

# PARALLEL HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

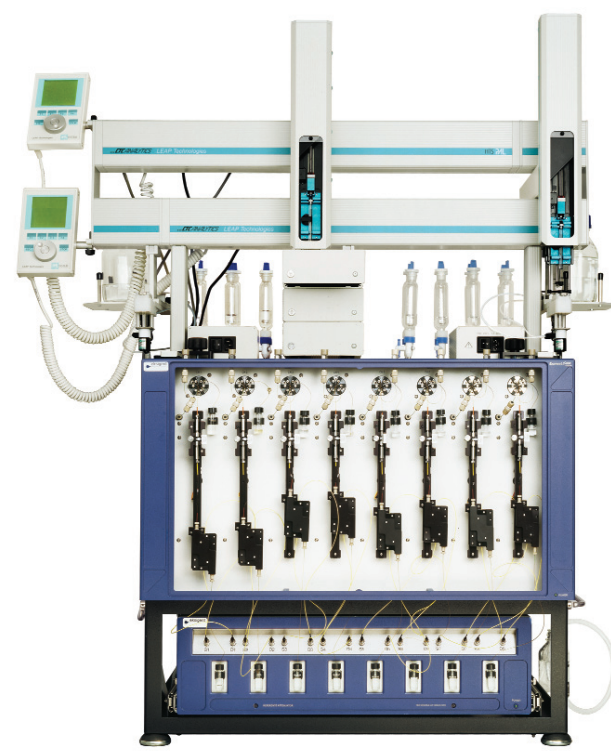
Ring-Ling Chien, Roger Farrow, Ken Hencken, Phil Paul, David Rakestraw, Jason Rehm  
Eksigent Technologies • 2021 Las Positas Court, Suite 161 • Livermore, CA 94551

