Technical Note

Performance Attributes of the Express-800 Multiplexed HPLC

While many analytical assays have been adapted to HPLC formats, widespread use of these assays has been limited by the relatively slow, serial nature of HPLC. The development of a rapid, multiplexed high resolution HPLC system provides a platform for dramatic increases in analytical HPLC throughput. By combining advances in microscale fluid delivery, small volume injections, mixing, and optical detection, a rapid gradient chromatography system has been developed. This system provides injection-to-injection cycle time improvements of as much as 5X compared to conventional (4.6 mm column) HPLC systems. By combining this system in an independent, 8-channel multiplexed format, sample throughput of 40X conventional HPLC has been achieved.

Materials and Methods

The assays described were run on the Eksigent Express-800 HPLC system. This system is a fully integrated 8-channel gradient HPLC system. Each channel includes separate mobile phase, pumps, injectors, columns, and array-based UV absorbance detectors. Sample input ports for each channel are individually addressable in the software system. The Express-800 system may be operated in high throughput (all samples run the same method) or high content mode (each channel is a different method). The system is optimized for use with 300 micron i.d. columns, which are now available as standard columns from most manufacturers. Sample is delivered to each injection port by filling a 300 nl sample loop in the injection valve. A timed injection delivers sample quantities ranging from 10 nl to the full loop volume. Detection is by UV absorbance through a microfabricated flow cell. Path length is 4 mm, and detection wavelength is fully dispersed UV from 200-380 nm.

Figure 1. The Eksigent Express-800 HPLC includes 8 fully independent HPLC systems and a high throughput autosampler. The system allows extremely rapid gradients in a multiplexed format, delivering sample through-put increases of as much as 40x compared to conventional HPLC systems.

Experimental and Results

Tests were run to determine flow control precision, sample throughput, and solvent consumption.

Flow control precision

Linear gradient flow precision tests were run to determine flow precision of 8 channels running simultaneous gradients. Flow precision was determined by monitoring and recording flow rate utilizing the system's internal flow meters. A and B mobile phase flow rates in each channel are monitored independently. A total column flow rate of 10 µl/min is programmed, with a gradient from 10% B to 90% B, then immediately back to 10% B. The system's ability to maintain precise flow rates in each mobile phase, with an ability to "turn corners" rapidly at transitions, is monitored. A second test that illustrates precise control is a rapid gradient run on 6 phenones. The gradient is run at 20 µl/min column flow rate, with a gradient from 45%-95% B in 15 seconds.

Flow rate recordings for all 8 channels running simultaneously are shown in Figure 3. The graphs show the measured flow rate in each mobile phase in each channel. Flow precision within each channel and between channels is clearly demonstrated. The Express system's ability to "turn the corner" at gradient transitions is a good example of the system's dynamic flow control responsiveness.

Rapid gradient flow control capability is shown in Figure 2, where 6 compounds are baseline resolved in under 20 seconds using a 15 second gradient from 45%-95% B.

Solvent consumption

Solvent consumption in the 8-channel system was determined by calculating total effluent volume from the system, based on continuous flow at 20 µl/min. This value is compared against the comparable value for 8 independent conventional systems utilizing 4.6 mm columns.

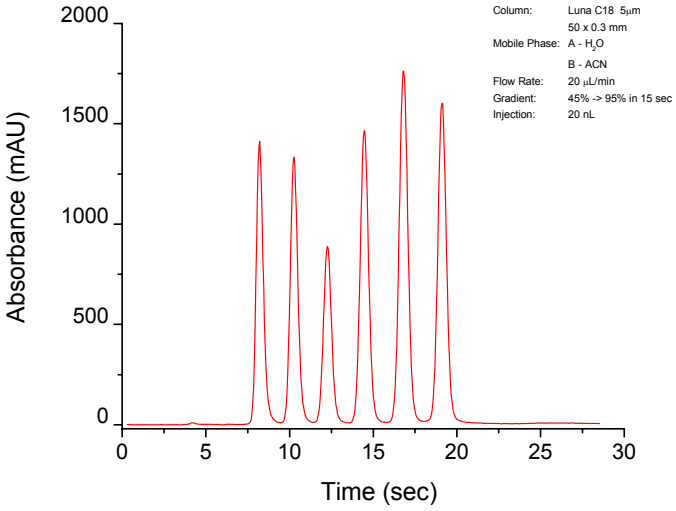

Figure 2. Demonstration of fast, high resolution gradient separation on the Express-800 system. Six phenones are baseline resolved in under 20 seconds.

Solvent consumption for the Express-800 system is 230 ml per day (20 µl/min x 8 x 1440 minutes/day). Equivalent solvent consumption on 8 4.6 mm systems is 54 liters (5 ml/min x 8 x 1440 minutes/day) at the same linear column velocity. This represents a 200-fold reduction in solvent consumption.

