



## enhanced peptide identification with the nanoLC systems

The NanoLC system for proteomics performs reproducible HPLC separations of peptides for analysis by a mass spectrometer. The system uses Microfluidic Flow Control (MFC) technology to generate precise LC gradients at nanoscale flow rates without flow splitting. This improves spray stability, increases mass spectrometer sensitivity, and improves identification of low abundance proteins. The system also incorporates Eksigent's Dynamic Flow Control capability, which allows the flow rate to be changed rapidly for higher speed sample loading or for extended MS/MS analysis (peak parking) to identify co-eluting and low abundance peptides.

### typical applications

Proteomics applications increasingly require higher reproducibility and sensitivity. The NanoLC system has been designed to address these needs.

- Phosphopeptide Analysis
- Biomarker Discovery
- Metabolomics

### nanoLC-1D™ plus system for 1-dimensional nano HPLC

The NanoLC-1D Plus system provides reproducible gradients at column flow rates of 20–1000 nL/min without flow splitting. The system includes an additional isocratic loading pump (1–30  $\mu$ L/min) for fast loading of large volume samples or a trap column. Binary solvent delivery, a loading pump, column switching valve, autosampler, and PC-based instrument control are provided in the compact NanoLC-1D Plus system.

### nanoLC-2D™ system for 2-dimensional nano HPLC

The NanoLC-2D system contains all of the features of the NanoLC-1D, plus the added capability of automated 2-dimensional chromatography. Two binary gradient pumps, sample loading and column switching valves and an autosampler are provided in the integrated NanoLC-2D system. The 10-port column switching valve for the second dimension accommodates 2 trap columns as well as an analytical column. The NanoLC software allows complete control of the pumps, valves, and autosampler and provides flexible sample injection modes. The system can be configured to run in either automatic 2-dimensional separation mode, or it can operate as 2 parallel, completely independent binary gradient HPLC systems.



## technology spotlight

### microfluidic flow control

Rather than utilizing positive displacement pistons or syringes to regulate flow, the Eksigent NanoLC system relies on Microfluidic Flow Control (MFC) technology. This pumping principle uses continuous feedback to an electronically controlled pressure source to maintain precise nanoscale flow rates as low as 20 nL/min without flow splitting. By combining true microfluidic pump control with direct feedback of flow rate, the system eliminates the flow inaccuracies and plumbing problems caused by splitter-based systems. Elimination of flow splitting through the use of Microfluidic Flow Control Technology makes the NanoLC system the industry standard for rugged, reliable nano LC/MS performance, run-to-run and day-to-day.

### dynamic flow control

Eksigent's Dynamic Flow Control (DFC) capability allows the flow rate to be changed rapidly for higher speed sample loading or for extended MS/MS analysis (peak parking) to identify co-eluting and low abundance peptides. After the gradient, DFC can automatically increase the flow rate to accelerate flushing and reduce the lag time between sample runs. Dynamic Flow Control can be triggered with one mouse click or by a signal from the mass spectrometer, eliminating the complexity of previous approaches requiring additional pumps or valves.

"We've been using this HPLC for nearly a year now and the performance and reliability are outstanding. We have not had a single service call and troubleshooting has been easily managed over the phone. For us, the flow control capabilities have been essential in getting high quality LC/MS/MS data."

– Cory Bystrom, Ph.D.  
Proteomics Shared Resource Director,  
Oregon Health and Science University

Figure 1. Flow meters continuously monitor the flow rate of each mobile phase with microprocessor feedback to a precise, rapid pressure source.

figure 1.