## Abstract

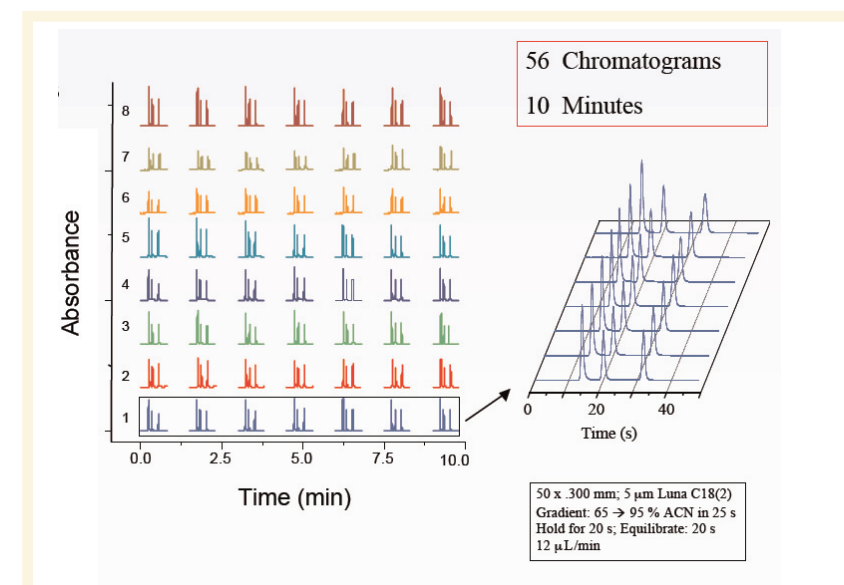
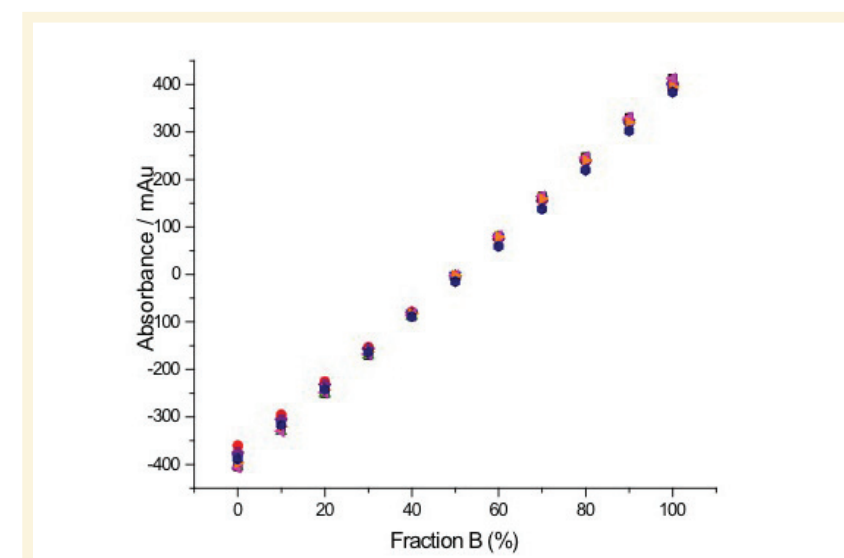
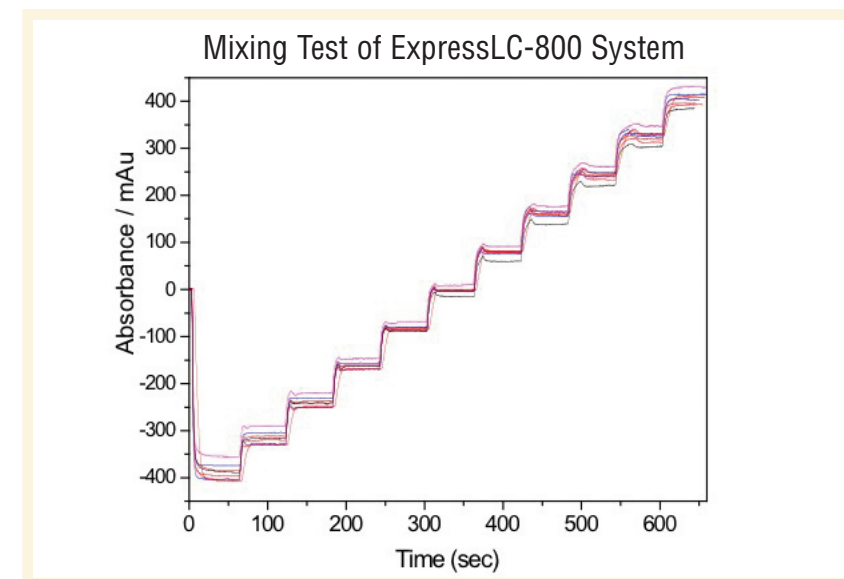
Capillary high performance liquid chromatography (HPLC) has gained lots of attention recently in the separation and analysis for materials of biological relevance. While many analytical assays have been adapted to HPLC formats, widespread use of these assays has been limited by the slow, serial nature of HPLC. Microscale separations provide increased separation efficiencies and speed while maintaining the quantitative accuracy and robustness of conventional HPLC. By combining recent advances in microscale fluid delivery and microfabrication techniques, a rapid, parallel high-resolution 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. A diverse set of pharmaceutical and industrial applications, as well as applications in drug discovery and biotechnology, will be presented to demonstrate the system performance.

## A New Generation of Parallel HPLC Systems

The assays described were run on the Eksigent ExpressLC™-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 ExpressLC-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.



