Reduction of Solvent Consumption in HPLC (2020 #2) –Rapid Analysis in Method Development without UHPLC
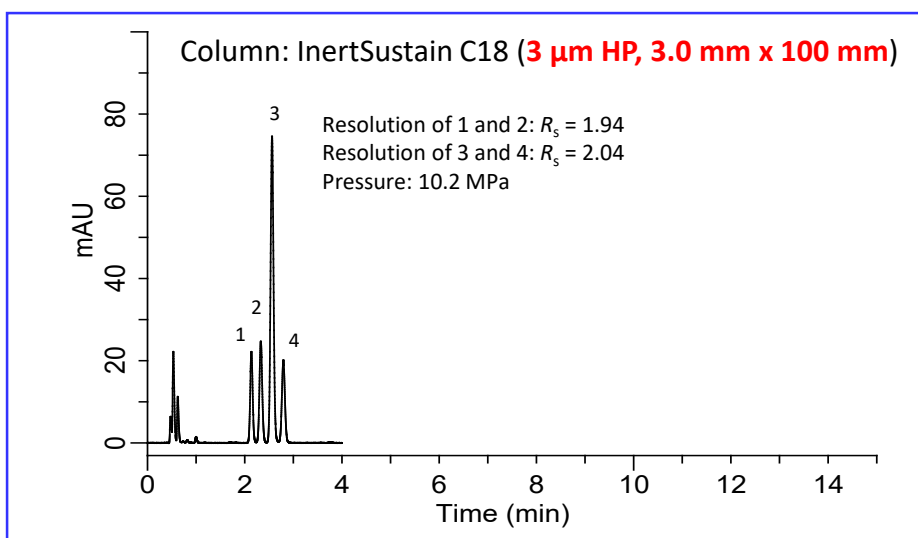
This technical note demonstrates how to reduce analysis time in method development without UHPLC. UHPLC columns packed with sub-2 µm particles generate high pressures above 50 MPa. Therefore, it takes long before the pressure is released for column switching or stop operation. This pressure release takes longer than the analysis itself in some cases. Rapid analysis at a relatively low pressure is possible with 3 µm particles without UHPLC.

(K. Suzuki)

3 µm HP, 3.0 X 100 mm is recommended for Method Development!

4.6 x 150 mm columns are popular for method development. These dimensions require an equilibration time of longer than 15 min and the analysis normally takes 15 - 30 min. On the other hand, the equilibration and analysis of 3 µm, 3.0 mm x 100 mm can be finished in 3 min and 5 min, respectively, because of its wide optimal linear velocity range. 3 µm HP series can be used on conventional HPLC systems, and consecutive analysis to test 10 different conditions can be shortened from a few hours to 1 hour.

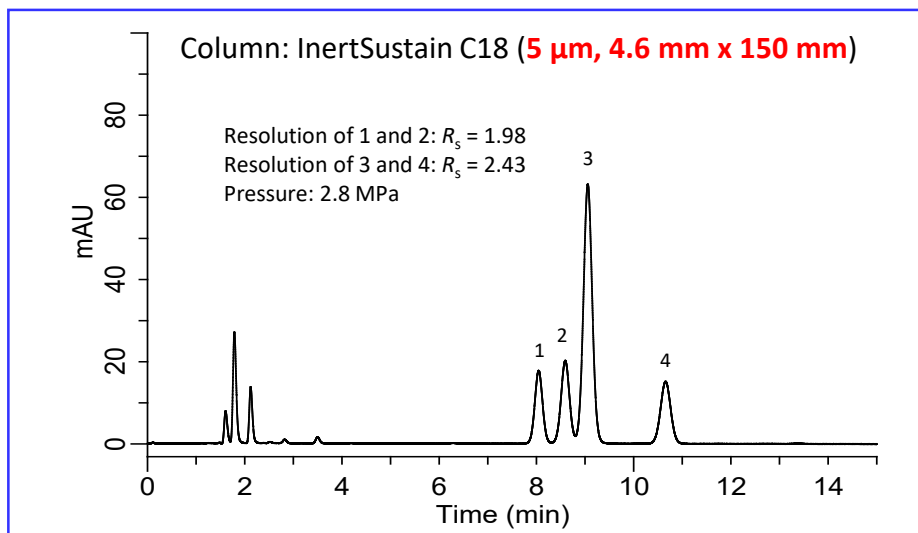### Rapid Analysis for Method Development