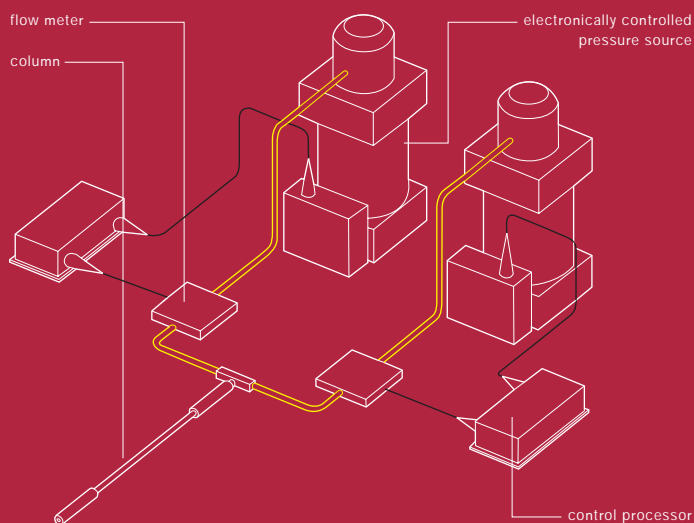
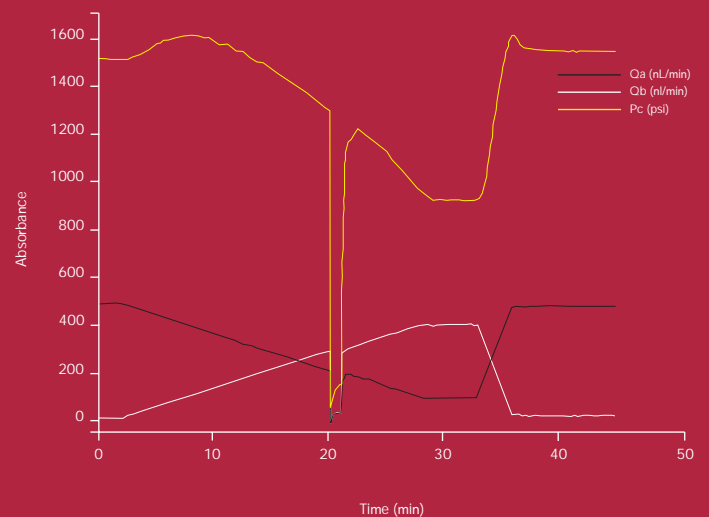


Figure 2. Flow profiles for mobile phase A (black, Qa), mobile phase B (white, Qb), and column backpressure (yellow, Pc) are plotted for a gradient at 500 nL/min. At 20 minutes, DFC was implemented to rapidly drop the flow rate to 50 nL/min. The gradient is paused during peak parking. Restoration of the flow rate to 500 nL/min shows that no interruption of the gradient profile occurs.

figure 2.



## exceptional reproducibility, sensitivity and resolution

### superior flow precision

Generate precise HPLC gradients at nanoliter-per-minute flow rates. Maintain flow rate precision at levels that have not previously been attainable. The NanoLC system is capable of responding to a 1 nL/min change in flow rate setpoint (Figure 4).

### unparalleled reproducibility

Splitter-based systems sacrifice reproducibility and limit the ultimate sensitivity level that can be achieved. Eksigent eliminated these problems by creating a new pumping principle eliminating the need for flow splitting. The NanoLC system uses Microfluidic Flow Control (MFC) technology to provide reproducible gradients at column flow rates of 20–1000 nL/min without flow splitting (Figure 5). The system actively measures and controls flow rate in both mobile phases and is unaffected by column backpressure changes. This results in retention time reproducibility an order of magnitude greater than that achieved with other nano HPLC systems (Figure 3, Table 1).

### best response time in the industry

The NanoLC system responds to changes in flow rate setpoint in less than 1 second, which is not achievable with other nano HPLC systems.

figure 3.

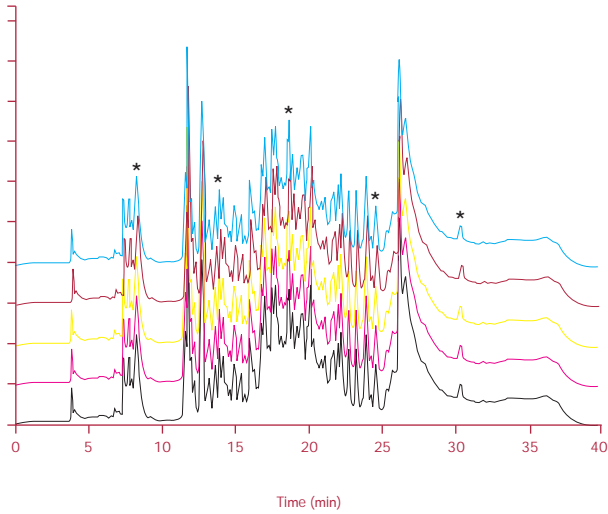


Figure 3. Reproducibility of splitter-based systems is compromised by column backpressure changes and differences in retention time between the splitter and the column. Because the NanoLC directly measures and adjusts flow delivery in each mobile phase, the system achieves excellent run-to-run reproducibility. Overlaid chromatograms of consecutive injections of a bovine serum albumin (BSA) tryptic digest run at 300 nL/min demonstrate the NanoLC system's excellent reproducibility, which is critical in differential expression studies where comparisons among samples are required.

table 1.

Retention time	% RSD
7.33	0.54
13.86	0.48
18.91	0.21
24.65	0.10
30.47	0.11

Column:	150 x 0.1 mm, 3 $\mu$ m C18
Mobile Phase:	Water/ACN +0.1% Formic Acid
Flow Rate:	300 nL/min
Detector:	Linear UV Detector
Injection:	500 nL

Table 1. Retention time reproducibility of peaks indicated with an asterisk in Figure 3.

