Applications and Use of High Pressure and Ultra High Pressure LC Columns

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Abstract

Modern chromatographic theory predicts that with decreased particle size comes increased efficiency. With the introduction of sub-2 µm particles several years ago, as well as LC instrumentation capable of pressures required for columns packed with these particles, there has been an increased interest in using these columns to achieve faster separations with increased efficiency. While operating sub-2 µm columns at conventional pressures (400-600 bar) provides some benefits of increased efficiency and sample throughput, newly introduced columns, capable of supporting extended use at pressures of 1000 bar, enable additional flexibility in chromatographic applications, including operating individual and coupled 2.1x100 mm and 2.1x150 mm columns at pressures greater than 1000 bar.

Relationship between Pressure and Capillary diameter, Particle Size and Column Length

Back pressure in an HPLC system comes primarily from the column and the tubing. It is caused by the friction of the solvent inside the capillaries and in the column.

The back pressure of a capillary *Δpcap* under the flow rates used in HPLC is proportional to the flow rate *F* and inversely proportional to the fourth power of the capillary diameter *dcap*:

$$\Delta p_{cap} \sim \frac{F}{d_{cap}^4}$$

Reducing the capillary inner diameter from 0.17 mm to 0.12 mm increases the backpressure of the capillaries (assuming same length) more than 4-fold!

The back pressure caused by the column *Δpcol* is proportional to the flow *F*, the column length *L* and the viscosity *η*, and is inversely proportional to the square of the column inner diameter *dc* and to the square of the particle size *dp*:

$$\Delta p_{col} \sim \frac{F \cdot L \cdot \eta}{d_c^2 \cdot d_p^2}$$

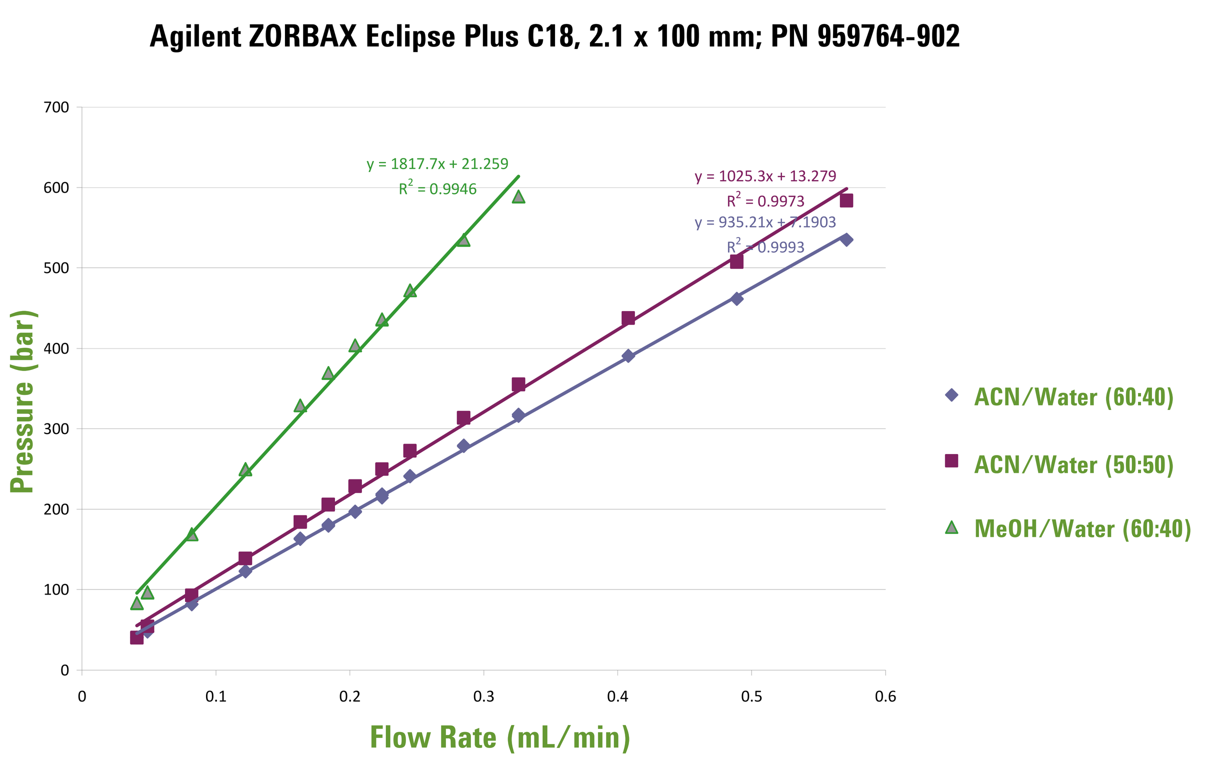
Higher pressure limits allow the use of longer column lengths *L* and smaller particle sizes *dp*, both of which increase efficiency *N* and thereby increasing resolution *Rs*:

