

# High Performance Capillary LC Analysis

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## Abstract

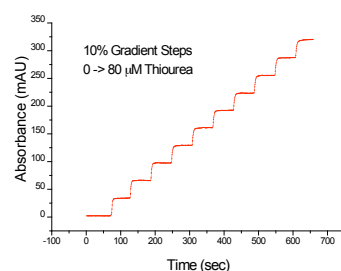
Capillary LC has tremendous potential for being the next major innovation in separatory analysis. The technique has demonstrated the ability to separate species with high resolution, unequaled speed and low sample and mobile phase consumption. Other techniques, notably capillary electrophoresis and supercritical chromatography, have historically shown superior resolution and speed when compared with conventional LC, but they have chronically suffered from inconsistent quantitative results. This presentation will address the advances that have been made in capillary LC and the ability to provide the quantitative data required in regulated pharmaceutical analysis.

## A New Generation $\mu$ Scale HPLC System

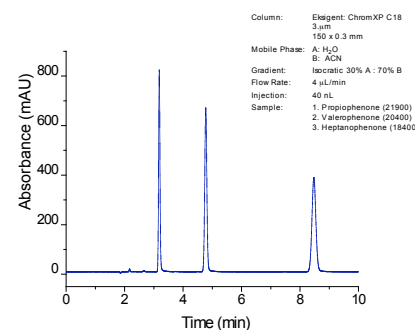
The assays described were conducted on the Eksigent ExpressLC-100 HPLC system which has been optimized for the use of 300  $\mu$ m ID columns. This HPLC system integrates mobile phase reservoirs, binary pumping with precision flow control, injector, column compartment, and array-based UV absorbance detector into a single package. Sample is introduced to a sample loop (typically 300 nL) on the injection valve using a high performance Leap/CTC autosampler. A timed injection delivers sample quantities ranging from 10 nL to the full loop volume. The system is optimized for use with 300 micron i.d. columns, which are now available as standard columns from most manufacturers. Detection via UV absorbance is accomplished using a microfabricated flow cell. The 45 nL flow cell has a path length of 4 mm, and a dual beam spectrometer monitors the UV absorption from 200-380 nm with 1 nm resolution. The output of the system can be easily coupled to a variety of MS interfaces while maintaining exceptional resolution.



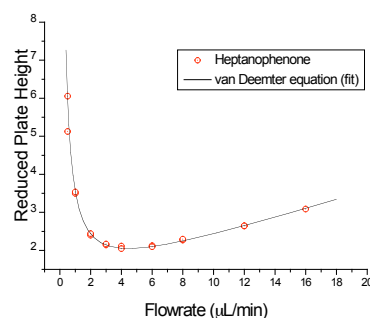
ExpressLC-100 capillary HPLC System.



Precision Flow Control

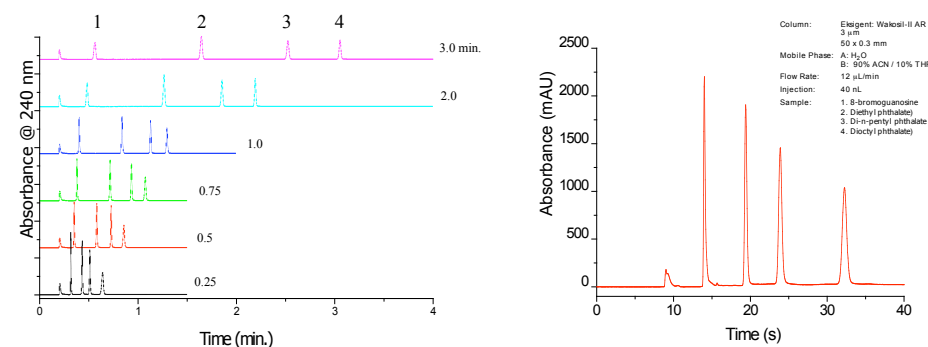


High Separation Efficiencies



## High Speed Separations

Rapid high pressure mixing, low dead volume injection valve and close component coupling provide delay volumes of ~ 300 nL. The small delay volumes combined with precision flow control and high separation efficiency of capillary columns allow fast analysis times.