Figure 4. Response of the NanoLC system to a 1 nL/min change in flow rate when the setpoint is changed from 400 nL/min to 399 nL/min, and a 2 nL/min change in flow rate when the setpoint is changed from 399 nL/min to 397 nL/min. Displayed is the flow rate setpoint (red) and the measured flow rate (blue).

figure 4.

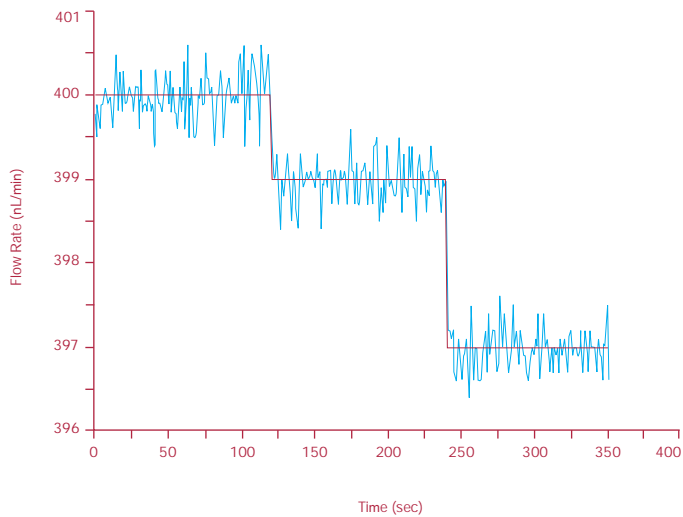
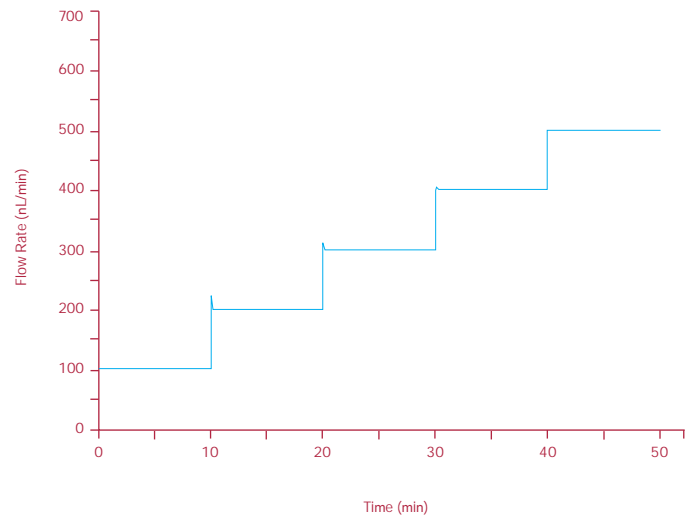


Figure 5. Stepped increase in flow rate from 100 nL/min to 500 nL/min. Response time of the system for each change in flow rate is < 1 sec.

figure 5.



## enhanced identification of low abundance peptides

## automated peak parking

Perform peak parking without the need for additional valves or external pumps and increase identification of co-eluting or low abundance peptides. Peak parking using the NanoLC's Dynamic Flow Control capability significantly extends MS/MS acquisition time while maintaining the resolution of downstream peaks (Figure 6). Reducing the flow rate allows for extended secondary analysis, increasing the number of unique peptides which can be identified per run.

Figure 6a,b. In this example for identification of phosphorylated peptides, DFC was initiated automatically by the mass spectrometer when a phosphorylated peptide was detected by neutral loss scanning using Information Dependent Acquisition (IDA). The data shows the extracted ion chromatograms with and without peak parking. Automated peak parking extends MS/MS acquisition by greater than a factor of five.

figure 6a.

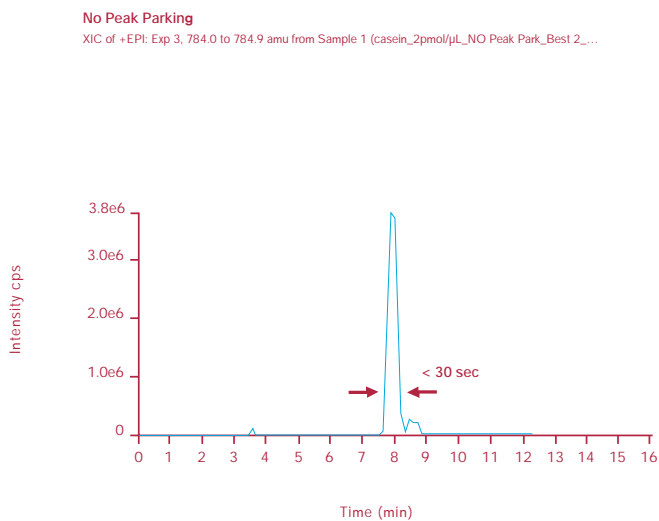


figure 6b.

