Lifetime Stability and Comparative Performance Analyses of Reversed-Phase Ultra High Performance Liquid Chromatography (UHPLC) Columns

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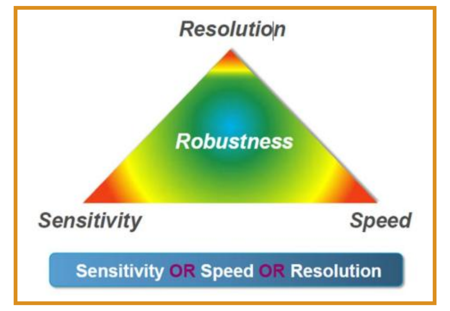
Introduction

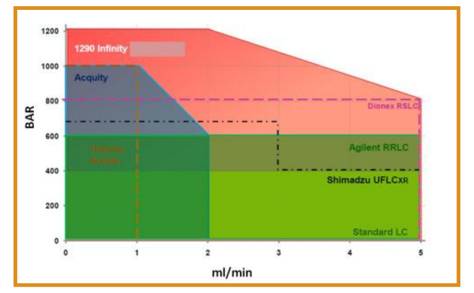
With the arrival of UHPLC, high efficiency separations with fast runtimes are being fully realized, so sacrificing resolution for speed is becoming more uncommon. However, with higher demands on sample throughput and faster separation times, which result in higher system pressures, the need for UHPLC columns with higher pressure tolerances and stable performance over time has never been greater.

An important and sometimes overlooked component of UHPLC column technology is lifetime reproducibility, column longevity and mechanical stability for extended periods of time under UHPLC pressure conditions. Also, current UHPLC column offerings have pressure limitations that can quickly compromise particle bed stability and fall exceedingly short in the number of expected injections, especially at high linear velocities (flow rates). In this study, we have analyzed and compared Agilent ZORBAX reversed-phase Rapid Resolution High Definition (RRHD) columns for chromatographic reproducibility, longevity and chemical and mechanical stability. The RRHD combination of a narrow particle size distribution and optimized loading conditions for increased bed stability, demonstrated superior high pressure LC performance benefits. Additionally, use on an Agilent 1290 Infinity at extended pressures (>1100 bar), has shown increased column robustness for expanding the utility of UHPLC chromatography

LC Instrumentation

UHPLC : Agilent 1290 Infinity

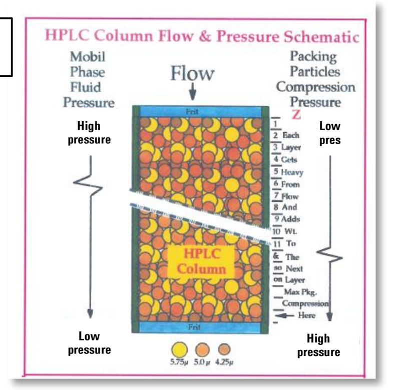




Packed Bed Stability

During the column packing process, compression on particles proportionately increases as column bed heights increase (cartoons A-B below). As such, there is a balance and limit to both loading pressure, slurry composition and particle compression to achieve a uniform tightly packed bed. These constraints require optimized loading and hardware technologies that are not typically encountered with traditional HPLC column development.

A.



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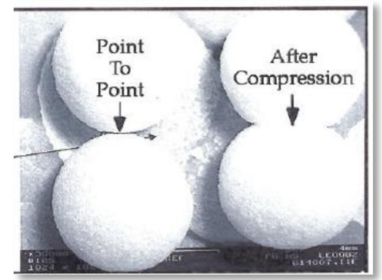
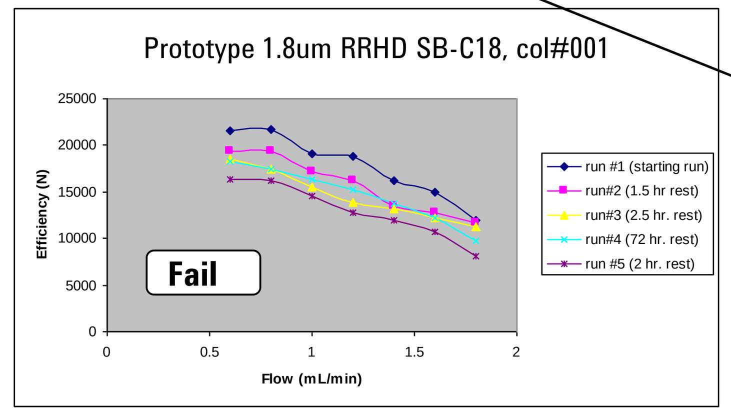


Panel A – Schematic of packing compression during hi-pressure column loading .
Panel B – Cartoon example of silica particle elasticity before and after compression.