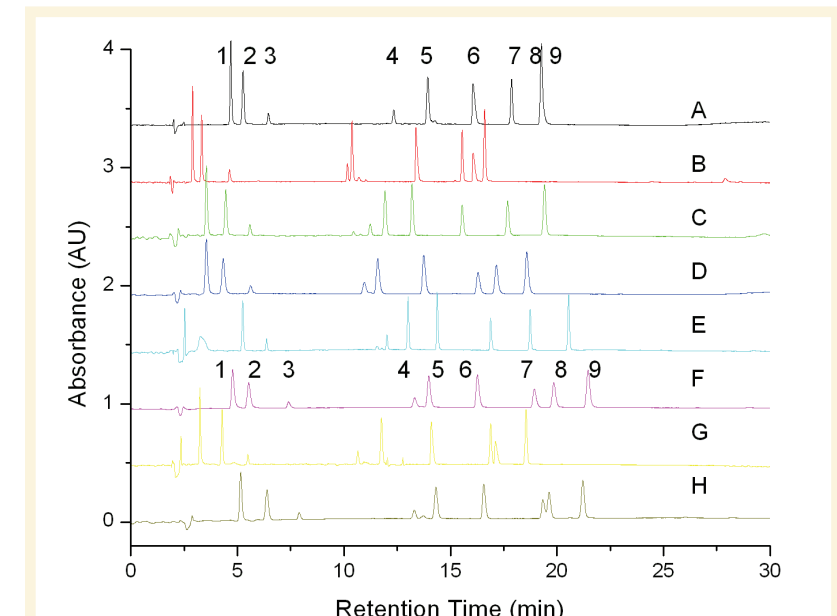
ExpressLC-800 HPLC system. Eight fully independent HPLC systems in a single enclosure. The multiplexed system is ideally suited to high throughput applications such as ADMET screening and method development.



## Parallel Method Development

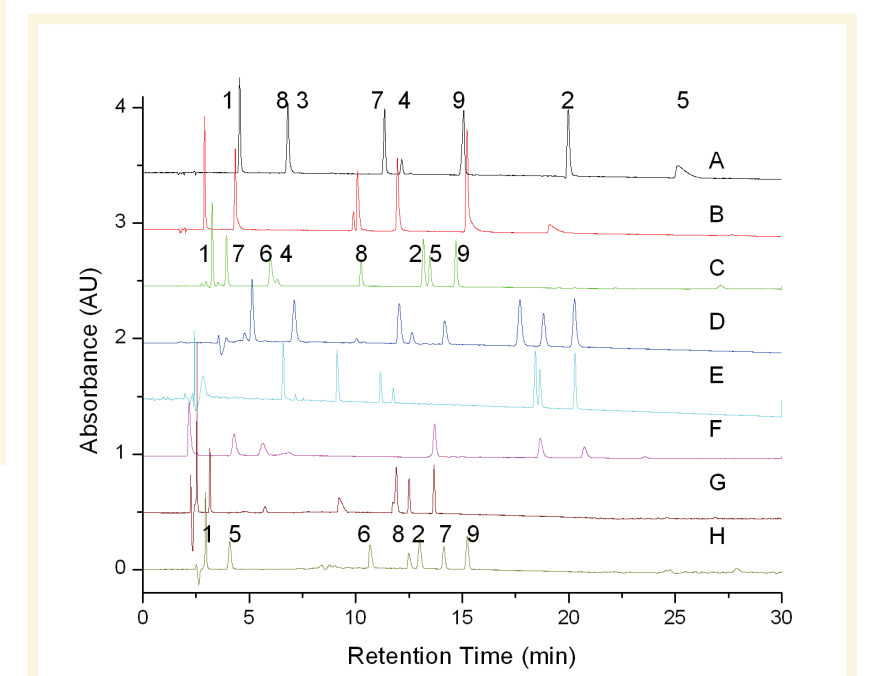
Channel	Column	Mobile Phase	Gradient
A	Aquasil C18, 3 µm (Thermo)	A: Water/0.1% TFA	0-30 min: B from 10-90% Flow rate: 4 µL/min
B	Zorbax C18 SB, 3.5 µm (Agilent)	A: Water/0.1% TFA	0-30 min: B from 10-90% Flow rate: 4 µL/min
D	Xterra MS C18, 5 µm (Waters)	A: Water/0.1% TFA	0-30 min: B from 10-90% Flow rate: 4 µL/min
E	Luna C18, 3 µm (Eksigent)	A: Water/0.1% TFA	0-30 min: B from 10-90% Flow rate: 4 µL/min
C	Symmetry Shield C18, 5 µm (Waters)	A: Water/0.1% TFA	0-30 min: B from 10-90% Flow rate: 4 µL/min
F	Xterra MS C18, 3 µm (Waters)	A: Water/0.1% TFA	0-30 min: B from 10-90% Flow rate: 4 µL/min
G	Zorbax C18 SB, 3.5 µm (Agilent)	A: Water/0.1% TFA	0-30 min: B from 10-90% Flow rate: 4 µL/min
H	Atlantis dC18, 5 µm (Waters)	A: Water/0.1% TFA	0-30 min: B from 10-90% Flow rate: 4 µL/min

Method development with 8 different columns using the same buffer and same gradient condition.