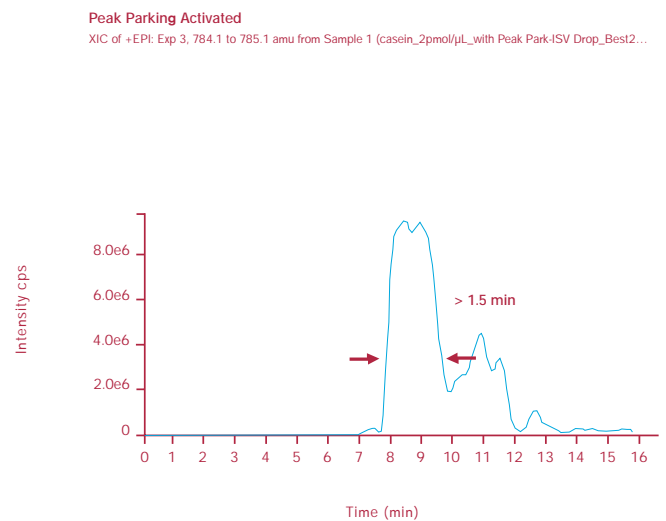


figure 7.

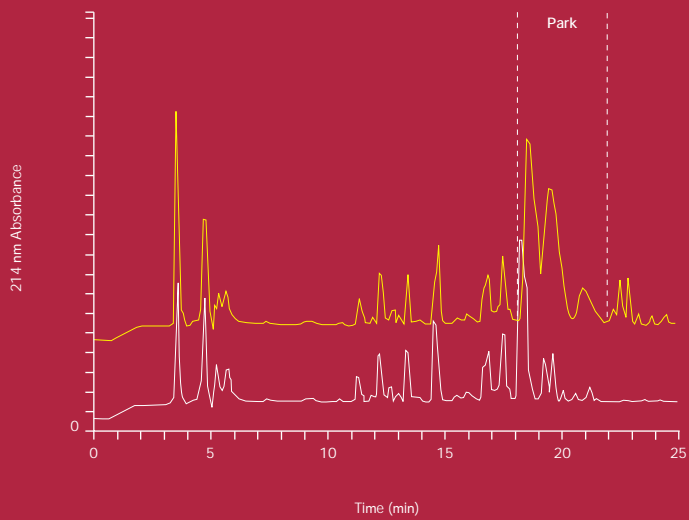


Figure 7. Peak parking from 300 nL/min to 50 nL/min implemented during a gradient separation of a cytochrome c tryptic digest resulted in improved resolution of a triplet of peaks, without any loss of chromatographic resolution after the park period.

figure 8.

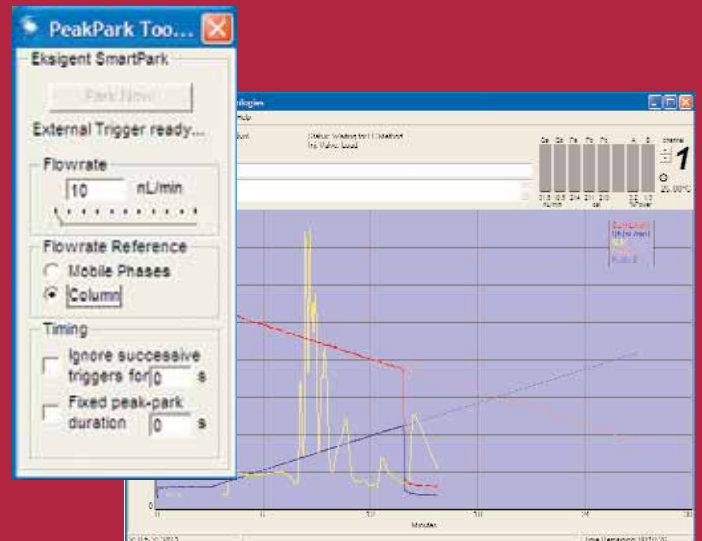


Figure 8. The peak parking window (enlarged to show features) is used to initiate and end peak parking. Alternatively, a signal from the mass spectrometer can be used.

