

Fast LC using a new generation micro-UHPLC system with array based UV detection

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Abstract

The commercial need for instrumentation for faster liquid chromatographic separation continues to grow as the demands for high resolution and throughput analytical methods increases. Higher operating pressures and smaller stationary phase particles currently available allow conventionally based separations to be performed in a fraction of the time formerly required. However, the theoretical repeatability and increases in efficiency are often not obtained with standard analytical columns (2.1 mm and greater IDs). In addition, the higher flow rates 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 designed specifically to work with small bore separation columns (between 0.5 and 1 mm IDs) which is capable of delivering 10,000 psi of column pressure. The ExpressLC-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. The system features a newly developed, microfabricated UV flow cell and CCD-based spectrometer that are optimized for sensitivity in microflow separations. Examples showing quantitative, low level impurity profiling using the new UV detector, and repeatable chromatography using small diameter (< 3 μm) particle chromatographic columns operated at a range of elevated column temperatures are presented.

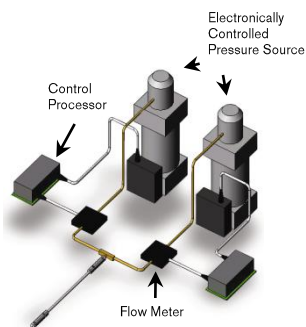
Introduction

A micro UHPLC system, the ExpressLC-Ultra, was developed that provides sensitive UV absorbance detection and fast, high performance separations in a compact instrument. The system consists of (1) a binary gradient pump with solvent selection option capable of delivering 5-50 μL/min flows (for use with 0.5 mm columns) at 10,000 psi, (2) an integrated injection valve capable of 0.1 to 10 μL injection volumes, (3) an industry standard CTC autosampler with high sample capacity and fast wash station, (4) a fast response column oven (temperatures up to 80°C), and (5) a sensitive and quantitative array-based UV absorbance detector with a microfabricated, small volume (90 nL) flow cell. With this system, many advantages of conducting separations at micro flow rates in small bore columns can be gained without sacrificing the performance required for today's separations.

Microfluidic Flow Control at ultra high pressures

The ExpressLC-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 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%.



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.

State-of-the-art analytical performance

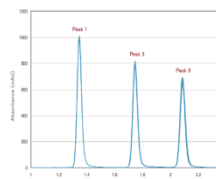
There are many advantages to using small bore columns and low flow rates for performing HPLC and UHPLC separations. These include (1) low volume gradient mixing leading to faster separations and cycle times, (2) higher linear velocities for a given pressure, (3) higher peak concentrations with limited sample mass, (4) reduced frictional heating challenges at UHPLC pressures, (5) and a great reduction in the volume of mobile phases and samples. However, micro-LC has often been accompanied by compromises in performance or ease-of-use. The ExpressLC-Ultra is designed to provide the advantages above, but while maintaining the state-of-the-art performance needed in today's pharmaceutical and analytical laboratories.



Photograph of the ExpressLC-Ultra system for ultra high pressure micro-LC. The system provides automated solvent selection for a 10,000 psi binary gradient delivery system, a unique small volume sample injection system, an industry standard autosampler with high sample capacity, a high performance column oven, and a new, sensitive CCD based UV detection system.

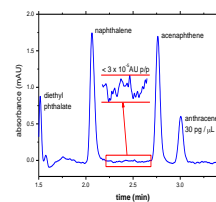


A microfabricated 90 nL (5 mm pathlength) flow cell employing fiber optic coupling provides sensitive detection for quantitative UV absorbance measurements in micro-LC. The cell, which is easily exchanged on the instrument, provides very low peak broadening. When combined with the newly designed CCD-based detector, the system provides low UV noise, and linear response to greater than 2AU. A 10mm pathlength cell (180 nL) has also been used.



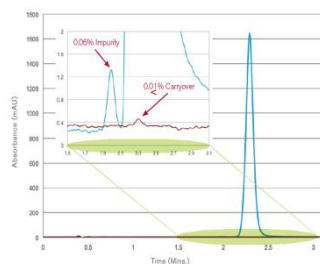
Peak	Peak Area (%RSD)	Retention Time (%RSD)
1	0.49	0.11
2	0.62	0.19
3	0.67	0.18

Overlaid chromatograms from thirty repeated separations conducted on the ExpressLC-Ultra. Excellent precision, as indicated in the table, is measured for both retention times and peak areas. Separation conditions: ChromXP C18-EP120 column (0.5 x 50 mm); 15 μL/min; mobile phase A: H₂O with 2% ACN, mobile phase B: ACN; gradient from 40 to 90%B in 3 minutes; 300 nL injection of acetophenone (peak 1), propiophenone (peak 2), and butyrophene (peak 3). UV data collection: 242 nm peak; 10 nm bandwidth; 10 Hz.