$$R_s = \frac{\sqrt{N}}{4} \cdot (\alpha - 1) \cdot \frac{k'}{k' + 1} \qquad \text{Where:} \quad N \sim \frac{L}{d_p}$$

Therefore: $\uparrow L = \uparrow N$, $\downarrow d_p = \uparrow N$

Scaling only the column internal diameter down to save solvent of course does not increase the backpressure since the flow rate of the method is also reduced accordingly.

Pressure Requirements of Current sub-2 µm Columns



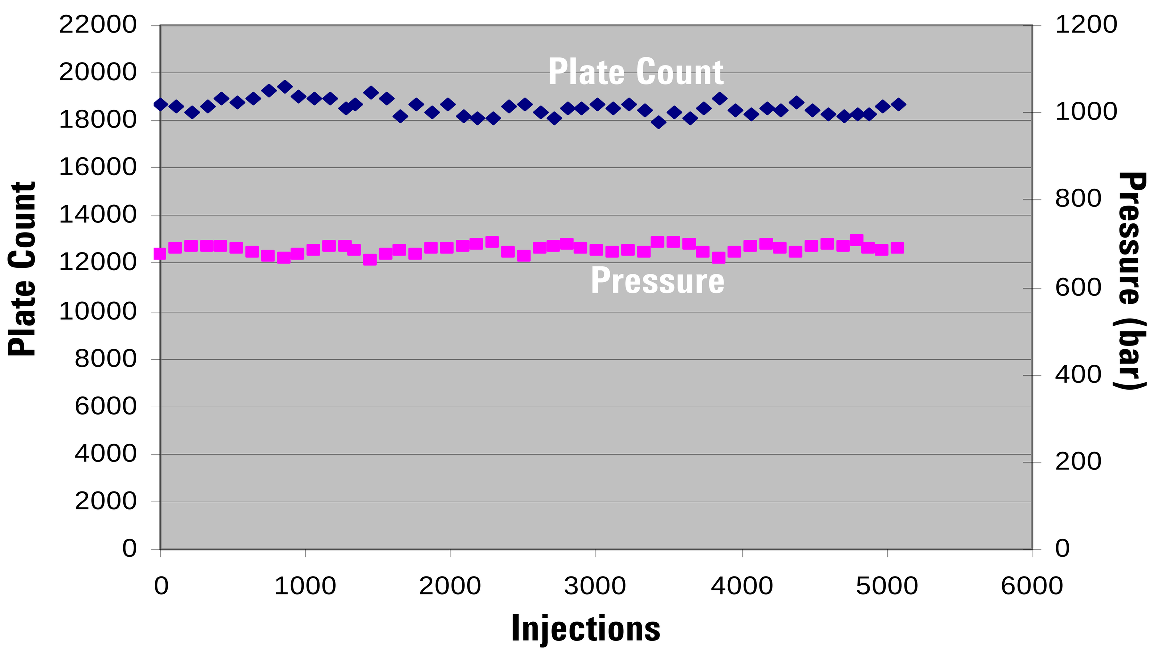
Operating at high flow rates or with more viscous mobile phase such as Methanol/Water require instrumentation capable of operating at those higher pressures. The newly introduced Agilent 1290 Infinity LC System, capable of 1200 bar operation, coupled with Agilent Rapid Resolution High Definition (RRHD) columns allow for sustainable operation under these ultra-high pressure conditions. Agilent's new RRHD columns are designed for operation at pressures up to 1200 bar with extended lifetime under those conditions.