RRHD (Rapid Resolution High Definition) columns contain 1.8 µm ZORBAX particles, specially packed to tolerate extreme pressures up to 1200 bar for continuous and efficient high pressure operation at high linear velocities.

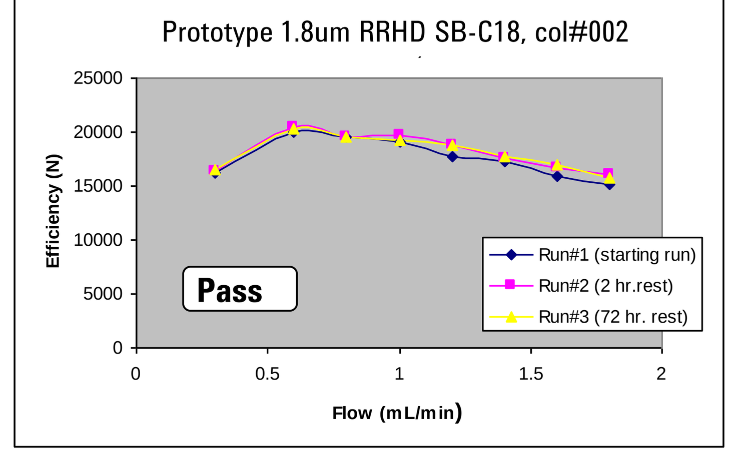
Optimizing Bed Stability – 3.0 x 100mm SB-C18

