

Hitachi High Technologies America

The Hitachi LaChrom *Elite* HPLC and Fast LC Pharmaceutical Applications: OTC Analgesics & Steroids

John K. Lim, Ph.D.*; Michael C. Riley; and Stephen T. Watts, M.S.;
Hitachi High Technologies America

Routine development of multi-component 'Fast Liquid Chromatography' (Fast LC) applications are rapidly and easily accomplished by integrating (a) the new Hitachi LaChrom® Elite HPLC system (1) (Figure 1), (b) Automated Method Development tools (ChromSword AUTO™) and (c) Hitachi Chromolith™ monolithic reversed-phase columns. These Fast LC applications allow for high throughput analyses leading to a significant reduction in run times contrasted to conventional C18 particulate column run times by several-fold. Examples presented herein demonstrate an integrated approach to Fast LC applications development of Over-the-Counter (OTC) analgesics and steroids.

We have found that analytical scale Fast HPLC applications are rapidly gaining interest among our HPLC users, especially where high throughput screening is desired for rapid analysis of multi-component samples.

Employing conventional particle column technology, fast analytical LC has been limited primarily to short (3 - 10 cm), small particle (3 μm), 4.6 mm i.d. columns, but with severe limitations on higher flow rate accessibility due to rapid build-up of column backpressure. This has effectively limited *high resolution* (column particle sizes < 5 μm) analysis to low flow rates (< 2-3 mL/min). Fast LC attempts on conventional columns will often (a) exceed the manufacturer's recommendations potentially voiding the column warranty, (b) accelerate column degradation, (c) increase likelihood

for leaks at fittings, especially if biocompatible PEEK (poly ether ether ketone) or other polymer tubing/fittings are used, and (d) may result in unacceptable chromatography (poor peak shape, low resolution, etc.). Other techniques applied to Fast LC include operating at higher temperatures with a column oven to lower solvent viscosity but this is not always successful as peak shapes may become distorted/broadened and thermally labile samples cannot be used.

High resolution, analytical scale Fast LC requirements (2) are summarized as follows:

- Highly accurate/precise HPLC isocratic & gradient solvent delivery system
- Autosamplers with excellent low carryover and excellent retention time reproducibility
- HPLC detectors capable of high sampling rates and fast response times
- Analytical HPLC columns (4.6 mm ID) capable of high flow rates (up to 9 mL/min) without appreciable backpressure buildup while offering excellent column characteristics (good resolution, high plate counts, etc.)

We will demonstrate with the applications described herein with the new Hitachi LaChrom *Elite* HPLC system, that analytical Fast LC can be routinely performed incorporating monolithic column technology and automated method development tools.

Experimental

Equipment	Hitachi LaChrom® <i>Elite</i> (LCE) system
Pump	L-2130 equipped with low pressure gradient kit & dynamic mixer unit
Autosampler	L-2200
Column Oven	L-2300 w/Peltier block
Column	Chromolith™ Performance (4.6 × 100) RP18e
Detector	L-2400 UV or L-2450 DAD
Acquisition CDS	EZChrom <i>Elite</i> ™ 2.8.3
Other	ChromSword Auto™ 2.0 Automated Method Development Software

Methods

<i>Steroids</i>	
Detector settings (UV)	Sampling Rate = 50 ms, Time Constant = 0.05 s
Wavelength	220 nm
Oven Temperature	30 °C
Flow Rate	4.0 mL/min
Eluents & Gradient	see Table I



Figure 1: The Hitachi LaChrom *Elite* HPLC System

Table I: Eluents and gradient

Time (min)	A: H ₂ O	B: CH ₃ CN
0.0	70	30
1.1	70	30
1.8	35	65
4.5	35	65
4.6	70	30
6.0	70	30

OTC Analgesics

Detector settings (UV) Sampling Rate = 50 ms,
Time Constant = 0.05 s

Wavelength 254 nm

Oven Temperature 30 °C

Flow Rate 2.0 mL/min

Eluents (Isocratic) 5.67 (0.1 N H₃PO₄) : 1 (CH₃CN)

Results and Discussion

The method development results for the *OTC Analgesics and Steroids Applications* were originally obtained on a Hitachi LaChrom HPLC system with the ChromSword AUTO 2.0 (CSA) Automated Method Development program. The gradients were recalculated for higher flow rates for the Hitachi Chromolith Performance column and reacquired on the Hitachi LaChrom *Elite* HPLC system to obtain the final Fast LC results.