Method development with 8 different columns using same buffer and same gradient condition. Flow rate: 4 mL/min. Detector wavelength: 210 nm. Sample: 1) Theophylline, 2) Sulfamerazine, 3) Amphetamine, 4) Hydrocortisone, 5) Diazepam, 6) Imipramine, 7) Bumetanide, 8) Thioridazine, 9) Fenpropfen

Channel	Column	Mobile Phase	Gradient
A	Aquasil C18, 3 µm (Thermo)	A: 10 mM NH4OAc pH ~7 in water	0-30 min: B from 10-90% 30-35 min: B was held at 90%
B	Zorbax C18 SB, 3.5 µm (Agilent)	A: 10 mM NH4OAc pH ~7 in water	0-30 min: B from 10-90% 30-35 min: B was held at 90%
D	Xterra MS C18, 5 µm (Waters)	A: 0.1% HCOOH pH ~2.7 in water	0-30 min: B from 10-90% 30-35 min: B was held at 90%
E	Luna C18, 3 µm (Eksigent)	A: 0.1% HOAc pH ~3.3 in water	0-30 min: B from 10-90% 30-35 min: B was held at 90%
C	Symmetry Shield C18, 5 µm (Waters)	A: 10 mM NH4OAc pH ~7 in water	0-5 min: 30%B isocratic 5-20 min: linear to 95%B 20-30 min 95%B isocratic
F	Xterra MS C18, 3 µm (Waters)	A: 10 mM Ammonium pH ~10 in water	0-5 min: 30%B isocratic 5-20 min: linear to 95%B 20-30 min 95%B isocratic
G	Zorbax C18 SB, 3.5 µm (Agilent)	A: 0.1% formic acid pH ~2.7 in water	0-5 min: 30%B isocratic 5-20 min: linear to 95%B 20-30 min 95%B isocratic
H	Atlantis dC18, 5 µm (Waters)	A: 0.1% formic acid pH ~2.7 in water	0-5 min: 30%B isocratic 5-20 min: linear to 95%B 20-30 min 95%B isocratic



Method development with 8 different columns using different gradient conditions. Sample: 1) Theophylline, 2) Sulfamerazine, 3) Amphetamine, 4) Hydrocortisone, 5) Diazepam, 6) Imipramine, 7) Bumetanide, 8) Thioridazine, 9) Fenpropfen

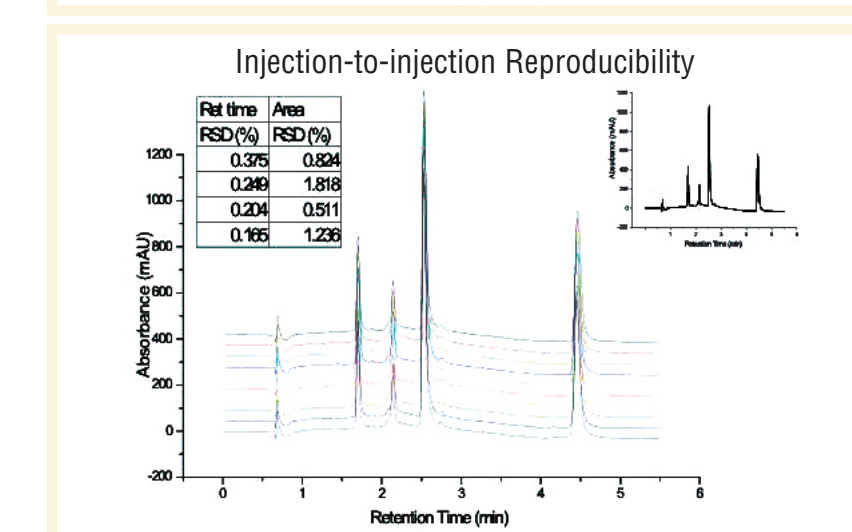
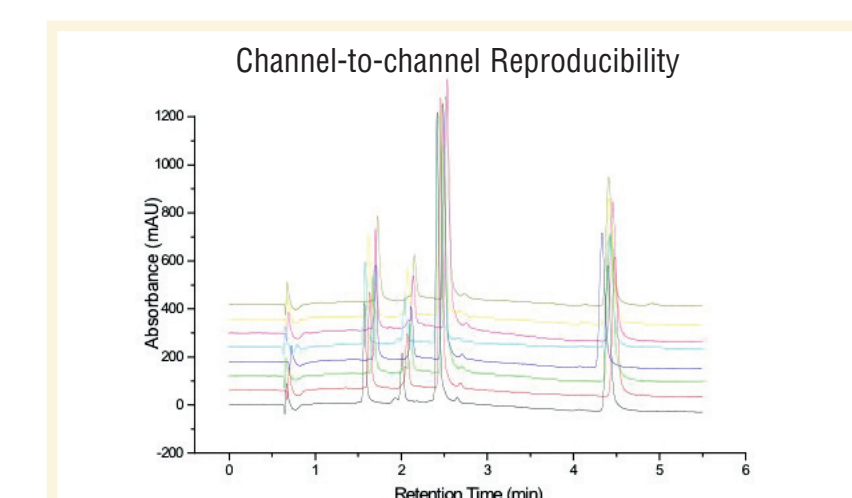
## System Reproducibility