Fig. 1



Larger Column Diameter = Need for Greater Bed Stability

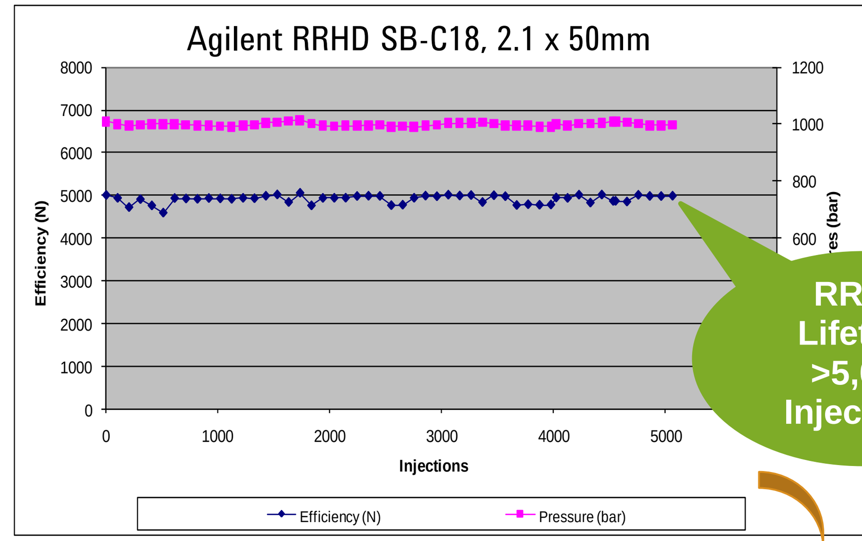
Fig. 2

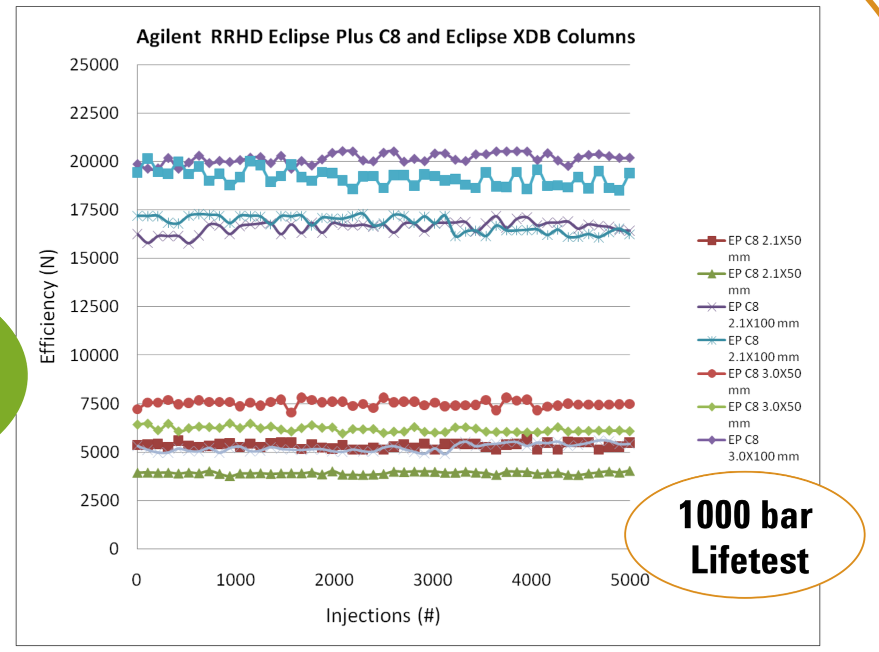


Prototype Rapid Resolution High Definition (RRHD) 1.8um columns were slowly ramped to achieve increasingly high pressures. After a run sequence was completed, the columns returned to ambient pressure for a defined length of time before another run sequence was started. As shown in figure 1, a column that had a sub optimally packed bed, displayed a drop in efficiency (N) after restarting the same run sequence. While the column shown in fig. 2 details stable efficiency (N) indicating a tightly packed bed and thus more stable column operation after hi-pressure operation.

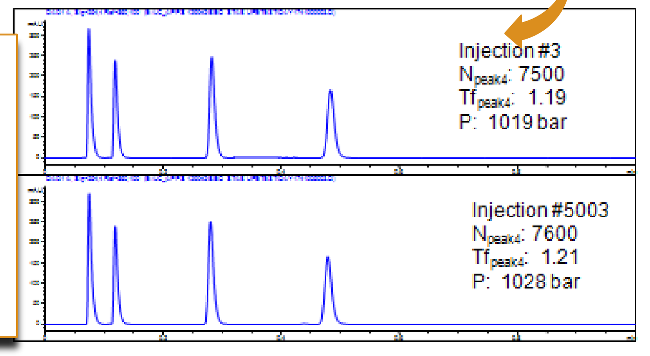
Column Lifetime & Stability

Low pH Lifetime Test (1000 bar)