Partial chromatogram from a 120nL injection of a mixture including 0.03 μg/mL anthracene; 0.5x150mm ChromXP C18 3 μm column at 20 μL/min, 254 ± 5nm, 10 Hz data rate, 5 mm pathlength flow cell. Inset shows an enlargement of the baseline noise levels.

Impurity profiling and low level carryover measurements

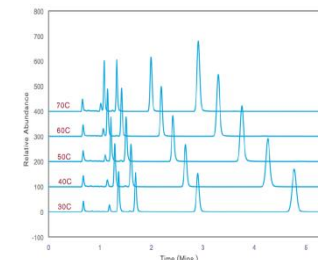


Combining the low noise and the highly linear response of the UV detection system in the ExpressLC-Ultra, low level impurity analysis can be conducted. In one separation, a main peak (naphthalene) with an intensity of 1600 mAU is measured along with a minor impurity peak with an intensity of 1.1 mAU shown in the inset. Peak quantitation by peak area indicates a 0.06% impurity. For this separation the impurity has a S/N of approximately 22 indicating a potential LLOQ of approximately 0.03% and a LLOD less than 0.01%. Also shown is a blank injection run immediately after the separation shown. Carryover for the naphthalene at a retention time of 2.3 minutes is measured to be less than 0.01%. Separation conditions: ChromXP C18-EP120 column (0.5 x 50 mm); 15 μL/min; mobile phase A: H₂O with 2% ACN, mobile phase B: ACN; isocratic at 60%B; 125nL injection; 15 μL/min. UV data collection: 214 nm peak; 10 nm bandwidth; 10 Hz acquisition.

Rapid thermal control for improved separations



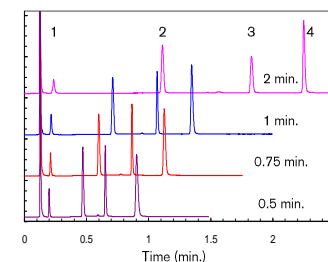
The front panel of the ExpressLC-Ultra is designed to allow easy connections of user configurable plumbing. The 1/2" OD tubing and fingertight fittings provide ease-of-use even for UHPLC pressure separations. Connections to the column oven and the UV flow cell can be made easily with very low volumes providing minimal bandbroadening even for the fastest, high performance separations.



Temperature effect on separation. Isocratic separation of test mix at five different column temperatures from 30 to 70 C conducted in an automated fashion. Separations were conducted sequentially at each temperature with excellent reproducibility. Less than 10 minute equilibration is needed between each temperature change.

Micro-LC for rapid gradient mixing and fast separations

Micro-UHPLC provides excellent opportunities for conducting fast separations and reducing injection-to-injection cycle times. The separations shown below demonstrate the separation power and speed of the ExpressLC-Ultra system. This reverse phase test mixture includes a very hydrophilic compound (8-bromoguanosine), a very hydrophobic compound (dioctyl phthalate), and a pair of compounds in between with similar hydrophobicities (diethyl phthalate and dipentyl phthalate). As shown, with the ExpressLC-Ultra one can achieve good peak shapes across the full range of compounds and excellent resolution between the two middle peaks. With the minimized dead volume in the system, the weakly retained 8-bromoguanosine elutes in ~0.2 minutes consistently. The reduced gradient delay also allows gradient focusing of the highly retained dioctyl phthalate even for the 30 second gradient. Note that the FWHM of the 8-bromoguanosine peak is only 0.4 seconds during the 30 second gradient.



Separation of chromatographic test mix as a function of gradient times for gradients from 2 to 0.5 minutes.
Conditions
Column 50x0.5mm, 2.7 μm Halo C18; col. temp. 30C
Flowrate 45 μL/min; gradient 5 to 95% ACN in time listed
Metered injection: 100nL
Detection: 250nm, 10nm bandwidth, 30Hz; 5 mm cell
Peak 1: 8-bromoguanosine
Peak 2: diethyl phthalate
Peak 3: dipentyl phthalate
Peak 4: dioctyl phthalate

Conclusions

An ultra high performance micro LC system for LC/UV analysis has been developed. The system uses Microfluidic Flow Control to provide accurate gradients from 5 to 50 μL/min at column pressures up to 10,000 psi. A newly developed UV absorbance detection system uses a spectrometer and CCD based detector to measure absorbance from 200 to 275 nm with acquisition rates up to 100 Hz. Coupled with a microfabricated flow cell the system provides very low UV baseline noise and is linear to over 2AU providing a large dynamic range for quantitation (including impurity measurements). Metered injections provide accurate, small volume injections over a settable volume range, and a column oven allows operation up to 80C and good thermal management—even for UHPLC separations. The system provides separation performance with high precision in both peak areas and retention times for isocratic and gradient separations. The small mixing and delay volumes of the micro LC system provide fast separations and fast cycle times while affording significant savings in mobile phase and solvent usage.