Column Robustness

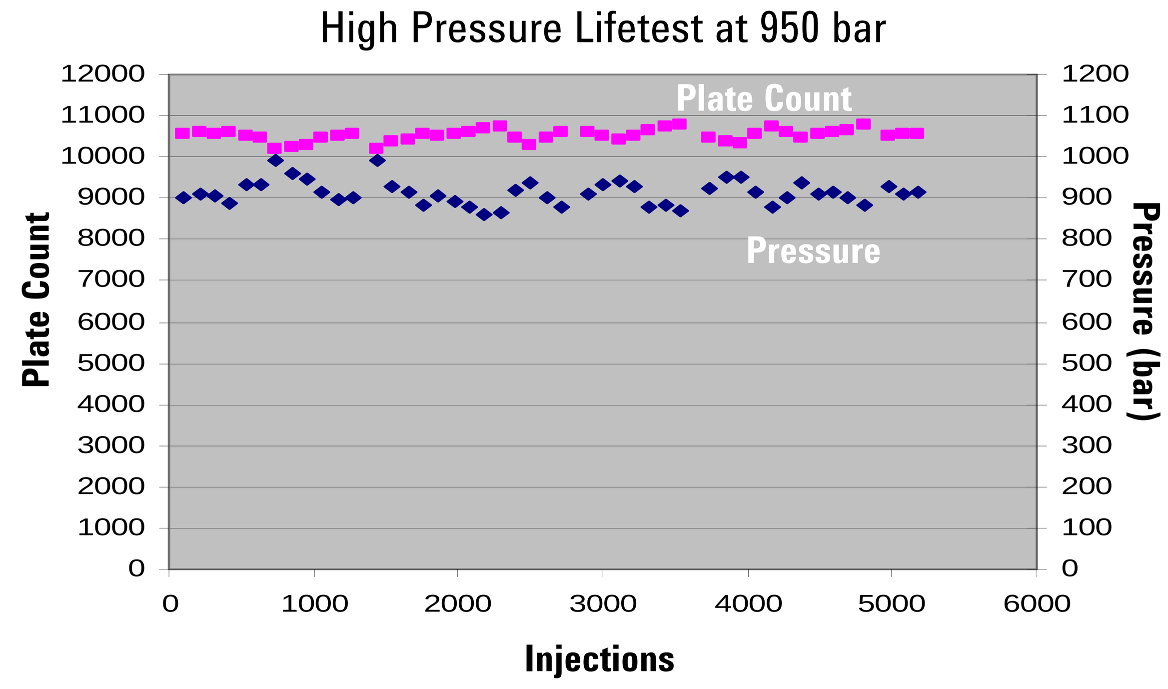
Agilent ZORBAX RRHD Column Lifetime

**Stationary Phase: ZORBAX RRHD Eclipse Plus C18
2.1 x 100 mm PN 959758-902**

Low Pressure Lifetest at 650 bar



High Pressure Lifetest at 950 bar

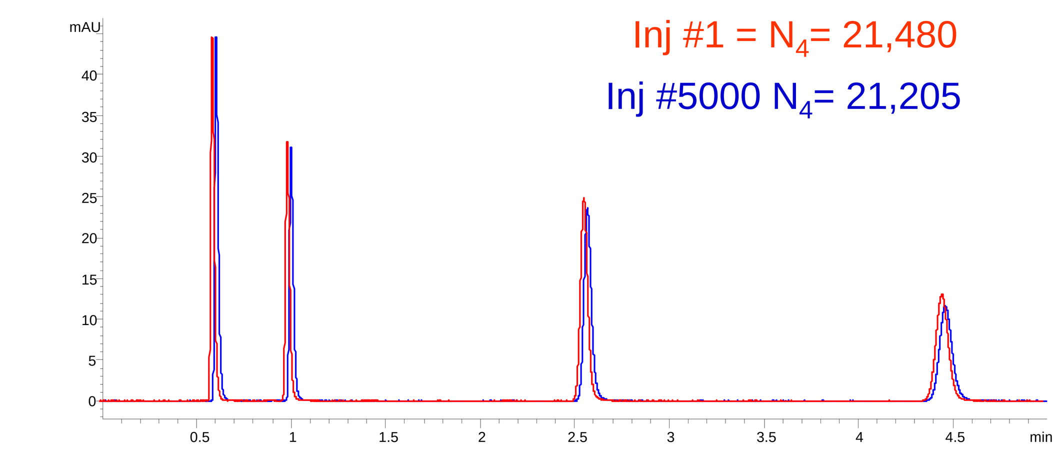


Column QC test (low pressure) performed before and after high pressure (1000 bar) 5000 injection lifetest

	Plate count	k'	Tf	Pressure (bar)