Figure 9a,b. Extracted ion chromatograms of four co-eluting peptides at a gradient flow rate of 500 nL/min is shown in the upper panel. Peak parking to 100 nL/min (lower panel) extends the elution times of the peaks, allowing for identification of the lower abundance peptides.

figure 9a.

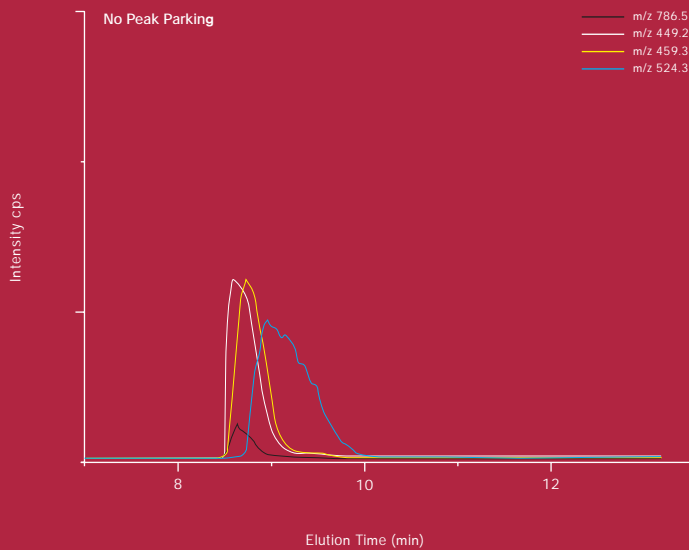
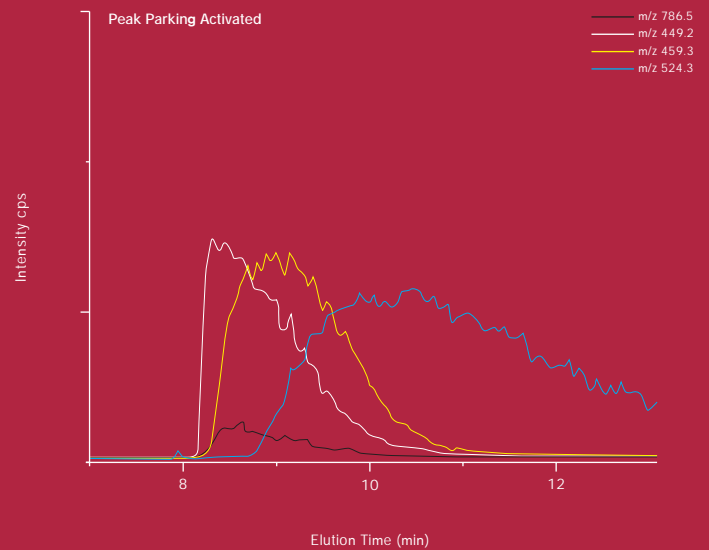


figure 9b.



# increased throughput and application flexibility

figure 10a.

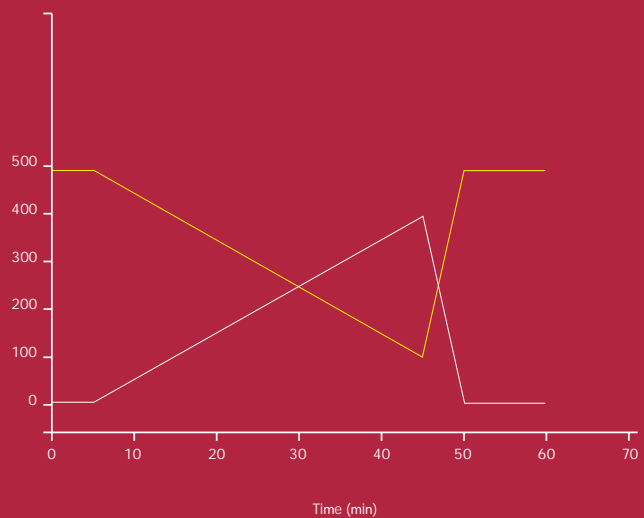


Figure 10a, b and c. The first dimension pumps can be used to form segmented gradients of a salt solution followed by wash steps onto an SCX column.

figure 10b.

