

Fast LC using a new generation of ultra high pressure, microflow HPLC instrumentation

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Abstract

The need for instrumentation for faster liquid chromatographic separation continues to grow as the demands for high throughput analytical methods increase. Higher operating pressures and smaller stationary phase particles currently available allow separations to be performed in a fraction of the time formerly required using conventional LC. The higher flow rates typically used for small particle columns lead to a substantial increase in solvent consumption and waste generation. We have developed an ultra-high pressure LC system (ExpressHT-Ultra) designed specifically to work with small bore columns (between 0.3 and 1 mm IDs), capable of delivering 10,000 psi of pressure. The ExpressHT-Ultra system incorporates Microfluidic Flow Control technology that ensures accurate flow rate and precise gradient delivery profiles. The system provides fast, low-flow separations with rapid cycle times that can save over 90% of the mobile phase used by a conventional LC system. Applications of the new UHPLC system which demonstrate increased throughput and performance with mass spectrometric detection are presented. A particular emphasis of the studies presented will be the generation of chromatography using small diameter ($\leq 3 \mu\text{m}$) particle columns.

Microfluidic Flow Control at ultra high pressures

The ExpressHT-Ultra's solvent delivery system is based on a binary gradient pumping system designed for HPLC columns with diameters of 1 mm or smaller. A benefit of small diameter columns is a reduction of the required mixing volume. Less volume means shorter gradient delays and faster separations. Column and system re-equilibration also take much less time. The net result is a system with cycle times as short as 60 seconds for high analytical throughput. The entire system is designed to operate at pressures up to 10,000 psi. This allows the use of columns with small diameter stationary phase particles (1.5 to 3 μm) delivering fast, high resolution separations

Eksigent's Microfluidic Flow Control ensures precise and stable flow rates. With MFC technology, direct pumping is used to deliver accurate, repeatable gradients. Each pressure source delivers fluid with the actual flow rate monitored using the flow modules in each channel. A feedback loop makes real-time adjustments to the flow rate. By continuously monitoring the flow from each of the binary system's pumps, the flow rate can be adjusted many times per second. The result is retention times with an RSD typically below 0.3%.



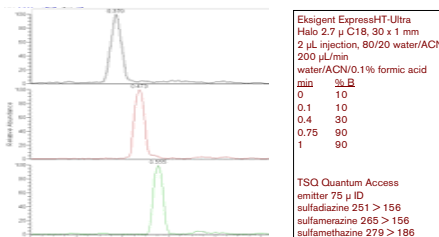
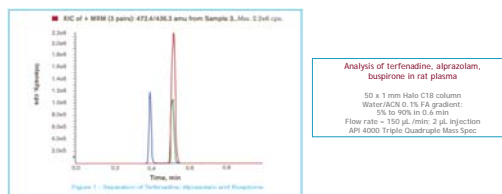
The ExpressHT-Ultra system for ultra high pressure LC/MS.

Schematic of the Microfluidic Flow Control (MFC) setup. In-line flow meters provide high speed measurements of the delivered flow rate to a built-in control processor. The processor performs real-time feedback control of the electronically controlled pressure sources to provide accurate flow for microflow LC.

High performance chromatography with rapid cycle times allows fast LC/MS/MS for bioanalysis

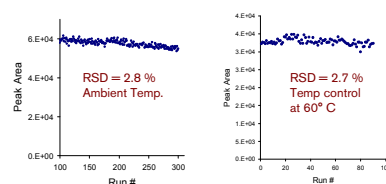
Fast LC/MS/MS using triple quadrupole mass spectrometers is used for many bioanalytical methods monitoring potential drug compounds. SRM monitoring of compounds in biological matrices is conducted in both discovery, preclinical, and clinical evaluation of compounds. Fast separations and cycle times allow for increased throughput to address an increasing sample load.

A typical measurement of three different compounds in precipitated rat plasma is shown below. Separations were conducted with a 1 mm x 50 mm column packed with 2.7 μm diameter HALO particles.



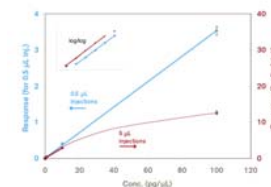
Baseline separation of three sulfa drugs in less than one minute demonstrating high chromatographic speed. A newly developed, high pressure active wash system is used to wash the injection valve, sample loop, and autosampler syringe. This allows one to maintain low carryover with cycle times less than 60 seconds.

Robust LC using microbore columns



The figures above demonstrate the robustness of the ExpressHT-Ultra system. Peak area repeatability over many injections at ambient and elevated temperatures are shown.

Variable injection volumes to extend quantitation

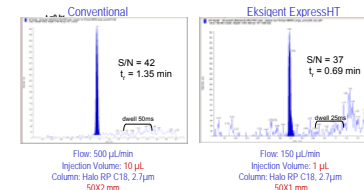


Calibration curves for haloperidol in 80/20 water/acetonitrile with verapamil as the internal standard. An ExpressHT-Ultra was used in conjunction with an ABI API 5000 triple quadrupole mass spectrometer. MRM transitions were m/z 376 > 165 and 455 > 165 for haloperidol and verapamil, respectively. The sample concentration range is from 1 $\mu\text{g}/\text{L}$ to 100 $\mu\text{g}/\text{L}$, and the internal standard concentration is 3 $\mu\text{g}/\text{mL}$.

Precise and accurate pump flow control, in combination with a high speed injection valve, allow for programmable metered injections with volumes as low as 100 nL. The filled injection loop is switched to the inject position for a pre-determined length of time to displace the programmed amount of sample. This feature can be extremely useful in cases where the upper dynamic range of a linear calibration curve is insufficient to cover samples of unexpected high concentration. Instead of diluting the entire series of samples and re-analyzing them, one can simply re-inject the same samples using a smaller injection volume. As illustrated at left, by reducing the injected volume from 5 μL to 0.5 μL , the upper linear dynamic range is effectively extended by an order of magnitude. There is no need for additional sample dilution steps or exchange of the injection loop.

High sensitivity and quantitation using smaller sample volumes and faster separations

S/N Ratio Comparison of Verapamil at 1 ng/mL



Comparable S/N ratios are measured comparing a more conventional separation using 2.1 mm columns and the ExpressHT-Ultra operating with a 1 mm diameter column. The separation on the ExpressHT-Ultra is conducted in half the time, with 1/10 of the sample volume, and substantially lower flow rates and solvent consumption. MS detection was conducted on an API 4000 equipped with a TurboV ESI interface. For the ExpressHT measurements, a newly developed low-volume ESI electrode assembly was used.

Data provided by H. Skor, D. Gale, and R. Rahavendran, Pfizer Global Research & Development-La Jolla, CA

Conclusion

An ultra high throughput micro LC system for LC/MS analysis has been developed. Variable injection volumes allow increased quantitation ranges without additional sample preparation. Excellent performance for bioanalytical methods has been demonstrated, providing sensitivity, robustness, and reliability while using less than 10% of the solvent used by a conventional system.

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