Initial QC	20722	6.52	1.07	359
Final QC	20653	6.67	1.12	370

Overlay of First and Last Injections of QC Test for Lifetime of RRHD 2.1 x 100 mm, 1.8 µm Column

Column QC Test: Mobile Phase: ACN:water Flow Rate: 0.3 mL/min Sample: Uracil, Phenol, 4-Chloronitrobenzene, Naphthalene

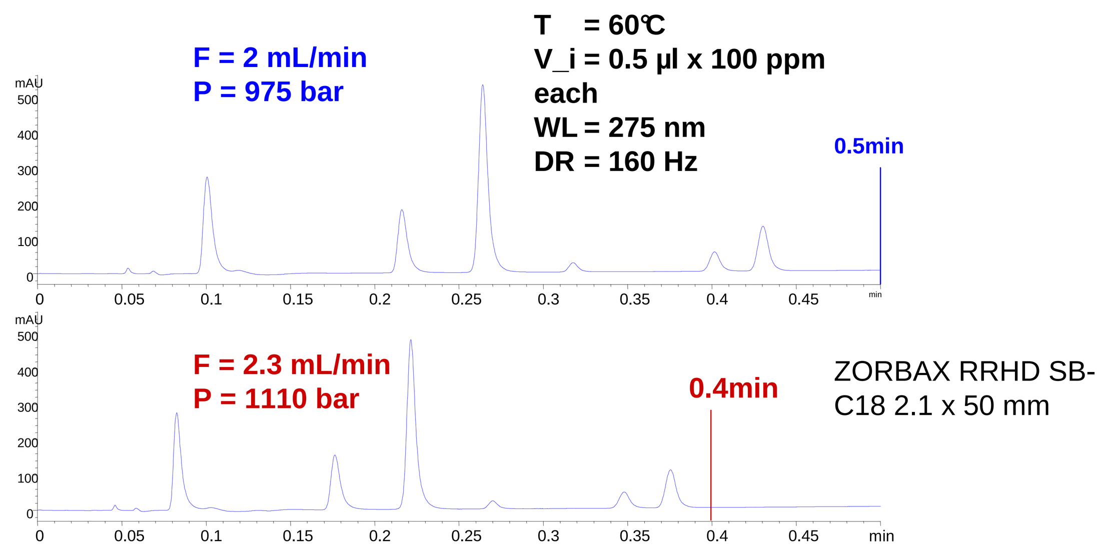


The QC chromatogram shows consistent performance over the 5000 injections.

