

## Use of the Eksigent ExpressLC System to Measure Chromatographic Hydrophobicity Index

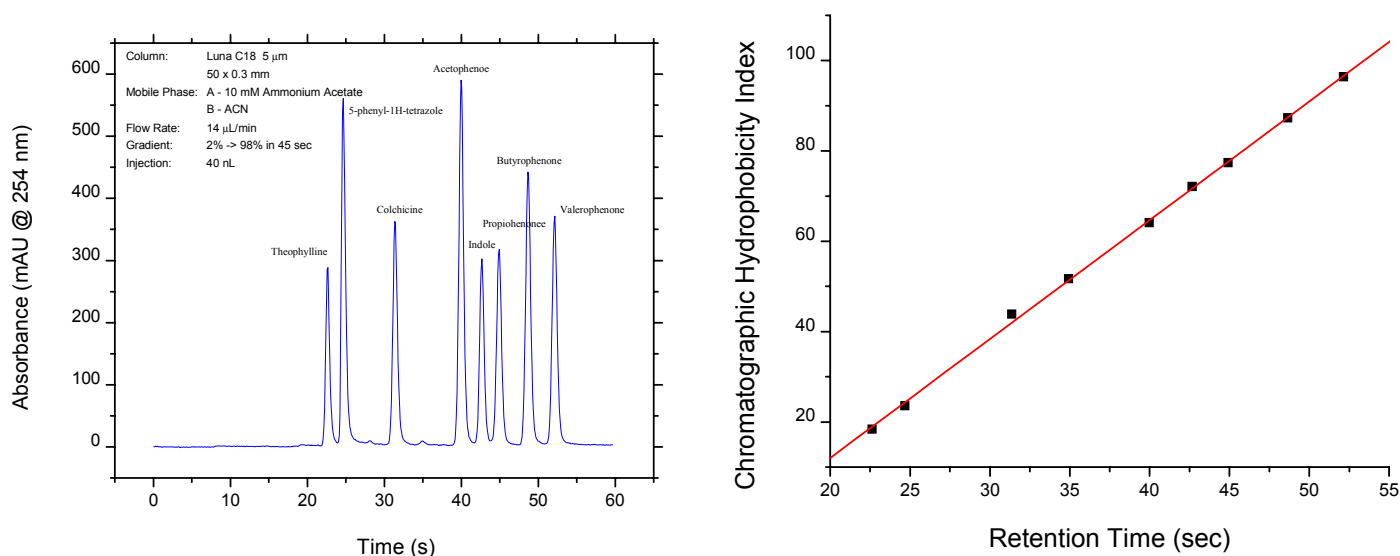
*Rapid HPLC provides high throughput profiling of compound lipophilicity*

### Introduction

Determination of the physicochemical and ADMET properties of compounds early in the drug discovery process can help prioritize leads from high-throughput screening and reduce the failure rate of drug candidates during development. One such profiling assay is compound lipophilicity, which is typically measured as octanol/water partitioning, or log P. This property is an important parameter for predicting oral absorption. An HPLC-based method for modeling octanol/water partitioning has been developed, called the chromatographic hydrophobicity index (CHI).<sup>1</sup> Using the Eksigent ExpressLC system, the throughput of this method can be greatly increased while also reducing the consumption of samples and solvents.

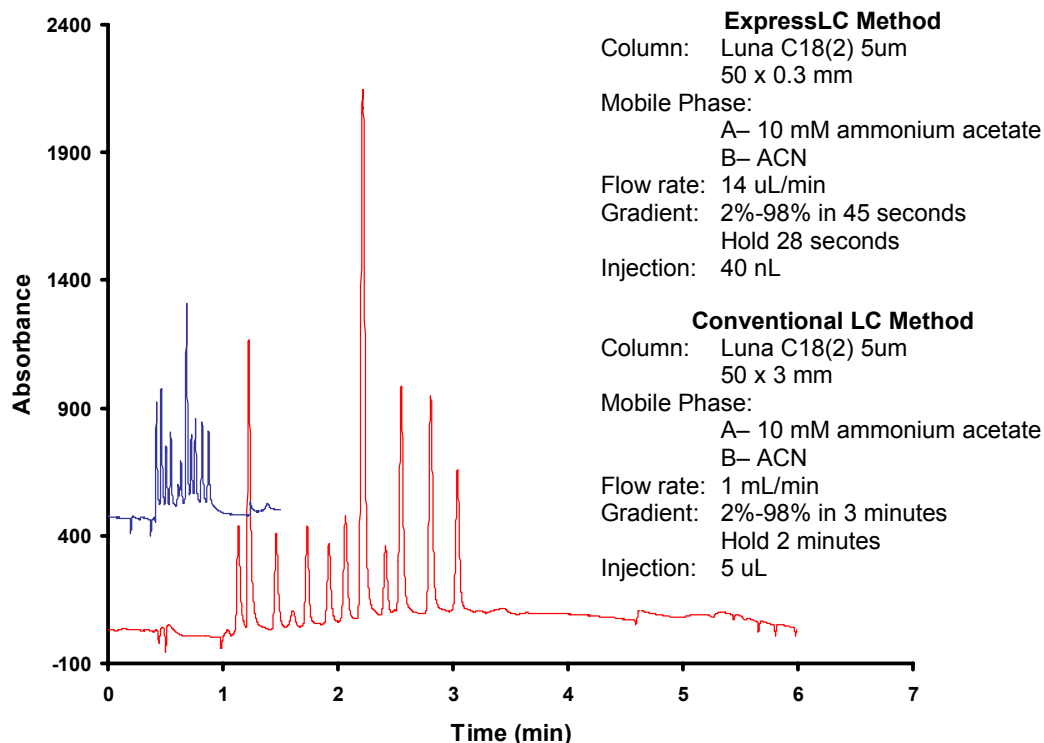
### Measurement of Chromatographic Hydrophobicity Index (CHI)