Column: InertSustain C18 (**3 µm HP, 3.0 mm x 100 mm**)

Resolution of 1 and 2: R_s = 1.94
Resolution of 3 and 4: R_s = 2.04
Pressure: 10.2 MPa

Conditions

System	: GL7700
Column	: InertSustain C18
Mobile phase	: A) CH_3CN
	B) H_2O
	A/B = 75/25, v/v
Flow rate	: 1.0 mL/min
Column Temp.	: 40 $^{\circ}$C
Detection	: UV 254 nm
Inj. Vol.	: 1.0 µL

1. *n*-Butylbenzene
2. *o*-Terphenyl
3. Triphenylene
4. *n*-Amylbenzene

Easy Method Transfer to Routine Analysis

A disadvantage of columns packed with 3 µm particles over those packed with 5 µm particles is that filter clogging is more likely to happen, which makes the column lifetime shorter. If 5 µm particles are desirable for routine analysis, an isocratic method can be transferred by simply switching to 5 µm 4.6 x 150 mm as shown below.

Column: InertSustain C18 (**5 µm, 4.6 mm x 150 mm**)

Resolution of 1 and 2: R_s = 1.98
Resolution of 3 and 4: R_s = 2.43
Pressure: 2.8 MPa

Same conditions, except for column dimensions.

Conditions

System	: GL7700
Column	: InertSustain C18
Mobile phase	: A) CH_3CN
	B) H_2O
	A/B = 75/25, v/v
Flow rate	: 1.0 mL/min
Column Temp.	: 40 $^{\circ}$C
Detection	: UV 254 nm
Inj. Vol.	: 1.0 µL

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● 3 µm HP Series for High Pressures

Regular columns packed with 3 µm particles can be easily deteriorated when the flow rate is high, the mobile phase contains a viscous solvent such as methanol, or the column length is long.
For such analysis, 3 µm **HP** (50 MPa max.) is desirable.
Note: the packing material is the same as the regular 3 µm series.

● Notes for Rapid Analysis

Peak distortion may occur when rapid analysis is tested. Note the below points in such a case. Please see LT068 and LT087 for more details. Points 1 and 2 can often be changed on the settings of the detector. Change them by following the manual of your system if necessary.

1. Data Acquisition Rate

Data acquisition rate below 100 ms is recommended for analysis shorter than 5 min.
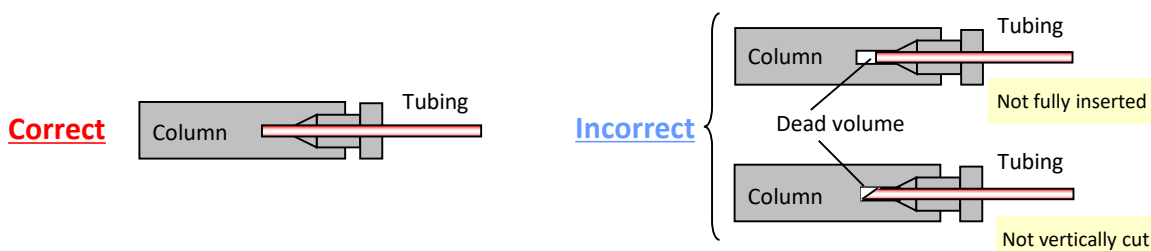
100 ms   800 ms
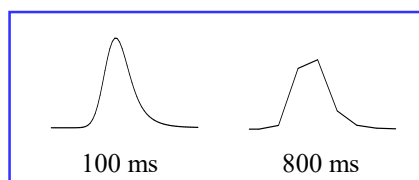
2. Response Time/Time Constant

Shorten the response (e.g. 0.1 s) or set the detector to the rapid analysis mode.

0.1 s   1 s
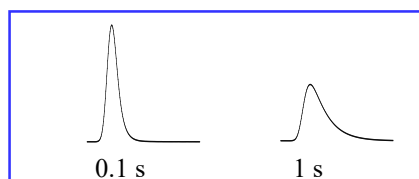
3. Tubing Connection

Incorrect connection between the column and tubing can distort peak shapes.
See below for the correct connection.

Correct Column / Tubing

Incorrect Column / Tubing — Not fully inserted
Dead volume
Column / Tubing — Not vertically cut

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