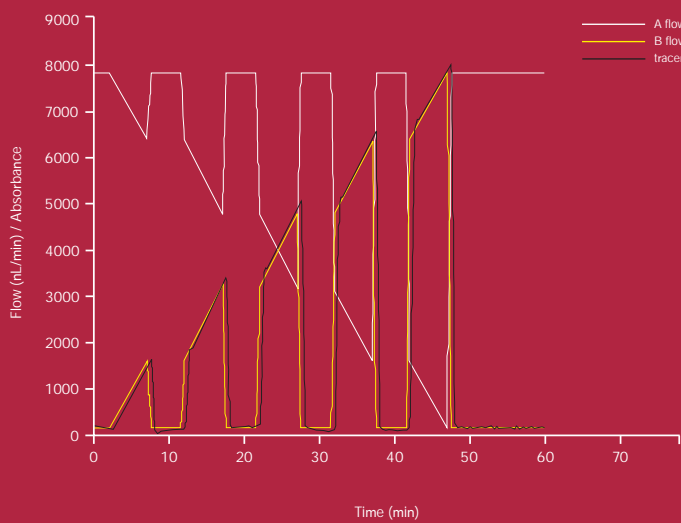
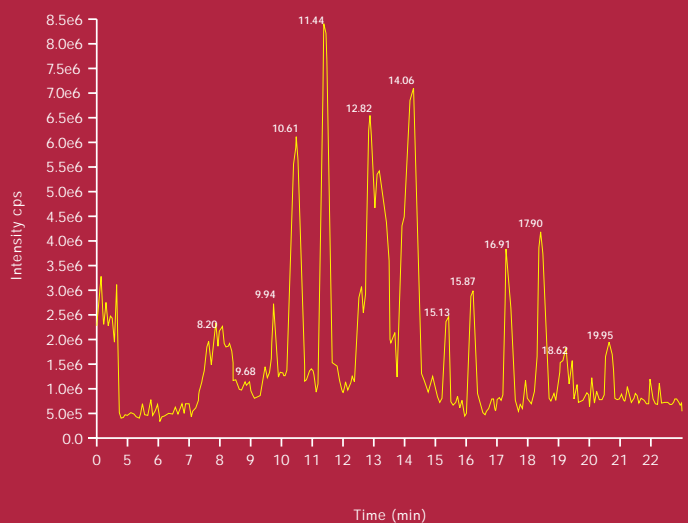


figure 10c.



### automated 2-dimensional separations

The NanoLC-2D system performs fully integrated 2-dimensional separations (such as MUDPi) with complete control of the pumps, valves and autosampler through the easy to use Eksigent Control Software.

In (Figure 10a) the flow rates of the A and B mobile phases (black and red, respectively) are shown along with a UV absorbance trace (blue) showing the B phase composition. Each segment of the salt gradient can be followed by a reversed phase separation using the second dimension pumps. The measured flow rates (Figure 10b) and base peak chromatogram (Figure 10c) for separation of a BSA tryptic digest at 500 nL/min are shown.

### rapid sample loading

The NanoLC-1D Plus or NanoLC-2D systems can be configured to perform rapid sample loading onto a trap column using the high flow first dimension pumps followed by a reversed phase gradient separation on an analytical column using the low flow second dimension pumps. The NanoLC-2D can also be configured to operate as 2 parallel, completely independent binary gradient systems, providing ultimate flexibility.

Figure 11a. Rapid sample loading configuration allows one trap to be loaded while a second trap elutes onto the analytical column. 5  $\mu$ L of a BSA tryptic digest was loaded onto the first trap (PepMap) at 5  $\mu$ L/min using the first dimension pumps and then eluted onto the analytical column and the gradient separation performed at 500 nL/min (Figure 11a).

figure 11a.

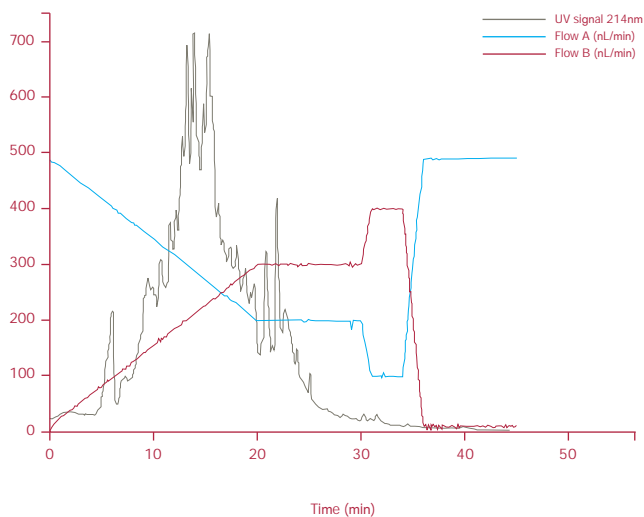
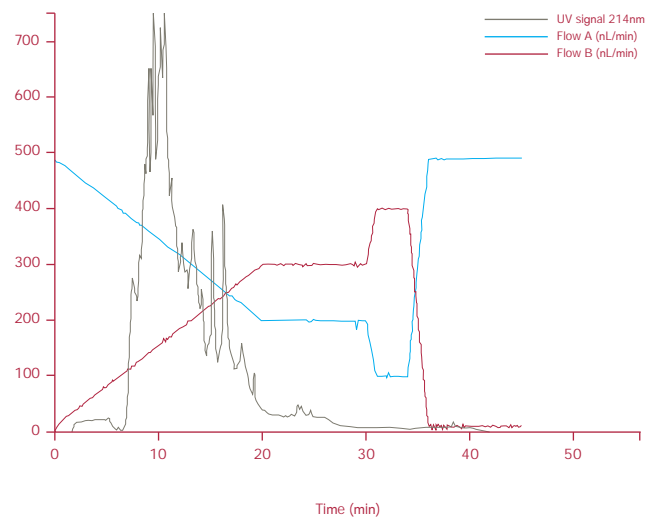