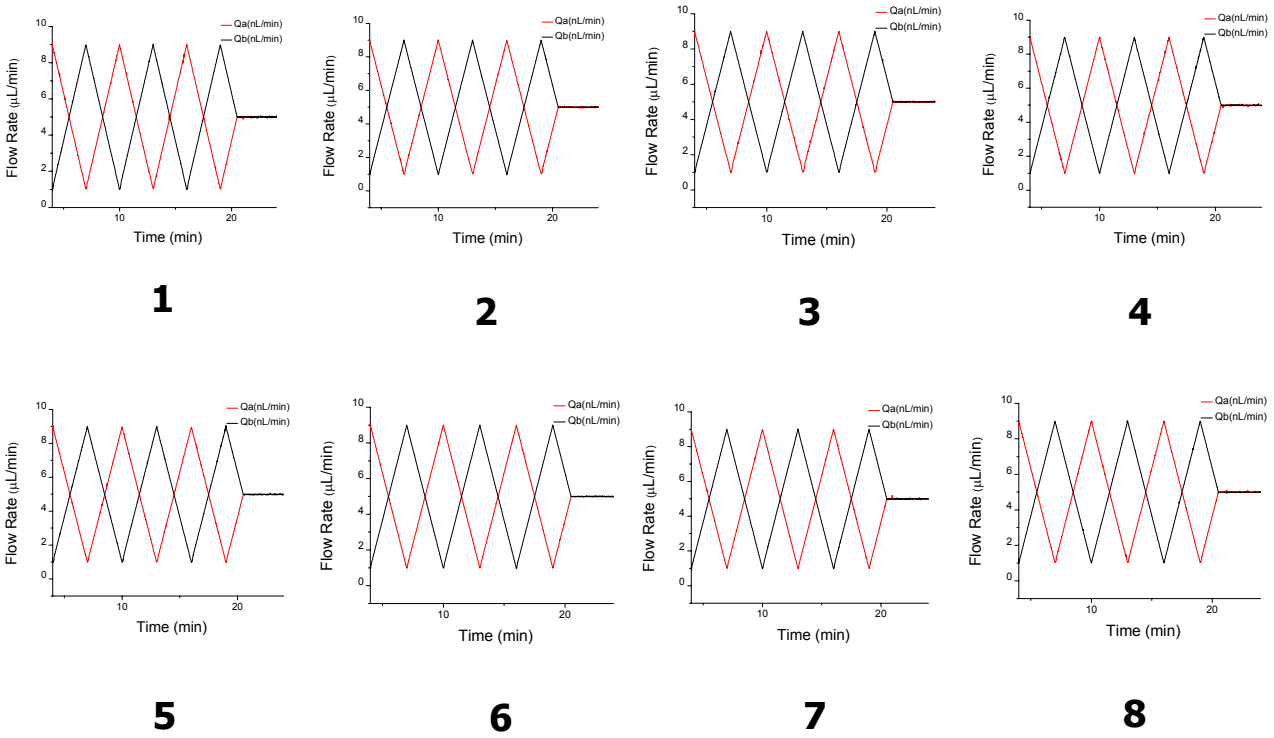

Figure 3. Flow rate data for all 8 channels running simultaneously is displayed. Total column flow rate is 10 µl/min, and the flow profile ramps from 10% B to 90% B and then back. Gradients ramps are run 5 times, with the final flow rate at 50% A/B. Precision within each channel and between channels is clearly evident. The ability for the system to rapidly "turn the corner" at gradient transitions is also demonstrated.