- Sample: 1 Aspartame 2 Cortisone 3 Reserpine 4 Diocetyl Phthalate/methanol 100ug/ml
- Injection: 80 nL
- Mobile Phase: A H<sub>2</sub>O / 0.1% TFA, B ACN / 0.08% TFA
- Flow rate: 4 uL/min
- Column: Same Agilent Zorbax C-18 3.5 mm column, 0.3 mm x 5 mm
- Gradient: 0-3min B 5 - 95% 3-6 min B 95%
- Three repeated runs were performed for channel-to-channel studies. Ten repeated runs were performed for injection studies
- Detection wavelength was set to 210+5 nm except for Peak2 which was set to 235+5 nm due to the interference of impurity at lower wavelength

Peak	Retention Time (min)								RSD %
	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	
Peak 1	1.60	1.65	1.68	1.71	1.60	1.70	1.63	1.73	2.97
Peak 2*	2.04	2.07	2.12	2.13	2.07	2.14	2.08	2.16	1.97
Peak 3	2.44	2.46	2.50	2.50	2.46	2.53	2.47	2.52	1.33
Peak 4	4.42	4.47	4.43	4.34	4.41	4.46	4.42	4.41	0.95

Peak	Peak Area (mAU-sec)								RSD %
	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	
Peak 1	1013	1000	1040	952	914	1012	973	959	4.2
Peak 2*	1600	1604	1599	1515	1469	1711	1600	1581	4.5
Peak 3	3386	3442	3422	3227	3134	3551	3348	3333	3.9
Peak 4	2730	2626	3110	2541	2507	2894	2774	2761	7.1