Increased Speed

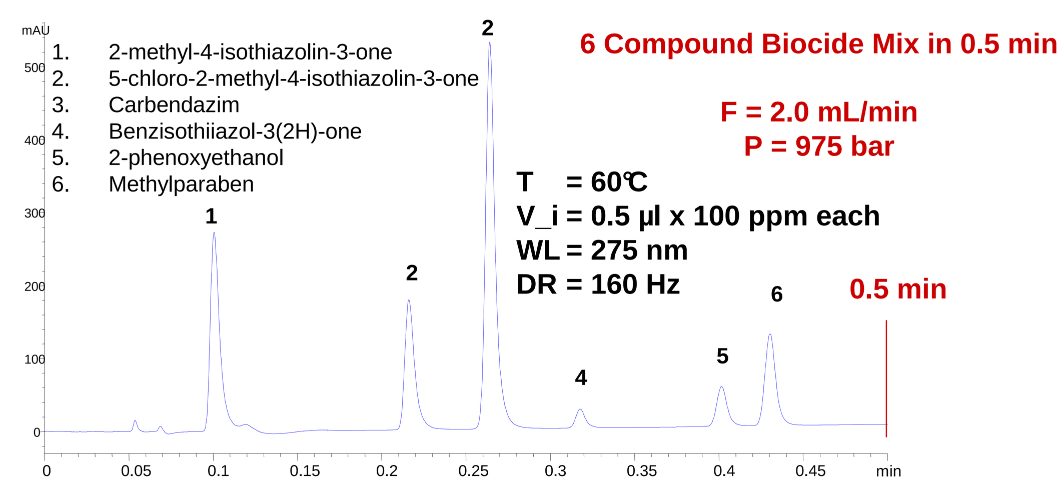
Increase Flow Rate for Fastest Separation on RRHD

Use Complete Pressure Range of Agilent 1290 Infinity LC System



Sub 1 Min Separations with ZORBAX RRHD Columns

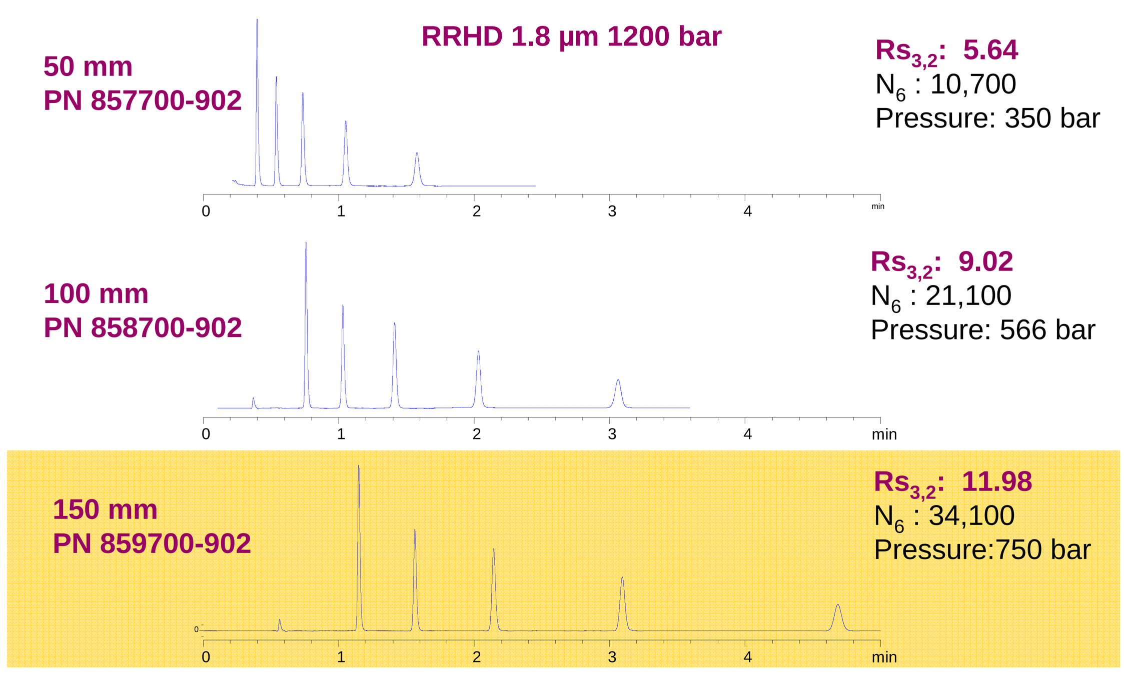
Column: ZORBAX RRHD SB-C18 2.1 x 50 mm 1.8 µm
Gradient: Water (0.05% trifluoroacetic acid) / 10-40 % ACN/1min