The chromatographic hydrophobicity index method uses fast-gradient reversed phase HPLC to model octanol/water partitioning of a compound by correlating the retention time with the percentage of acetonitrile required to achieve an equal distribution of the compound between the mobile and stationary phases.<sup>1</sup> A calibration curve is generated using a set of reference compounds by plotting their CHI values versus retention time. The slope and intercept of this calibration curve can then be used to determine the CHI values of unknown compounds based on their retention times.



Figures 1 (left) and 2. Nine reference compounds were analyzed using the Eksigent ExpressLC system and a calibration curve of their CHI values versus retention times was plotted. Despite using a rapid 45 second gradient, the assay exhibits excellent linearity.

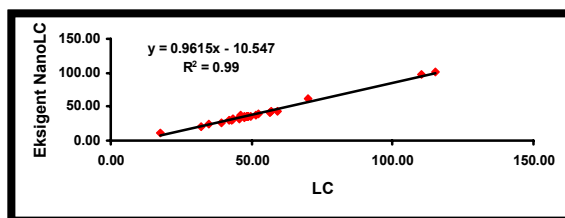
A set of compounds were analyzed on the Eksigent ExpressLC system. For comparison, the same compounds were also analyzed on conventional HPLC using a 5 cm, 3 mm i.d. column at a flow rate of 1 mL/min and an injection volume of 5  $\mu$ L. The optimized method on the conventional system required an injection-to-injection cycle time of 6 minutes. The method on the Express system has an injection-to-injection cycle time of 1 minute 30 seconds, or 4 times faster, without a loss in performance.<sup>2</sup>



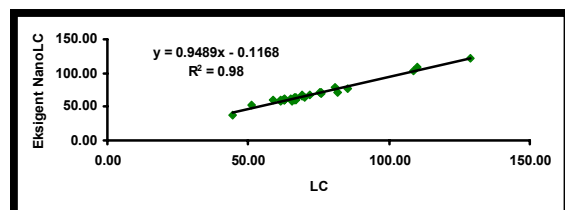
**Figure 3.** Despite a dramatically faster injection-to-injection cycle time, the ExpressLC system delivers resolution and precision comparable to a 4X slower conventional method. A gradient is made up of 3 phases: mixing delay, the gradient itself, and reset (defined as hold time plus re-equilibration). In each phase the ExpressLC system is significantly faster:

	ExpressLC	Conventional LC
Mixing delay	2 seconds	30 seconds
Gradient	45 seconds	3 minutes
Re-set	43 seconds	2.5 minutes
<b>Total cycle time</b>	<b>1.5 minutes</b>	<b>6 minutes</b>