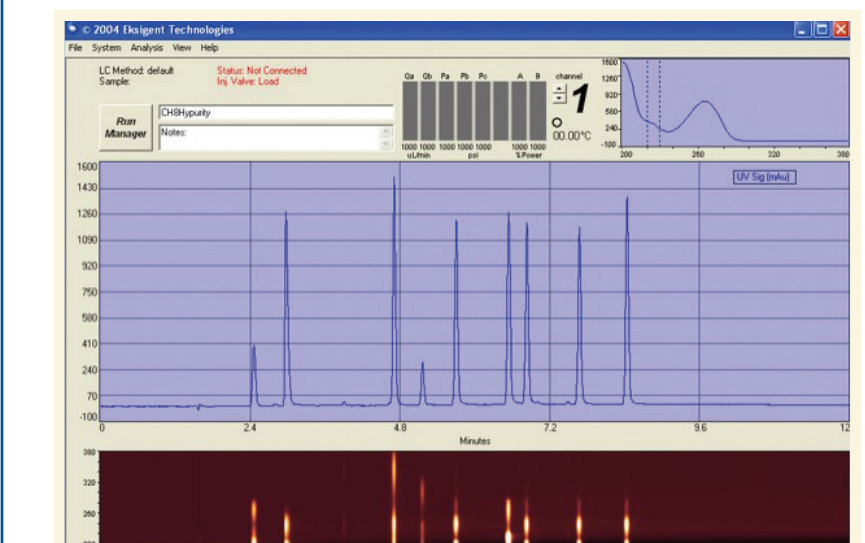
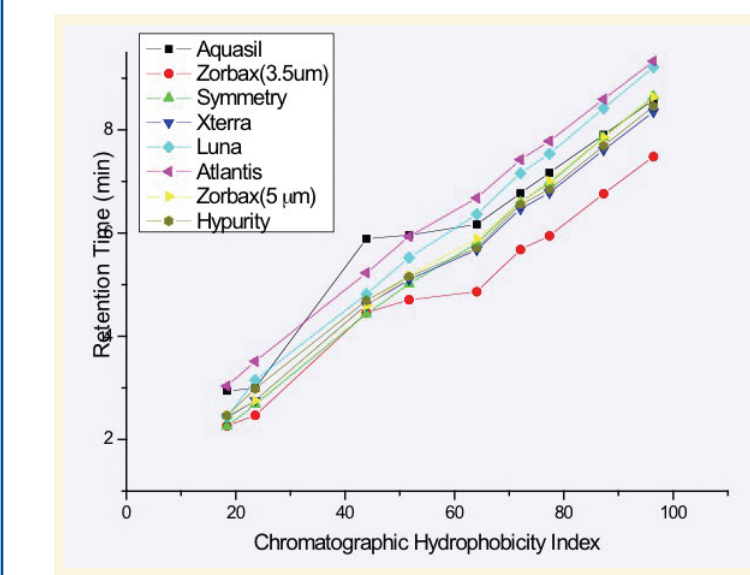
## Chromatographic Hydrophobicity Index

Determination of the physiochemical 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. The chromatographic hydrophobicity index (CHI) 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. 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. A parallel CHI method development was demonstrated using ExpressLC-800 system.

