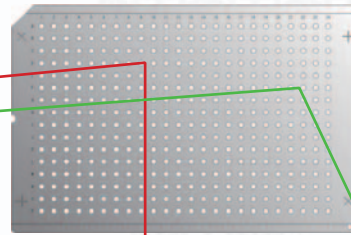
Easy to combine with MALDI-TOF MS systems – AccuSpot MALDI spotting device –

The Prominence nano system can be used with an online connected nano-ESI mass spectrometer or with a MALDI TOF mass spectrometer with a MALDI plate spotting device.

For the accurate spotting of each chromatographic peak onto a MALDI plate, flow rates at about several hundreds of nL/minute are commonly applied; thus, LC with low flow rate precision is required. The Prominence nano is an ideal system for this purpose.



Separation of digested BSA sample (500 fmol as protein) (UV chromatogram)



| | Analytical conditions |
|-----------------|--|
| Detection | UV 220 nm |
| Column | MonoCap for Fast-flow (250 mm L. x 100 μm I.D.) |
| Mobile phase | A) Water / Acetonitrile / TFA = 95/ 5/0.1 (v/v) B) Water / Acetonitrile / TFA = 10/90/0.1 (v/v) Gradient elution |
| Flow rate | 1 μL/min |
| Temperature | Ambient |
| Trapping column | ODS (1 mm L. x 0.5 mm I.D.) |
| Spotting period | Every 12 seconds |

Spotting volume for one spot: 200 nL except matrix solution

Proteome analysis system combined with a Shimadzu mass spectrometer – Providing maximum performance and a total solution –

When connected to a Shimadzu mass spectrometer, the Prominence nano system demonstrates its maximum performance.

A total system configuration, comprising the Prominence nano and either the LCMS-IT-TOF MS with a nano-ESI interface or a MALDI-TOF MS with the AccuSpot MALDI spotter, provides authentic results and a total solution for proteomic analyses.

* Prominence nano can operate stand-alone or with a mass spectrometer, including those manufactured by other vendors.



Prominence nano modules

LC-20ADnano Solvent Delivery Unit



| | LC-20ADnano (228-45121-3x) |
|-----------------------------|---|
| Solvent delivery method | Reflux flow control system |
| Flow-rate setting range | 1 nL/min – 5 µL/min (stand-alone operation) 10 nL/min – 5 µL/min (controlled by the Nano-Assist) 100 nL/min – 5 µL/min (controlled by CBM-20A, Solution software) |
| Safety measures | Liquid-leakage sensor, High-pressure/low-pressure limits |
| Operating temperature range | 16°C - 28 °C |
| Dimensions, Weight | 260 (W) x 140 (H) x 420 (D) mm, 11 kg |
| Power requirements | AC 110 V, 230V, 150 VA, 50/60 Hz |

FCV nano Switching Valve



| | FCV nano (228-45123-91) |
|-----------------------------|--|
| Valve type | 6-ports 2-position high-pressure switching valve |
| Port-to-port volume | approximately 25 nL |
| Inner diameter of flow pass | approximately 0.1 mm |
| Operating pH range | 1 - 14 |
| Maximum operating pressure | 20 MPa |
| Dimensions, Weight | 110 (W) x 110 (H) x 250 (D) mm, 4 kg |

Combination of the Prominence nano System and a Shimadzu Mass Spectrometer

The AccuSpot MALDI spotting device, coupled to the Prominence nano, is a powerful tool when combined with Shimadzu MALDI-TOF MS Series instruments.



(Typical system configuration including the AccuSpot)
Prominence nano 1-dimensional system with the
AccuSpot MALDI spotting device

The high-grade LCMS-IT-TOF mass spectrometer, coupled to the Prominence nano through the nano-ESI interface, provides outstanding performance for proteomic / metabolomic analyses.

(Typical system configuration including the LCMS-IT-TOF)
Prominence nano 2-dimensional system with the LCMS-IT-TOF mass spectrometer (including
LCMSsolution software) and the nano-ESI interface, NES-100



System Configurations

Prominence nano 1-dimensional System



| P/N | Item | Qty |
|--------------|---|-----|
| 228-45012-xx | CBM-20A, System Controller | 1 |
| 228-45121-xx | LC-20ADnano, Solvent Delivery Unit for analysis | 2 |
| 228-45000-xx | LC-20AD, Solvent Delivery Unit for trapping | 1 |
| 228-45019-xx | DGU-20As, On-line Degasser | 1 |
| 228-45007-xx | SIL-20AC, Autosampler | 1 |
| 228-45060-xx | Option Boxvp | 1 |
| 228-45123-91 | FCV nano, Switching Valve | 1 |
| 223-03507-91 | PC-55N, A/D Board | 2 |
| 228-45041-91 | Preserver Tray | 1 |
| 228-45124-92 | Start-up Kit for nano LC | 1 |

Prominence nano 2-dimensional System



| P/N | Item | Qty |
|--------------|---|-----|
| 228-45012-xx | CBM-20A, System Controller | 1 |
| 228-45121-xx | LC-20ADnano, Solvent Delivery Unit for 2 nd dimension | 2 |
| 228-45002-xx | LC-20AB Solvent Delivery Unit for 1 st dimension | 1 |
| 228-45000-xx | LC-20AD, Solvent Delivery Unit for trapping | 1 |
| 228-45018-xx | DGU-20A3, On-line Degasser | 2 |
| 228-45007-xx | SIL-20AC, Autosampler | 1 |
| 228-45060-xx | Option Boxvp | 1 |
| 228-45123-91 | FCV nano, Switching Valve | 2 |
| 223-03507-91 | PC-55N, A/D Board | 2 |
| 228-45041-91 | Preserver Tray | 1 |
| 228-45124-91 | Start-up Kit for nano LC | 1 |
| 228-45312-91 | Mixer Housing | 1 |
| 228-45312-93 | Mixer Cartridge | 1 |

* In addition to the items listed above, columns, sample vials and some other items are required.



JQA-0376

Founded in 1875, Shimadzu Corporation, a leader in the development of advanced technologies, has a distinguished history of innovation built on the foundation of contributing to society through science and technology. We maintain a global network of sales, service, technical support and applications centers on six continents, and have established long-term relationships with a host of highly trained distributors located in over 100 countries. For information about Shimadzu, and to contact your local office, please visit our Web site at www.shimadzu.com



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