Figure 11b. While the gradient separation was running, a second 5  $\mu$ L sample was loaded onto a second trap (CapTrap) and then eluted onto the analytical column at 500 nL/min (Figure 11b).

figure 11b.



easy to learn,  
maintain and operate

amazing software

Eksigent's Control Software allows rapid setup of experiments. Methods can be created or modified in less than a minute. For automated 2D methods simply enter the beginning and ending salt concentration and total number of steps and the intuitive control software generates an entire run table in seconds (Figure 13). Additional specialized diagnostic tools make the system easy to learn, maintain and operate.

- Continuous monitoring of pressure, flow rate and solvent consumption
- Program gradients graphically or in table format
- View gradient composition and column backpressure in real-time
- Control of NanoLC pumps and autosampler under Xcalibur®, Analyst®, and HyStar® software packages
- Built-in automated diagnostics (Figure 14)
- Works with any mass spectrometer

Figure 12. The main screen displays the gradient, both programmed (gray) and actual (blue and red). This screen shot was taken mid-run during peak parking.

figure 12.

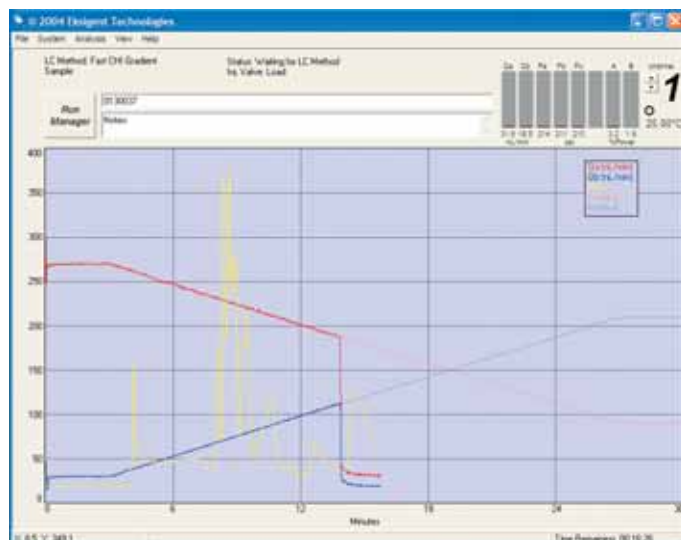
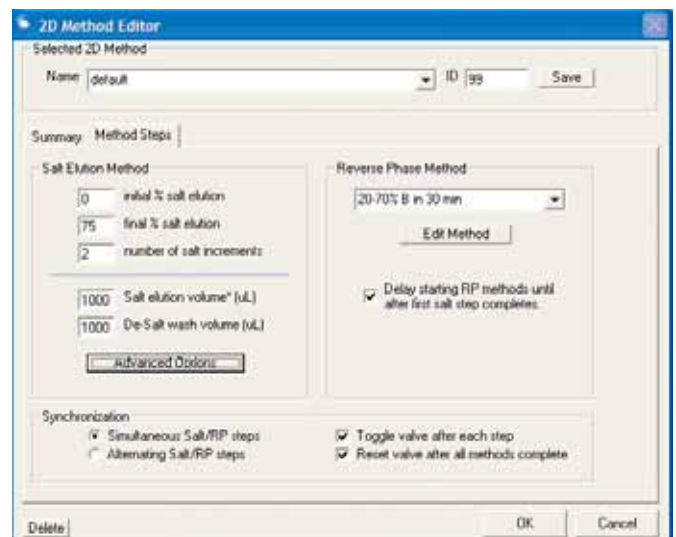


Figure 13. The 2D Method Editor makes it easy to program both channels for a 2D separation. A 2D method can be created simply by specifying the desired initial and final percentages of salt and the total number of steps, as well as the method to be used for the reversed phase separation in the second dimension.

figure 13.