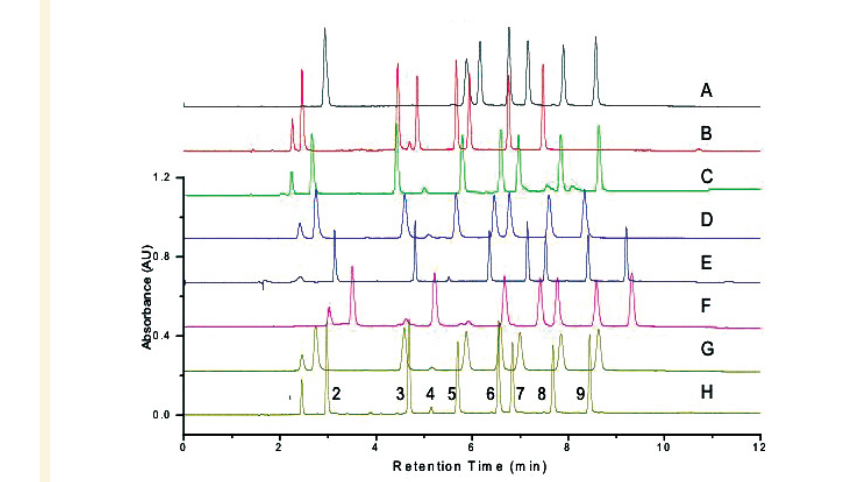
Parallel Method Development I

Channel	Column	Mobile Phase	Gradient
A	Aquasil C18, 3 µm (Thermo)	A: 10 mM NH4OAc pH ~7 in water B: AcCN	0-9 min: B from 8-98% 9-12 min: B was held at 98% Flow Rate: 6 µL/min
B	Zorbax C18 SB, 3.5 µm (Agilent)	A: 10 mM NH4OAc pH ~7 in water B: AcCN	0-9 min: B from 10-90% 9-12 min: B was held at 90%
C	Symmetry Shield C18, 5 µm (Waters)	A: 10 mM NH4OAc pH ~7 in water B: AcCN	0-5 min: 30%B isocratic 5-20 min: linear to 95%B 20-30 min 95%B isocratic
D	Xterra MS C18, 5 µm (Waters)	A: 0.1% HCOOH pH ~2.7 in water B: AcCN	0-30 min: B from 10-90% 30-35 min: B was held at 90%
E	Luna C18, 3 µm (Eksigent)	A: 0.1% HOAc pH ~3.3 in water B: AcCN	0-30 min: B from 10-90% 30-35 min: B was held at 90%
F	Atlantis dC18, 5 µm (Waters)	A: 10 mM Ammonium pH ~10 in water B: AcCN	0-5 min: 30%B isocratic 5-20 min: linear to 95%B 20-30 min 95%B isocratic
G	Zorbax C18 SB, 3.5 µm (Agilent)	A: 0.1% formic acid pH ~2.7 in water B: 0.08% formic acid in AcCN	0-5 min: 30%B isocratic 5-20 min: linear to 95%B 20-30 min 95%B isocratic
H	Atlantis dC18, 5 µm (Waters)	A: 0.1% formic acid pH ~2.7 in water B: 0.08% formic acid in AcCN	0-5 min: 30%B isocratic 5-20 min: linear to 95%B 20-30 min 95%B isocratic

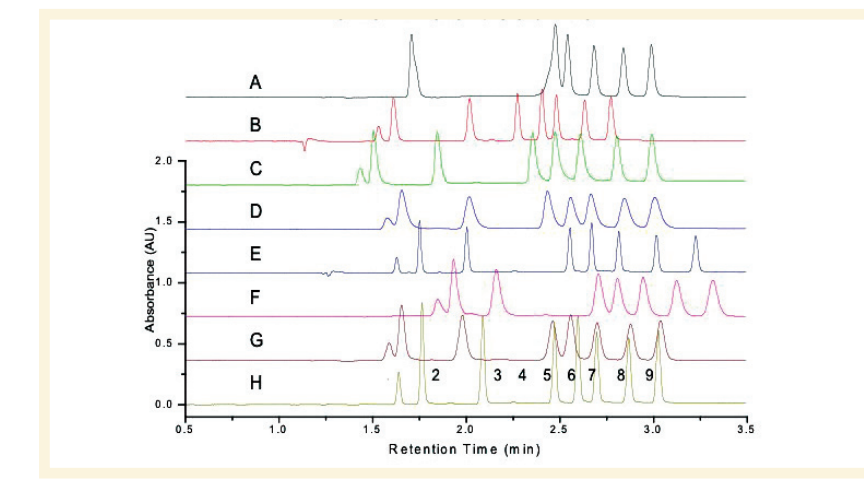
\*For method development all column dimensions are: 0.3 x 150 mm



## CHI Assay Using Same Gradient on 8 Different Columns



Conditions: Mobile Phase: A: 10 mM NH4OAc, B: Acetonitrile. Gradient Time (min): 0-9 min: %B: 8-98%, Flow Rate: 6 µL/min. Wavelength: 225 nm. Sample: 1 theophylline, 2 5-phenyl-1H-tetrazole, 3 colchicine, 4 8-phenyl-theophylline, 5 acetophenone, 6 indole, 7 propiophenone, 8 butyrophenone, 9 valerophenone



Conditions: Mobile Phase: A: 10 mM NH4OAc, B: ACN. Gradient: Time (min): 0-9 min: %B: 8-98%, Flow Rate: 8 µL/min. Wavelength: 225 nm. Sample: 1 theophylline, 2 5-phenyl-1H-tetrazole, 3 colchicine, 4 8-phenyl-theophylline, 5 acetophenone, 6 indole, 7 propiophenone, 8 butyrophenone, 9 valerophenone

## Conclusion

- A rapid, high resolution, multiplexed system has been developed for high throughput HPLC.
- The Chromatographic Hydrophobicity Index method has been successfully transferred from conventional HPLC format to the ExpressLC-800 system.
- CHI plots from eight different columns were obtained and compared in a single run.
- Faster gradient could shorten the sample run from 10 minutes to 3 minutes, without a loss in performance.

