

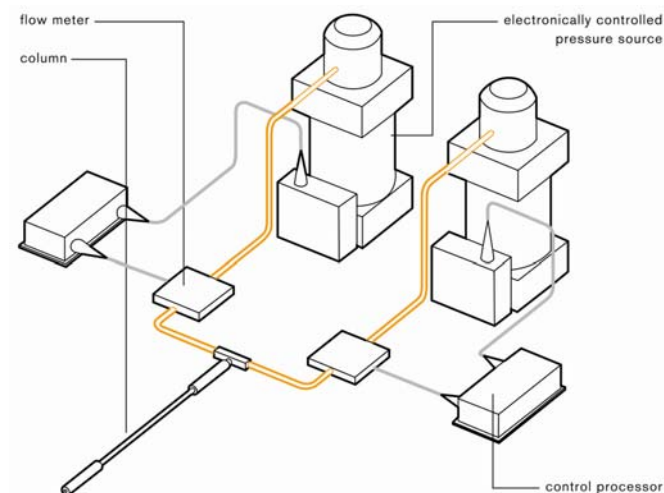
description of operation

introduction

Eksigent HPLC systems incorporate microfluidic flow control (MFC) to generate precise LC gradients at nanoscale and capillary flow rates. MFC has two primary benefits; first, it provides precise gradients at nanoliter-per-minute rates without flow splitting; and second, it can respond extremely rapidly to setpoint changes.

Flow meters in each mobile phase continuously monitor flow rate and feed a proportional signal back to a microprocessor (see Figure 1 for details). The microprocessor is a tunable PID (proportional/integral/derivative) controller that in turn sends out a voltage signal to the controller at the pressure source for each mobile phase. The signal is proportional to the pressure required in each mobile phase to achieve the desired flow rate or gradient. Pressure in the system is generated by connecting laboratory air or nitrogen to a pneumatic amplifier that produces a 36-fold amplification in pressure. The MFC controller regulates this pressure to generate the required flow rate. For example, 100 psi incoming air pressure from the laboratory air system can be used to produce a hydraulic pressure range extending from 0 psi to 3,600 psi.

Figure 1. Illustration of Microfluidic Flow Control (MFC) system components. The system allows precise, rapid control of nanoscale and capillary flow rates. Two MFC systems combine to form a binary gradient LC system.



microfluidic flow control

flow rate calculation

Flow rate in each mobile phase is determined by measuring differential pressure across a calibration module of known geometry, giving it a fixed “flow conductance”. The flow rate is given as:

$$Q = k \Delta P / \mu$$

Where Q = flow rate
 k = flow conductance
 ΔP = differential pressure
 μ = viscosity of mobile phase

The flow rate measurement is taken on pure mobile phase only. While these mobile phases may themselves be mixtures (e.g., water and acetonitrile), the mixture fractions, and therefore mobile phase viscosity, remain constant. To determine the viscosity in each mobile phase, functional forms that are suggested by theory are used to fit mixture dependent viscosity data and temperature corrections. System software stores mixture viscosity parameters in a database that includes a wide variety of frequently used mobile phases. The temperature at each flow meter is measured to allow determination of temperature-corrected viscosity. The viscosity calculations are accurate within the 20-30 °C limits of ambient temperature encountered in a typical laboratory environment. To verify system operation, the flow conductance k is measured and corrected using a software-automated flow meter calibration routine. This calibration is a standard part of the system maintenance program.

determination of pressure required to generate a gradient

The user specifies the total flow rate through the separation column, Q_{COL} , and volume fractions of mobile phase A, $v_A(t)$ as a function of time.

The volume fraction of phase B, $v_B(t)$, is:

$$v_B(t) = 1 - v_A(t)$$

The flow rate of each phase ($Q_A(t)$ and $Q_B(t)$) is given by the volume fraction of each phase multiplied by Q_{COL} .

The pressure at the head of the separation column, P_{COL} , is measured using a microscale pressure transducer.

Knowing the viscosities, resistances, and flow rates of each mobile phase along with the measured $P_{\text{COL}}(t)$, the set point pressure of each phase can be calculated:

$$P_A(t) = \mu_A / k_A Q_A(t) + P_{\text{COL}}(t)$$

$$P_B(t) = \mu_B / k_B Q_B(t) + P_{\text{COL}}(t)$$

The microfluidic flow controllers maintain these set points and adjust the pressure to run the designated gradient. As an example, a linear gradient from 95% A to 20% A over 30 minutes (see Figure 2 on the next page).

microfluidic flow control

dynamic flow control capability

In addition to precise control of gradients at nanoscale flow rates, the rapid pressure control of MFC also allows the flow rate to be changed dynamically during a gradient run. For example, the mass spectrometer may see multiple co-eluting peptides that will require more than the typical peak width to allow MS/MS on all peptides. The mass spectrometer can then send a signal to the NanoLC, and the NanoLC can lower the gradient flow rate rapidly, or peak park, to increase the mass spectrometer's acquisition time. Because the mass spectrometer in nano electrospray is concentration sensitive rather than mass sensitive, this change in flow rate does not degrade sensitivity. After the peptides have been analyzed, the NanoLC can resume the gradient's normal flow rate, without degrading the resolution of downstream peaks. See Figure 3.