### autosampler integration

A compact, flexible and easy to operate autosampler mounts on top of the NanoLC system. The Eksigent control software can be used to fully program and control the Eksigent NanoLC-AS1 Autosampler. Eliminate the problems that come with the large sample volumes in proteomics by integrating rapid and metered sample injection functions directly in the software.

### integration with mass spectrometer software

Full integration of instrument control for the NanoLC-1D Plus system, NanoLC-2D system and NanoLC-AS1 autosampler is available for Thermo Electron's Xcalibur® software, versions 1.3 or higher, and for ABI/Sciex's Analyst® software, version 1.4.1, and Bruker Daltonics' HyStar® software, version 3.2.

### low solvent waste

In addition to minimized mobile phase preparation and waste generation, direct pumping allows experimental conditions (e.g. column format) to be changed easily without the need to reconfigure flow-splitting hardware.

Figure 14. Internal diagnostics monitor and alert the user to system problems, including leaks and blockages. The system tracks usage information such as number of sample injections and flow meter usage. Automated diagnostics are provided for flow controller tuning and flow meter calibration.

figure 14.



Figure 15. The NanoLC-AS1 autosampler accommodates 96-well trays or a 48-vial holder. The autosampler includes Peltier cooling, biocompatible flow paths, and a variety of sample methods to handle any proteomics applications.

figure 15.





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## nanoLC system specifications

### configuration

**nanoLC-1D plus 1-Dimensional system:** Includes binary gradient pump, dedicated sample loading pump, 10-port column switching valve, and temperature controlled autosampler with 6-port bio-compatible sample injection valve.

**nanoLC-2D 2-Dimensional system:** Includes 2 independent binary gradient pump systems with a 10-port column switching valve accommodating 2 trap columns and an analytical column, and an integrated temperature controlled autosampler with 6-port bio-compatible sample injection valve.

### flow rate range

**nanoLC-1D plus system:** 20–1000 nL/min for gradient pump, 1-30  $\mu$ L/min for sample loading pump

**nanoLC-2D system:** First dimension 1–20  $\mu$ L/min, second dimension 20–1000 nL/min

### pump type

Microfluidic direct pumping system with independent flow control feedback for each mobile phase. Retention time RSD < 0.5% at 500 nL/min

### gradient formation

High pressure gradient mixing, no flow splitting

### delay volume

**nanoLC-1D plus system:** 65 nL from mixer to column. Delay time <25 sec at 500 nL/min using 5 cm column and low port-to-port volume injection valve.

**nanoLC-2D system:** First dimension 300 nL from mixer to column. Low flow dimension 65 nL from mixer to column.

### mobile phase compatibility

All mobile phases compatible with 316 stainless steel, PEEK, and silica

### injections

Standard, metered and rapid injection modes. Sample injection volumes 200–50  $\mu$ L. Option for larger sample volumes available.

### columns

**nanoLC-1D plus system:** Optimized for use with 50–150  $\mu$ m i.d. columns, 5–15 cm in length

**nanoLC-2D system:** First dimension is optimized for use with 300–500  $\mu$ m i.d. traps or columns (5 cm). Second dimension 50–150 micron id columns, 5–15 cm in length

### peak parking

Peak parking flow rate user settable, as low as 5 nL/min. Triggered manually or through mass spectrometer.

### peak parking response

Response time from 500–100 nL/min, typically <4 seconds at spray tip. Response time can vary with external hardware configuration.

### autosampler

One 96- or 384-well plate capacity. Peltier type temperature maintained sample compartment, 5–40 °C; cooling capacity 12 °C below ambient. Dual needle design for seal piercing. Integral 6-port bio-compatible injection valve.

### system control

Computer with graphical user interface for control of all system parameters. Software allows import of run tables and creates CDF, text, and Excel files for data export and analysis. Tracking of instrument runtime, column usage, total injections, solvent usage, and error codes. System drivers available for Thermo Electron's Xcalibur and Applied Biosystems/MDS SCIEX Analyst 1.4.1 mass spectrometer software.

### dimensions

**nanoLC-1D plus system:** 14" (35 cm) wide, 15" (38 cm) deep, 8" (20 cm) high  
**nanoLC-2D autosampler:** Additional 18" (45 cm) in height

**nanoLC-2D system:** 18" (46 cm) wide, 22" (56 cm) deep, 8" (20 cm) high  
**nanoLC-2D autosampler:** Additional 18" (45 cm) in height

### computer

Additional lab space needed for keyboard, mouse and monitor