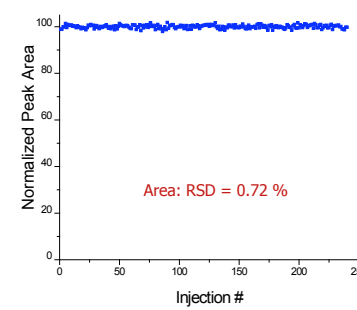
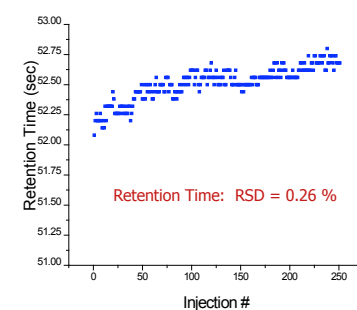
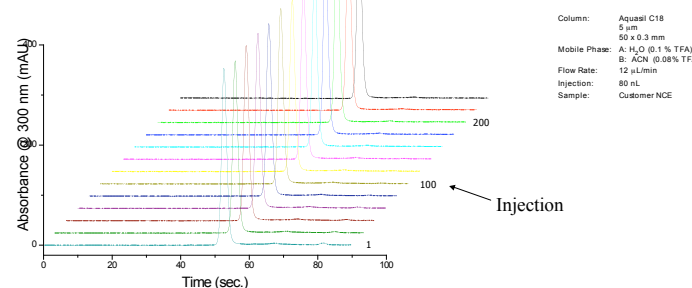


Varying the gradient slope allows optimization of methods for desired speed and resolution. Flow rate: 16  $\mu$ L/min. Gradient 2  $\rightarrow$  98% in selected times (shown in plot).

Sample: 1) 8-bromoguanosine, 2) Diethyl phthalate, 3) Di-n-pentyl phthalate, 4) Diocetyl phthalate

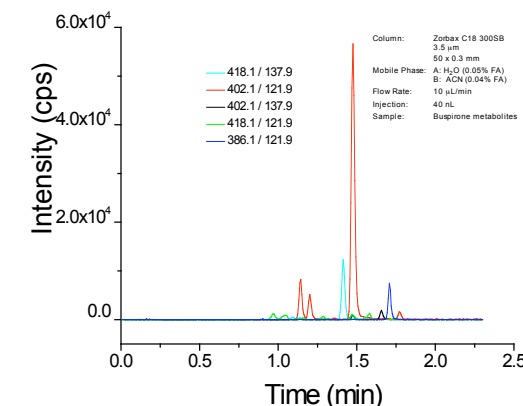
## System Repeatability

The combination of precision flow control and timed injections provides unprecedented repeatability for capillary HPLC. The long term repeatability is demonstrated below for 250 injections conducted over a 12 hour period.



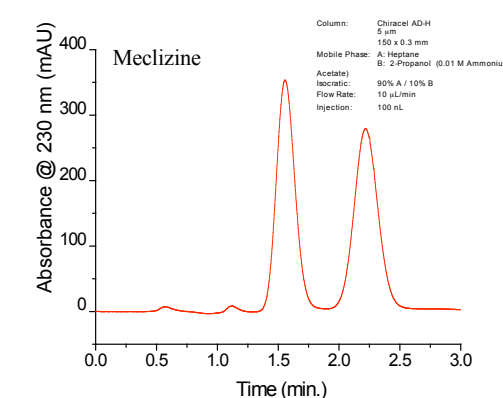
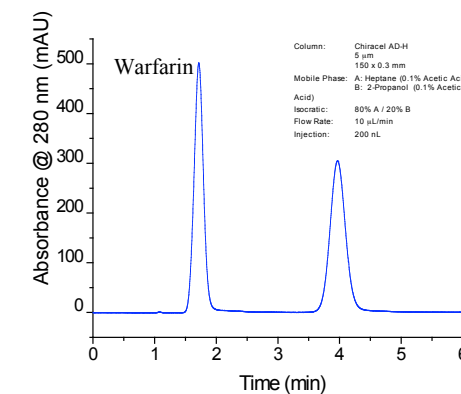
## LC/MS

LC/MS analysis has become increasingly important throughout drug discovery and development. Direct coupling of the full eluent from the UV detection cell to an electrospray interface can be accomplished with minimal loss of resolution to obtain exceptional mass sensitivity. Multiple reaction monitoring data collected on an API 2000 is shown for buspirone metabolites.



## Chiral Analysis

Increased sophistication of LC chiral analysis has come to the forefront of pharmaceutical analysis due to unique bioactivity of specific enantiomers. Often, the limited availability of pure enantiomers, high cost of conventional LC columns, low peak efficiencies, lengthy analysis times, and frequent conversion to normal phase chromatography has made pharmaceutical chiral analysis an analytical specialty field unto itself. Chiral analysis of a wide range of compounds can be conducted in less than 6 minutes, with efficient peaks and very low sample size requirements. In addition, integrated mobile phase reservoirs provide for the rapid conversion to normal-phase eluents.



## Conclusion

Capillary high performance liquid chromatography (HPLC) offers significant performance benefits over conventional HPLC using 4.6 or 2.1 mm columns. These benefits are now possible without sacrificing retention time and peak area repeatability. This high level of performance combined with the ability to use a wide range of stationary phases, couple directly to MS and greatly reduce the amount of solvent usage makes capillary HPLC a very attractive choice for pharmaceutical analysis.

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