Increased Resolution

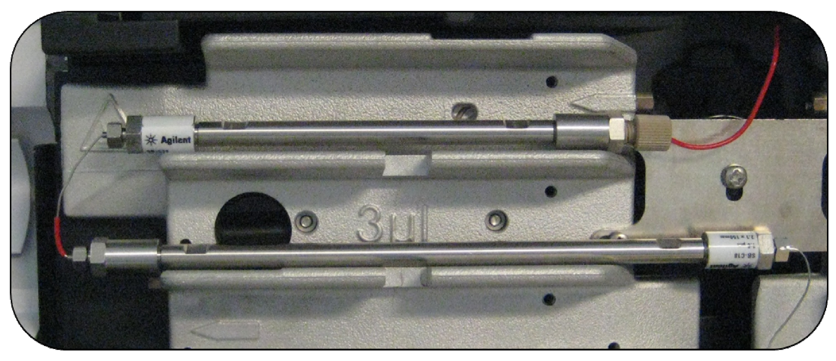
Choose Column Length for Analysis Time and Rs

Mobile Phase: 40:60 water:acetonitrile, Flow Rate: 0.5 mL/min, Temp: ambient, Columns: RRHD SB-C18, 2.1 mm ID, Lengths as noted Peaks: 1: uracil, 2 acetophenone, 3 propiophenone, 4 butyrophenone, 5 valerophenone, 6 hexanophenone



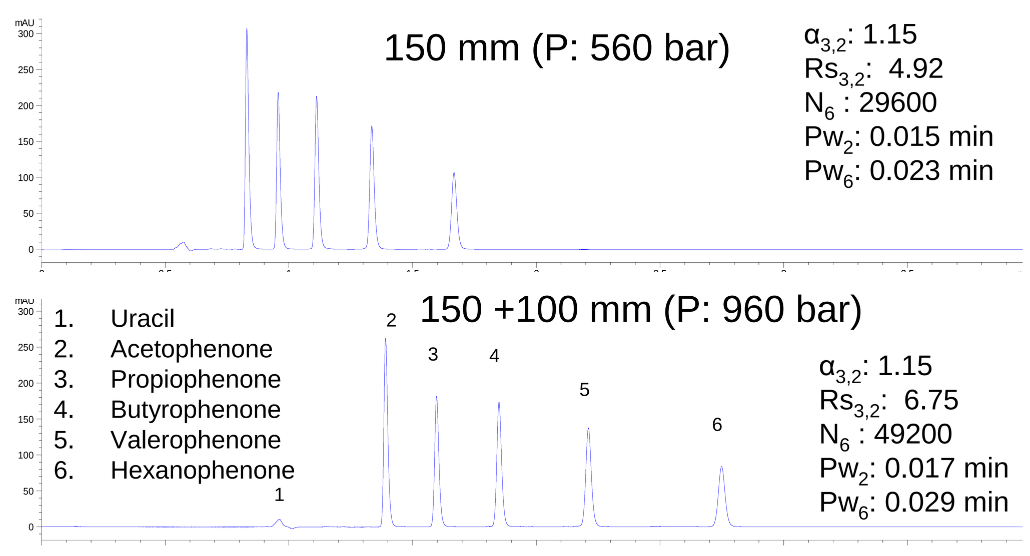
The 150 mm length RRHD column becomes a good option for high resolution with the 1290 Infinity LC.