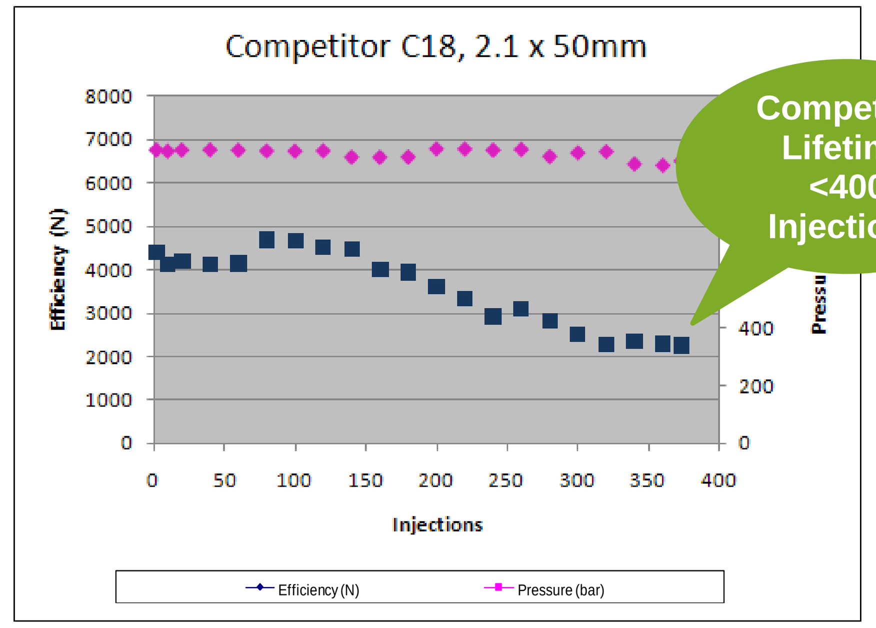




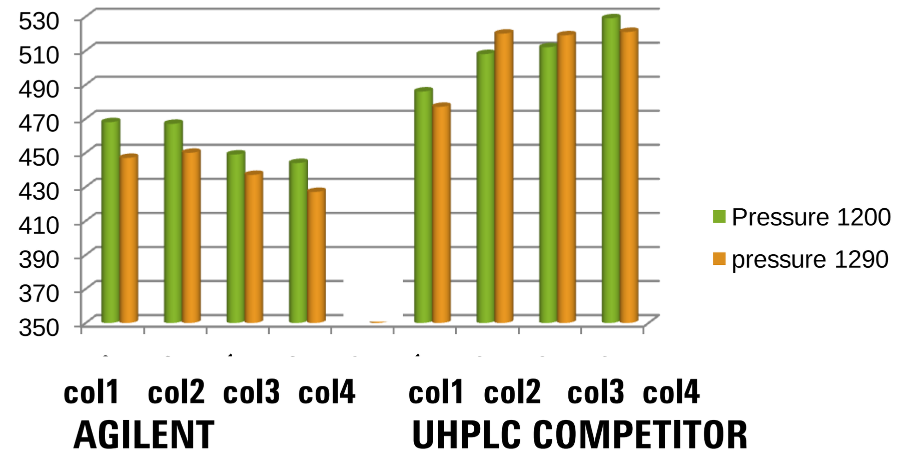
Chromatographic performance was evaluated (4-component test mix) at the start and completion of the lifetest. At injection #3 and #5003, column efficiency, tailing and back pressure were maintained indicating a stable and well packed column bed.



➢RRHD 1.8um Zorbax particle technology, robust loading conditions and optimized sub 2um hardware enable continuous RRHD column use at high system pressures (1000 bar) in all column size configurations.



Column Back Pressure Comparison



Agilent and competitor 2.1 x 100mm C18 columns were evaluated for pressure performance under ACN/water conditions. Agilent columns exhibited less pressure drop thus expanding the UHPLC range for achieving higher linear velocities and lower operational pressures.

Low pH Lifetest Conditions/columns:

Sample: 4 component mix (uracil, phenol, 4-chloronitrobenzene, napthalene) in 55/45 ACN/water

Method: alternating 5 sample injections with 99 blank injections. Run time per sample injection = 1.2 min.
Run time per blank injection = 0.3 min.
plot napthalene efficiency/injection (10% decrease in N = failure)

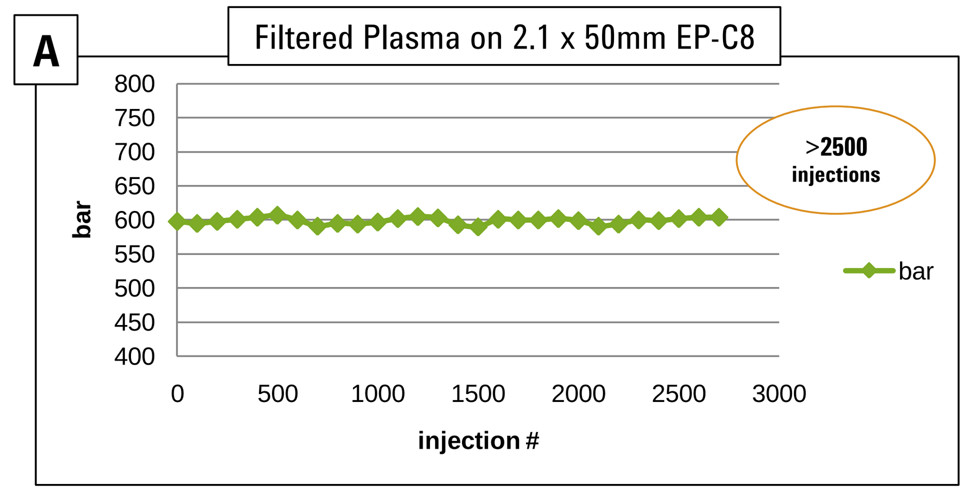
Mobile phase: 55/45/0.1 ACN/H_2O/FA, pH =2.7
Temp: 25 C
Flow (to deliver 1000 bar backpressure): 1.68 ml/min = 2.1 x50mm, 1.02 ml/min = 2.1 x 100mm, 2.93ml/min. = 3.0 x 50mm, 1.9 ml/min. = 3.0 x 100mm