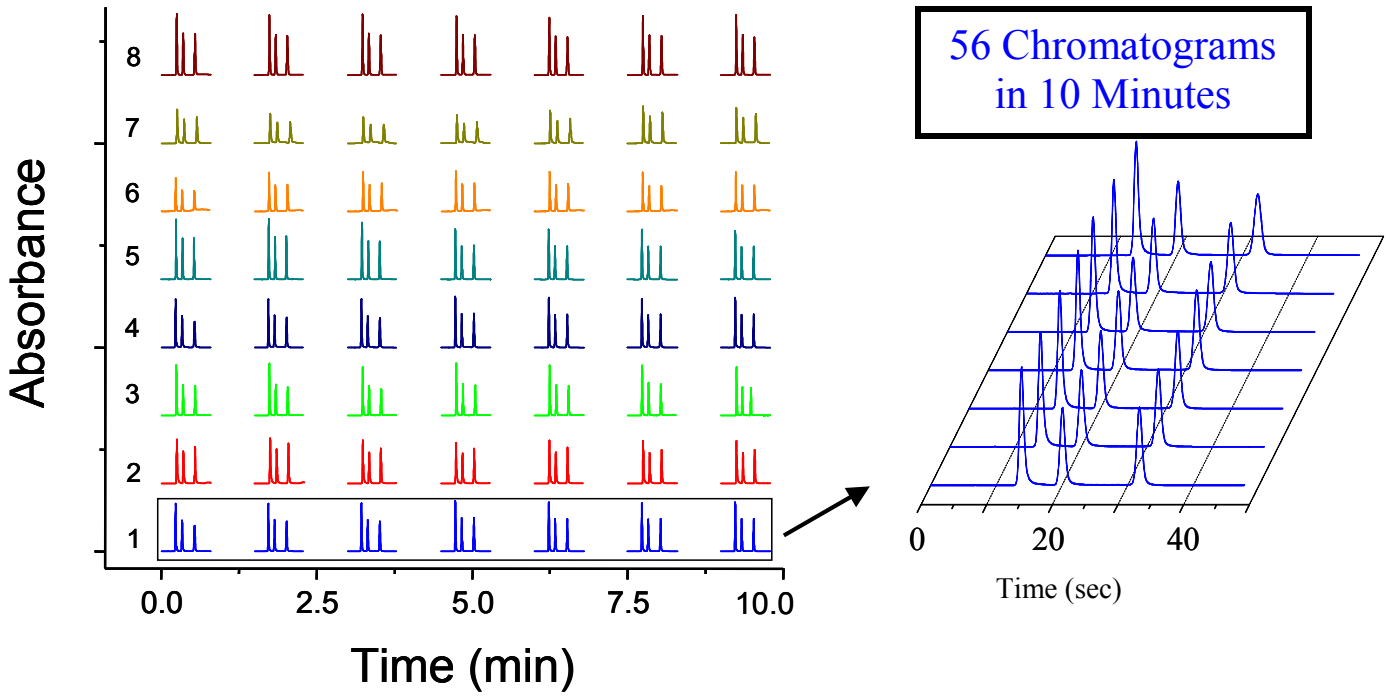

Figure 4. Data from a rapid (25 second) gradient with 20 second hold and 20 second equilibration was collected on all 8 channels simultaneously. The 65 second injection-to-injection cycle on each channel resulted in a total of 56 separate runs during 10 minutes of operation. This translates to more than 300 separations per hour.

Sample Throughput

Sample throughput was determined by operating the Express-800 in high throughput mode, running the same method on each channel. A 30 nl sample of 3 phenones was injected into each channel using the Express system's software-programmed injection system. The method utilized for these experiments uses a 5 cm long column with 5 micron Luna C18(2). The flow rate is 12 µl/min, with a gradient from 65 to 95% ACN in 25 seconds, with a 20 second hold and a 20 second equilibration. Total cycle time from injection to injection is 65 seconds. The system was operated for 10 minutes, with 7 injections occurring in each channel.

Chromatograms for 10 minutes of operation across all 8 channels are shown in Figure 4. The system completed 56 chromatograms in this period. Operating fulltime, this yields more than 300 runs per hour, and more than 8,000 separations per day.

Conclusion

A rapid, high resolution, multiplexed system has been developed for high throughput HPLC. The system offers dramatic increases in separation speed, and, when combined with multiplexing, delivers throughput increases of as much as 40 times when compared to conventional HPLC.

Eksigent Express-800 HPLC System Specifications

Feature	Specification
Configuration	8 independent HPLC systems including binary gradient pump, injection valve, column temperature control, UV detection system, integrated with high throughput autosampler.
Flow rate range	200 nl/min to 20 µl/min.
Pump type	Microfluidic direct pumping system with independent flow control feedback for each mobile phase. Tunable PID flow rate feedback control. Retention time precision <0.3% RSD.
Gradient formation	High pressure gradient mixing, no flow splitting. System can run full gradients as rapidly as 8 seconds. Maximum gradient length 2 hrs. at 5 µl/min.
Delay volume	300 nl from mixer to column.
Mobile phase compatibility	All mobile phases that are compatible with 316 stainless steel, PEEK, and silica. Mobile phase stored in removable glass storage vials.
Injection valve	Eksigent Variable Injection System. Sample injection volume 10 nl to 300 nl (software selectable). Injection precision 0.5% RSD at 40 nl.
Columns	System optimized for use with 300 µm i.d. columns, from 2.5 cm to 15 cm
Column temperature control	Temperature stabilized at 27-40 °C based on 22 °C lab temp. Software selectable. Temperature stability +/- 0.1 ºC
Detection	Fully dispersed UV absorbance detection at 200 – 380 nm using linear array detector. Detector noise 5 x 10^{-5} AU rms @ 250 nm, 1 s averaging. Detector drift less than 4 x 10^{-4} AU/hr. Linear dynamic range > 10^{4}.
Flow cell	45-nL microfabricated flow cell with integral fiber optics, 4 mm path length.
Autosampler	Integrated autosampler available on both systems.
System control	Computer with graphical user interface for control of all system parameters. Software creates CDF, text, and Excel files for data export and analysis.
Enclosure size	Approximate dimensions 30” (76 cm) on all sides.
Report features	System automatically generates printable reports including method conditions, chromatogram, peak retention time and area, and absorbance map for full chromatogram.
GLP Features	Tracking of instrument runtime, column usage, total injections, solvent usage, lamp hours, and error codes. System is 21 CFR Part 11 compliant.

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