The two applications demonstrating the integrated Fast LC concept with the Hitachi LaChrom *Elite* HPLC and Hitachi Chromolith columns are presented in Figure 2. Each of these Fast LC applications is easily and reliably reproduced with the LaChrom *Elite* system equipped with a dynamic mixer and Chromolith Performance column. The fast detector time constants (0.05 s) and high sampling rates (50 ms) of the Hitachi LaChrom *Elite* HPLC system provides excellent fast peak data handling, essential for obtaining accurate and reproducible chromatographic results.

Steroids: Figure 2a compares the results obtained on the Hitachi LaChrom *Elite* HPLC system with the Chromolith Performance column running at a 'typical' flow rate of 1 mL/min so that all of the components are separated under 12 min with a maximum column backpressure of ~ 500 psi. When the flow rate is increased to 4 mL/min, along with the recalculated gradient, the peaks are separated in under 4 min with a maximum column backpressure of < 2000 psi. Minimal resolution loss was observed for the critical peak pair, prednisolone and cortisone (peaks 2 & 3, respectively), and requires only minor gradient modification in the 1.8-4.5 min. range to increase the R_s value.

OTC Analgesics: Figure 2b illustrates the final results obtained under the Fast LC conditions for a variety mixture standard of OTC analgesic ingredients. This application was performed isocratically at 2 mL/min, under 4 min with column backpressures under 1000 psi, a substantial decrease from a 15 min run time at flow rate of 0.5 mL/min.

Summary and Conclusions

The applications presented in this article demonstrate the utility of the Fast LC concept for high throughput analyses integrating the

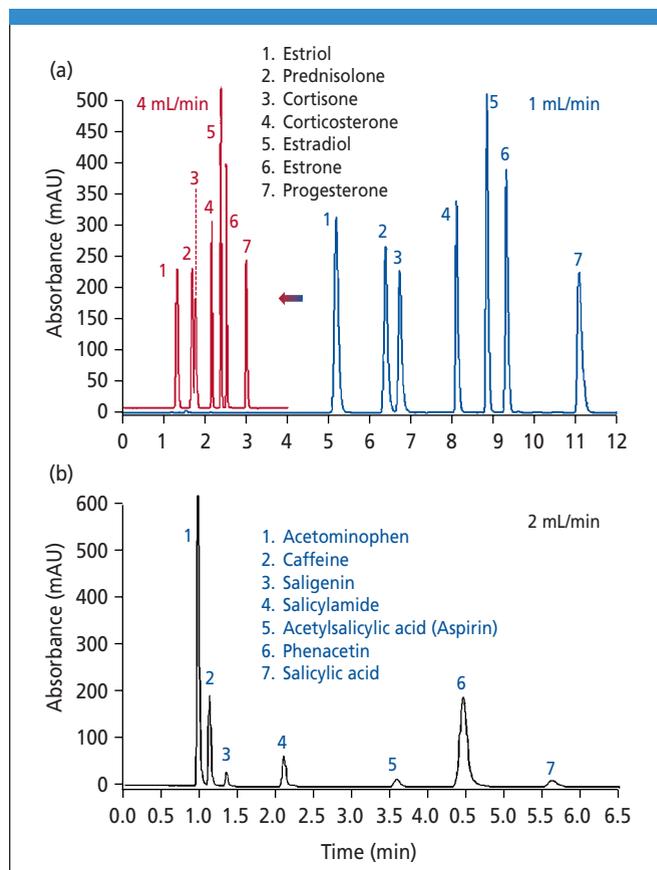


Figure 2: Hitachi LaChrom *Elite* Applications with Chromolith Performance columns. (a) *Steroid Application:* the acquisition time for this complicated mixture of steroids has been reduced from 12 min (at 1 mL/min flow) to less than 4 min (at 4 mL/min flow). (b) *Over the Counter (OTC) Analgesics:* this isocratic separation is acquired in less than 6 min with a flow rate of 2.0 mL/min.

Hitachi LaChrom *Elite* HPLC system, monolithic column technology and HPLC method development tools (CSA) for pharmaceutical applications. Excellent peak resolution, peak symmetry, and high theoretical plate count numbers (*N*) with very low column backpressures are the final results. The substantial reduction in run times translates into higher sample throughput without sacrificing quality results in the analyses.

References & Endnotes

- (1) The new Hitachi LaChrom *Elite* HPLC system, CSA and Chromolith columns are described in the HHTA web site www.ls.hitachi-hhta.com.
 - (2) A general overview of fast GC peak handling is described and is equally applicable for HPLC: J.V. Hinshaw, *LCGC* **19**(11), 1136-1140 (2001).
- * To whom correspondences should be addressed.

Hitachi High Technologies America

Life Sciences Division,
3100 North First Street, San Jose, CA 95134
tel. (800) 455-4442, fax (408) 432-8258
e-mail John.Lim@hitachi-hhta.com
www.hitachi-hhta.com/LSHome