Columns :
2.1 x50mm EC-C18, part#959757-902
2.1x50mm SB-C18, part#857700-902
2.1x50mm SB-C8, part#857700-906

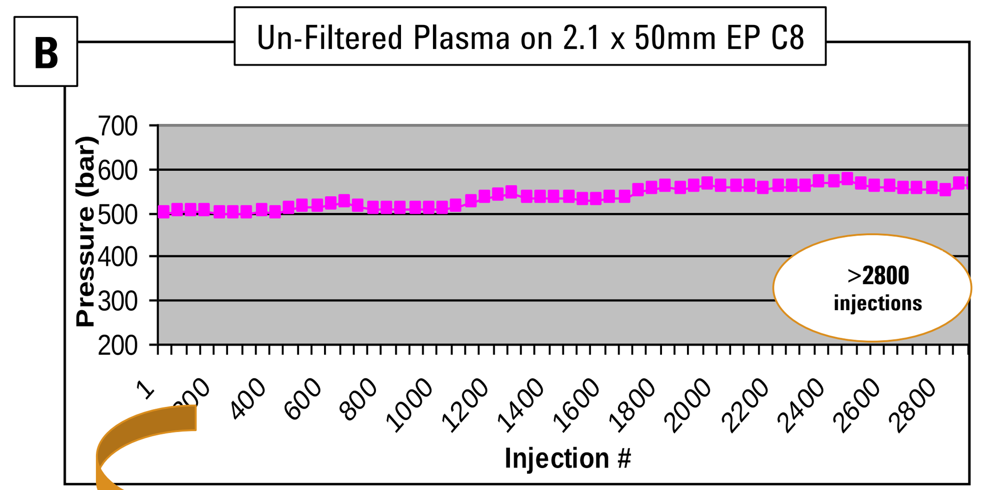
2.1 x100mm EC-C18, part#959758-902
2.1x100mm SB-C18, part#858700-902
2.1x100mm SB-C8, part#858700-906

Human Plasma Test

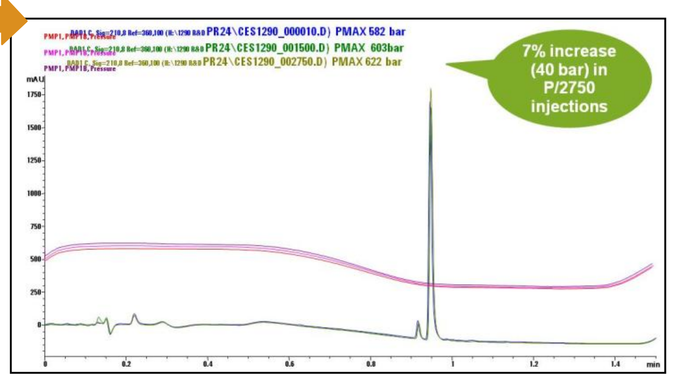
A



B



C



Pressure profiles comparisons of filtered (fig. A) and unfiltered (fig. B) human plasma samples repeatedly injected onto Agilent RRHD Eclipse Plus C8 columns,: 2.1 x 50mm, ACN/water conditions. Fig C shows reproducible pre and post pressure /plasma traces after 2800 repeated injections of unfiltered human plasma.