Coupling ZORBAX RRHD Columns for Increased Resolving Power



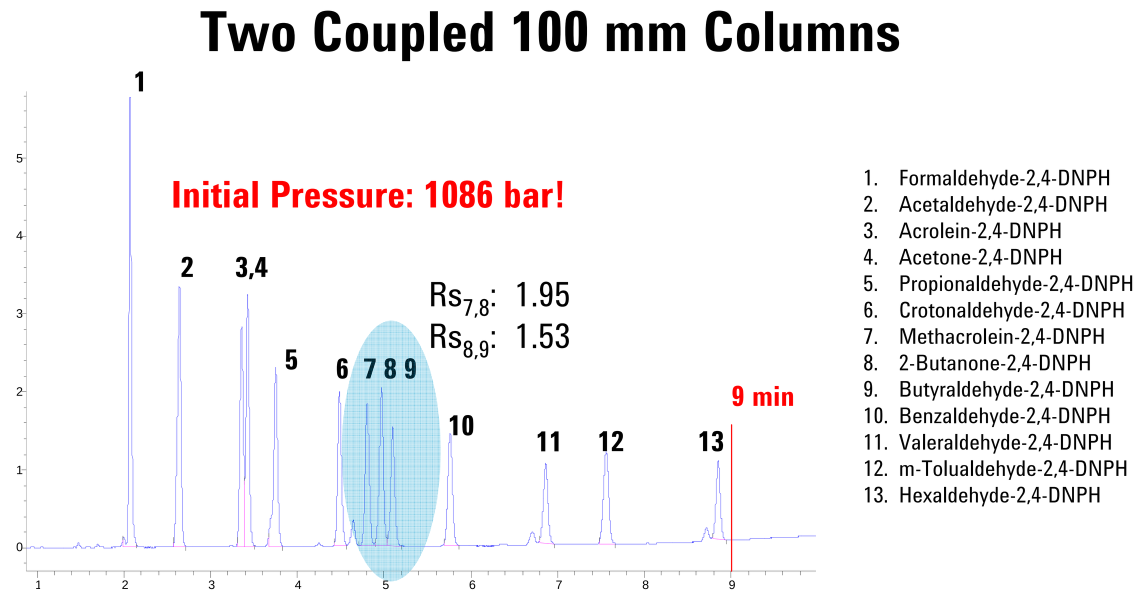
Custom length columns (>15 cm) can be created by joining together using a 0.12 x 70 mm tube (PN G1316-87303). This tube only adds 0.79 µL volume and allows for a straightforward arrangement of the columns within the G1316 column heating compartment.

Gain N, Resolution with Two Columns in Series 150 +100 mm = 250 mm effective length



20% Water, 80% Acetonitrile, Flowrate=0.5mL/min, ambient Temperature, 2.1x 100 mm & 150 mm ZORBAX RRHD SB-C18 columns

Separation of DNPH Derivatized Aldehydes via Two Coupled 100 mm Columns



Gradient 60%-70% Acetonitrile over 8 minutes, Flowrate=0.5mL/min, Ambient, temperature, 2.1 x 100mm ZORBAX RRHD SB-C18 columns

Conclusion

- Smaller particle columns generate higher efficiency but at the cost of higher pressure.
- Longer columns increase resolution.
- Improved resolution can lead to better quality information.
- Higher available pressure allows use of smaller particles, increasing resolution while decreasing analysis time.
- New ZORBAX RRHD Columns offer excellent stability and performance at pressures up to 1200 bar.

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