pH 2



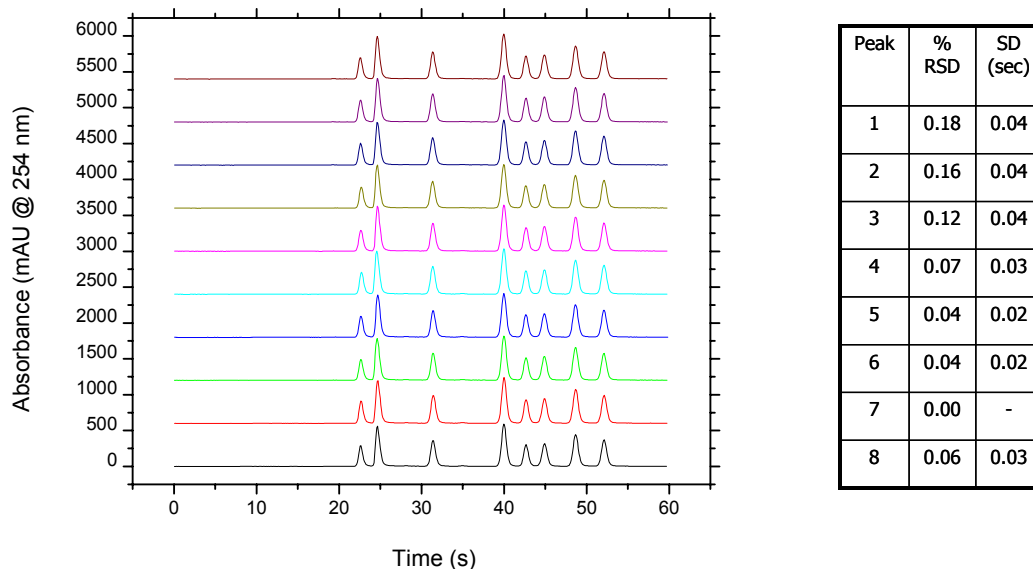
pH 7.4



**Figure 4.** Correlation plots of CHI plots for the ExpressLC system vs. a conventional HPLC system at 2 different pH levels. The correlations show excellent agreement, despite the ExpressLC system's use of a method that was fully 4 times faster.

## High Throughput Analysis

To determine quantitative performance over multiple injections, 10 samples were run sequentially during a period of 15 minutes (1.5 minutes per sample). The resulting chromatograms were analyzed for retention time precision. The overlaid chromatograms and retention time RSD's are shown below.



**Figure 5.** Overlaid chromatograms and retention time RSD calculation for 10 consecutive runs completed in 15 minutes. The retention time precision is excellent, even for early-eluting peaks. The maximum % RSD, occurring on the first peak, is only 0.18%.

## Summary

- The Chromatographic Hydrophobicity Index method has been successfully transferred from conventional HPLC format to the ExpressLC system.
- The ExpressLC system shortens the sample run from 6 minutes to 1.5 minutes, without a loss in performance. Generated CHI values show close agreement between the two systems.
- The ExpressLC system requires less than 1% sample than the conventional system per injection.
- Retention time precision over the course of 10 consecutive runs in 15 minutes shows excellent reproducibility, with the highest recorded RSD of 0.18% for the first peak.
- CHI sample throughput on the Express-100 single-channel system will exceed 40 samples per. On the Express-800 system throughput will exceed 320 samples per hour.

## Reference

<sup>1</sup> Valko, K., Bevan, C. & Reynolds, D. Chromatographic Hydrophobicity Index by Fast-Gradient RP-HPLC: A High-Throughput Alternative to log P/log D. *Anal. Chem.* **69**, 2022-2029 (1997).

<sup>2</sup> Data from both systems courtesy of Katherine B. Smith, Ph.D., Department of Computational, Analytical, and Structural Sciences, GlaxoSmithKline.

## Eksigent ExpressLC System Specifications

Feature	Specification
<b>Configuration</b>	Binary gradient pump, injection valve, column temperature control, UV detection system, integrated with high throughput autosampler. Single channel (Express-100) and 8 channel (Express-800) versions available.
<b>Flow rate range</b>	200 nl/min to 30 µl/min.
<b>Pump type</b>	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.
<b>Gradient formation</b>	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.
<b>Delay volume</b>	300 nl from mixer to column.
<b>Mobile phase compatibility</b>	All mobile phases that are compatible with 316 stainless steel, PEEK, and silica. Mobile phase stored in removable glass storage vials.
<b>Injection valve</b>	Eksigent Variable Injection System. Sample injection volume 10 nl to 300 nl (software selectable). Injection precision 0.5% RSD at 40 nl.
<b>Columns</b>	System optimized for use with 300 µm i.d. columns, from 2.5 cm to 15 cm
<b>Column temperature control</b>	Temperature stabilized at 27-40 °C based on 22 °C lab temp. Software selectable. Temperature stability +/- 0.1 °C
<b>Detection</b>	Fully dispersed UV absorbance detection at 200 – 380 nm using linear array detector. Detector noise $5 \times 10^{-5}$ AU rms @ 250 nm, 1 s averaging. Detector drift less than $4 \times 10^{-4}$ AU/hr. Linear dynamic range $> 10^4$ .
<b>Flow cell</b>	45-nL microfabricated flow cell with integral fiber optics, 4 mm path length.
<b>Autosampler</b>	Integrated high throughput CTC PAL autosampler with tray storage system and autowash station.
<b>System control</b>	Computer with graphical user interface for control of all system parameters. Software creates CDF, text, and Excel files for data export and analysis.



**Express-100**  
Single channel integrated HPLC system, shown with CTC autosampler.

**Express-800**  
Eight channel integrated HPLC system, shown with CTC autosampler.



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