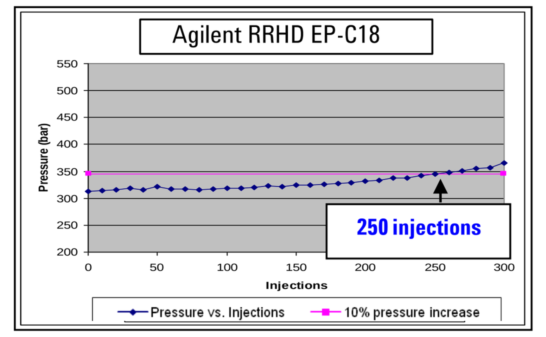
Precipitated Human Plasma Test Conditions:

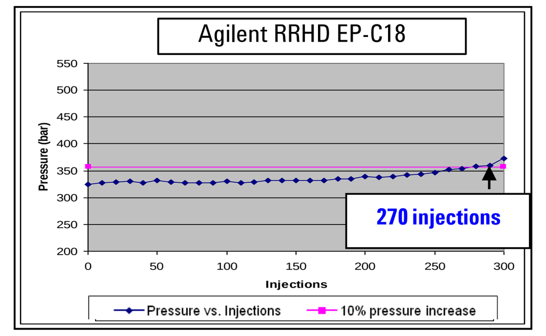
sample prep: Precipitate 1mg/ml plasma in ACN /filter w/0.22um cellulose spin tube. Dilute 50% w/ napthalene (100ug) mix (75/25 acn/water). Final conc. = 0.5 mg/mL plasma
Sample: 0.5mg/ml plasma + 0.1mg/ml naphthalene in 75/25 ACN/H2O solution (filtered).
Inj. Amt: 5ul
Method: flow = 0.8 ml/min; temp = ambient; detector = 210 nm;
Mobile phases: A – H2O + 0.1% TFA; B – ACN + 0.08% TFA

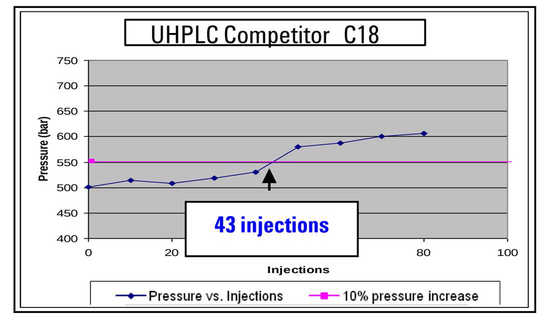
Gradient table

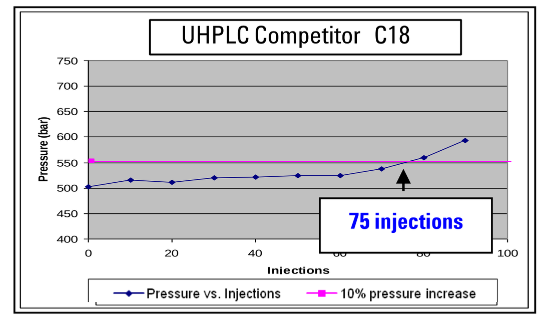
Time (min)	0	0.2	0.7	1.2	1.3	1.5
B%	20	20	90	90	20	Stop

Microparticulates Test









Repeated injections of an unfiltered microparticulate sample (microcrystalline cellulose) onto 2.1 x 50mm Agilent RRHD Eclipse plus C18 columns and 2.1 x 50mm UHPLC competitor columns. Column sequences were run until a 10% increase in back pressure was observed. RRHD columns demonstrated a higher tolerance to incoming sample microparticulates.

Particulates Test Conditions:

Particulates suspension (microcrystalline cellulose, Carboxymethylcellulose sodium, Xanthan gum, Carrageenan, Citric acid, sodium phosphate, Potassium sorbate and methylparaben in methanol/water)
Sample: Particulates suspension (vortex 30s. prior)
Inj. Amt.: 1ul
Method: flow = 0.55 ml/min (2.1 x 50mm col.); temp = ambient; detector = 254 nm
Mobile phases: 55/45/0.1 ACN/H_2O/FA

Conclusions

- ➢ **Agilent's family of 1.8um Rapid Resolution High Definition (RRHD) columns are specially packed and engineered to remain stable under extreme pressures, up to 1200 bar, for extended periods of column use.**
- ➢ **1.8um RRHD columns deliver robust and reproducible chromatographic performance after repeated sample injections (>5000 injections) at high operational pressures.**
- ➢ **RRHD columns display a high resistance to premature plugging and subsequent back pressure increases from incoming samples containing microparticulates such as plasma and cellulose .**