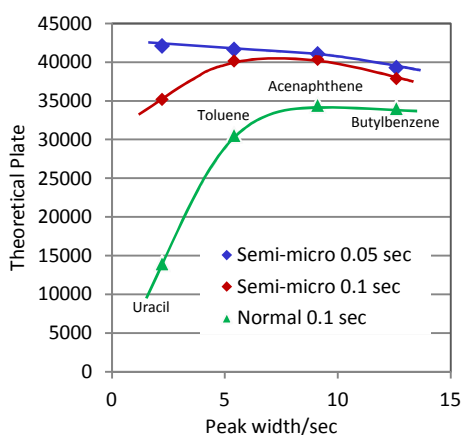
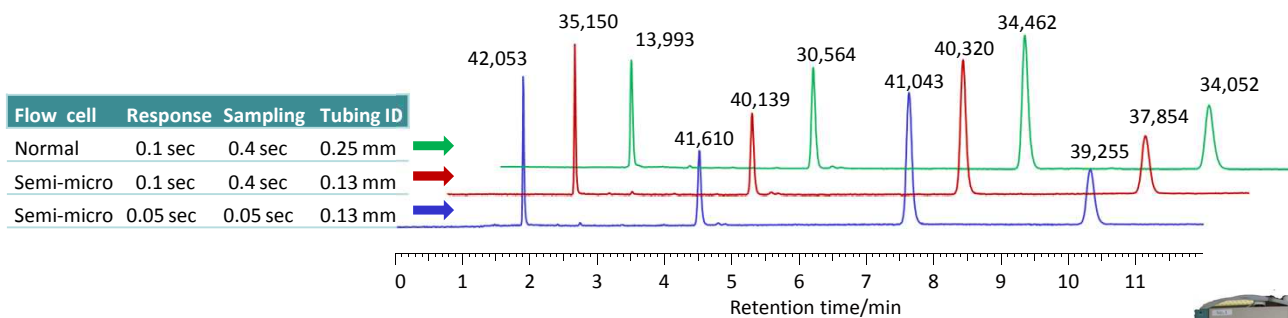


## Comparison between normal and semi-micro HPLC



Comparison of chromatograms

Column: SunShell C18, 5  $\mu$ m 250 x 4.6 mm  
 Mobile phase: CH<sub>3</sub>CN/H<sub>2</sub>O= 70/30  
 Flow rate: 1.0 mL/min  
 Temperature: 40 °C  
 Pressure: 6.7 MPa  
 Detection: UV@250 nm  
 Sample: 1 = Uracil  
 2 = Toluene  
 3 = Acenaphthene  
 4 = Butylbenzene

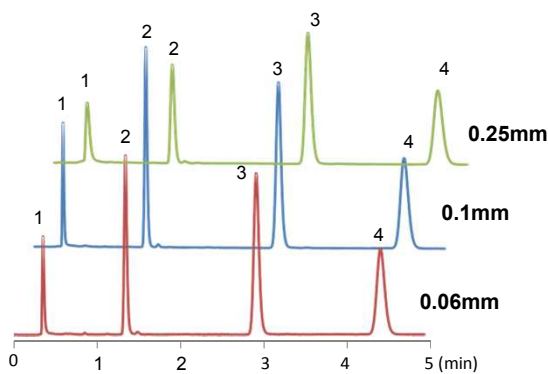
HPLC: Hitachi LaChrom ELITE



Semi-micro HPLC derives near 100% performance of a core shell column. Even if normal HPLC is used, it derives 80% performance except for a narrow peak whose width is less than 5 second

Relationship between Peak width and theoretical plate

## Effect of inner diameter of tubing



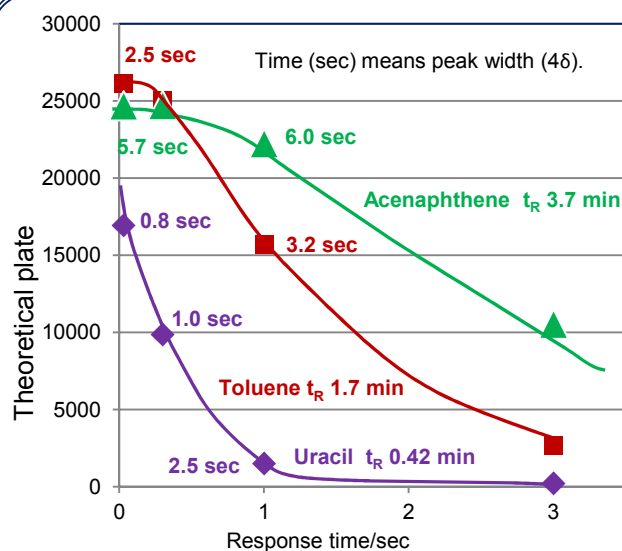
Average of theoretical plate (n=3)

Inner diameter of tubing	0.06mm	0.1mm	0.25mm
Peak (1)	792	785	246
Peak (2)	7790	7652	3535
Peak (3)	10704	10345	7998
Peak (4)	10113	9772	7689

Column: SunShell C18, 2.6  $\mu$ m 50 x 2.1 mm  
 Mobile phase: CH<sub>3</sub>CN/H<sub>2</sub>O=60/40  
 Flow rate: 0.3 mL/min Temperature: Ambient  
 Tube length: 30 cm (Peek, from the column to the flow cell)  
 Instrument: X-LC(JASCO) Response time: 0.01 sec

The above theoretical plate was compared changing the inner diameter of tubing between a column and a flow cell of the detector. A tubing with a large inner diameter has a large dead volume, so that it makes the peak width be wide. As a result, theoretical plate decreases. I recommend to use the tubing with 0.1 mm or less than 0.1 mm inner diameter for core shell columns.

## Effect of response time of detector



Column: SunShell C18, 2.6  $\mu$ m 100 x 4.6 mm  
 Mobile phase: CH<sub>3</sub>CN/H<sub>2</sub>O=60/40  
 Flow rate: 1.8 mL/min Temperature: Ambient  
 Sample: Toluene Tube: i.d.0.1mm x 20 cm Peeksil  
 Instrument: X-LC(JASCO)

The response time of a detector is important. Regarding uracil, the real peak width is less than 0.8 sec. When the peak width is less than 1 sec, 0.03 sec of response time is needed. Furthermore, the sampling rate of an integrator should be set to be 0.1 sec.