Figure 2. Example of pressures used to generate gradient flow rate. This is typical of water/acetonitrile gradients, where the column experiences a maximum pressure partway through the gradient.

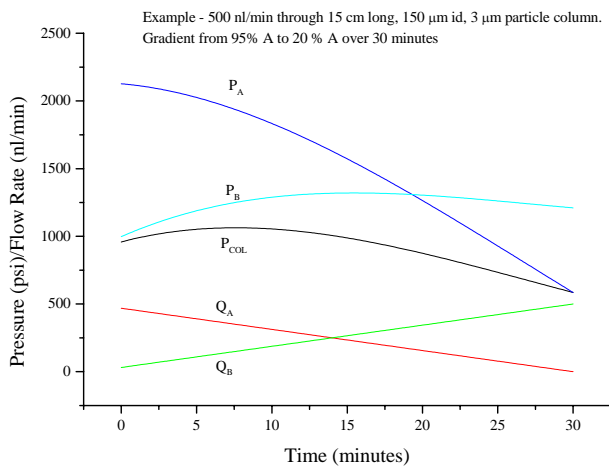
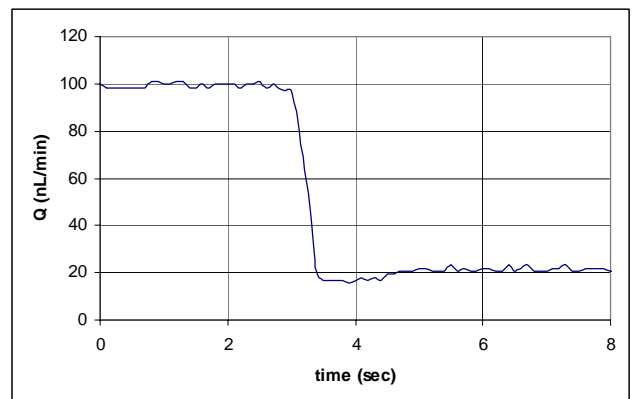


Figure 3. Flow rate lowered from 100 nL/min to 20 nL/min in less than 1 second at the flow meter. Typically column capacitance delays this effect less than 2 seconds at the spray tip.





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expressLC system specifications

configuration

expressLC-100 single-channel system: Includes binary gradient pump, electronic injection valve, column temperature control, and array-based UV detection system. Optional high-speed autosampler available.

expressLC-800 8-channel parallel system: Includes 8 binary gradient pumps, 8 electronic injection valves, 8 column temperature control compartments, an array-based UV detection system and high-throughput autosampler.

flow rate range

0.20–30 $\mu\text{L}/\text{min}$

pump type

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

gradient formation

High pressure gradient mixing. System can run full gradients as rapidly as 8 seconds. Maximum gradient length 2 hrs. at 5 $\mu\text{L}/\text{min}$.

delay volume

< 500 nL from mixer to column.

mobile phase compatibility

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

injection valve

Eksigent Variable-Volume Injection System (software selectable). Standard injection volume 10–250 nL (larger injection volumes available).

columns

System optimized for 2.5–15 cm, 300 μm i.d. capillary LC columns

column temperature control

Software selectable from 27–40°C; stability within $\pm 0.1^\circ\text{C}$

detection

UV absorbance detection from 200–380 nm using linear CCD array detector. Detector drift $\leq 4 \times 10^{-4}$ AU/hr Non-linearity $\leq 5\%$ @ 2 AU.

flow cell

45 nL microfabricated flow cell with integral fiber optics, 4 mm path length

autosampler

High-throughput CTC autosampler available

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, lamp hours, and error codes. System drivers available for Thermo Electron's Xcalibur and Applied Biosystems/MDS SCIEX Analyst 1.4.1 mass spectrometer software.

report features

Generates reports that include method conditions, chromatograms, peak retention times and areas, and spectral absorbance map.

dimensions

expressLC-100 system:

21" (53 cm) wide, 20" (51 cm) deep, 18" (46 cm) high

expressLC-100 autosampler:

Additional 14" (36 cm) high and 6" (15 cm) wide

expressLC-800 system:

30" (76 cm) wide, 34" (86 cm) deep, 40" (102 cm) high

expressLC-800 autosampler:

Additional 16" (41 cm) high and 16" (41 cm) wide

computer

Additional lab space needed for keyboard, mouse and monitor