

New HPLC Column Options for UHPLC

Rapid Resolution High Definition
Columns and More



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UHPLC – Why Use Different Columns?

UHPLC instruments include a variety of high pressure LC choices including those designed for high throughput/fast LC.

These instruments operate at pressures >400 bar/6000 psi.

The desire is to have small particle size columns <2-3 μ m for improved efficiency, resolution and productivity on these instruments.

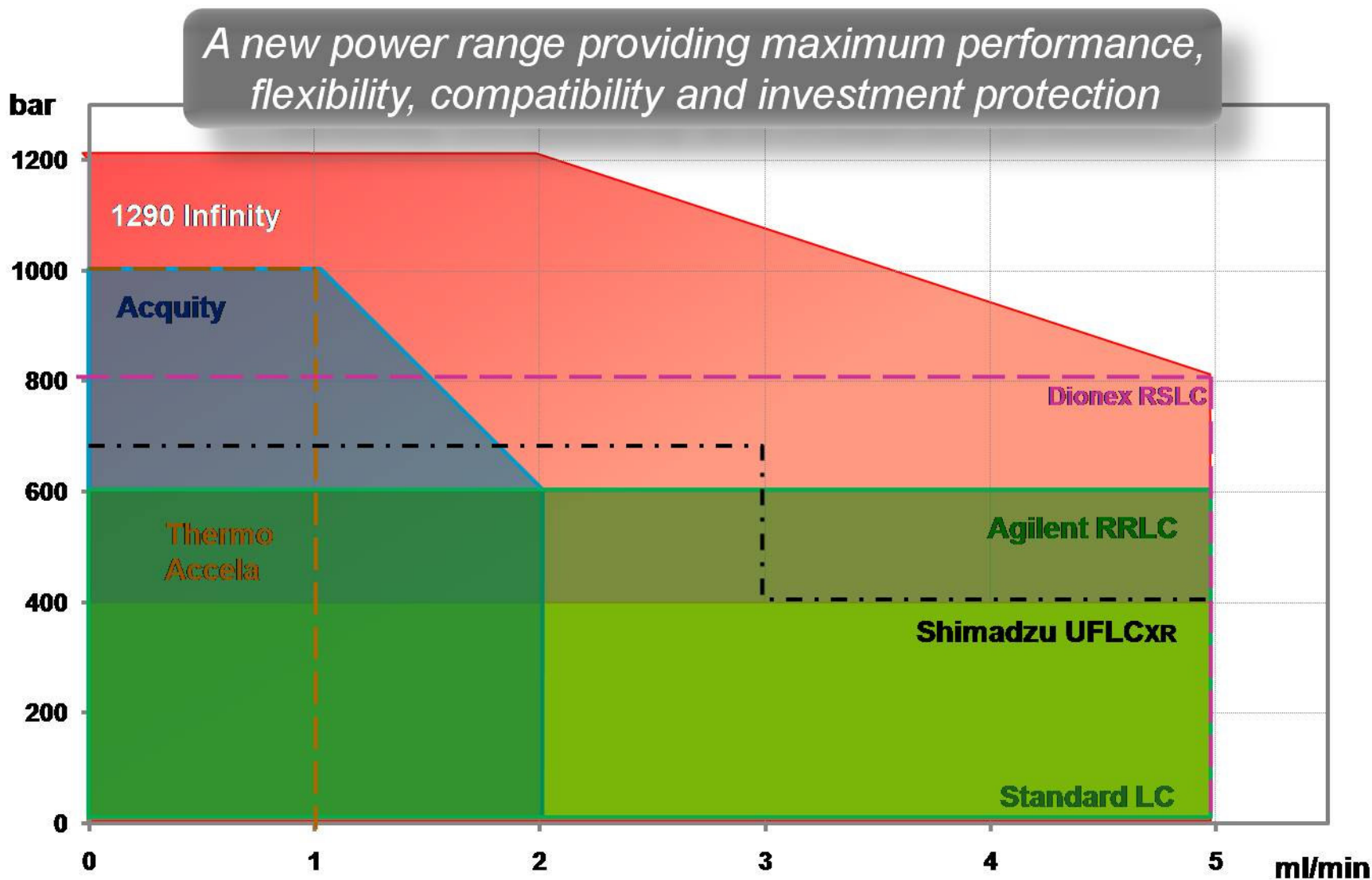
These provide the flexibility to operate above 400 bar for many applications – **for speed or for enhanced resolution with long 1.8 μ m columns**

Agilent's new 1290 Infinity LC can operate up to 1200 bar.

This requires columns designed for up to 1200 bar operating conditions – the new RRHD Columns!



The 1290 Infinity Has a Wide Useable UHPLC Range



Productivity that Can Be Achieved Using RRHD Columns with the 1290 Infinity LC

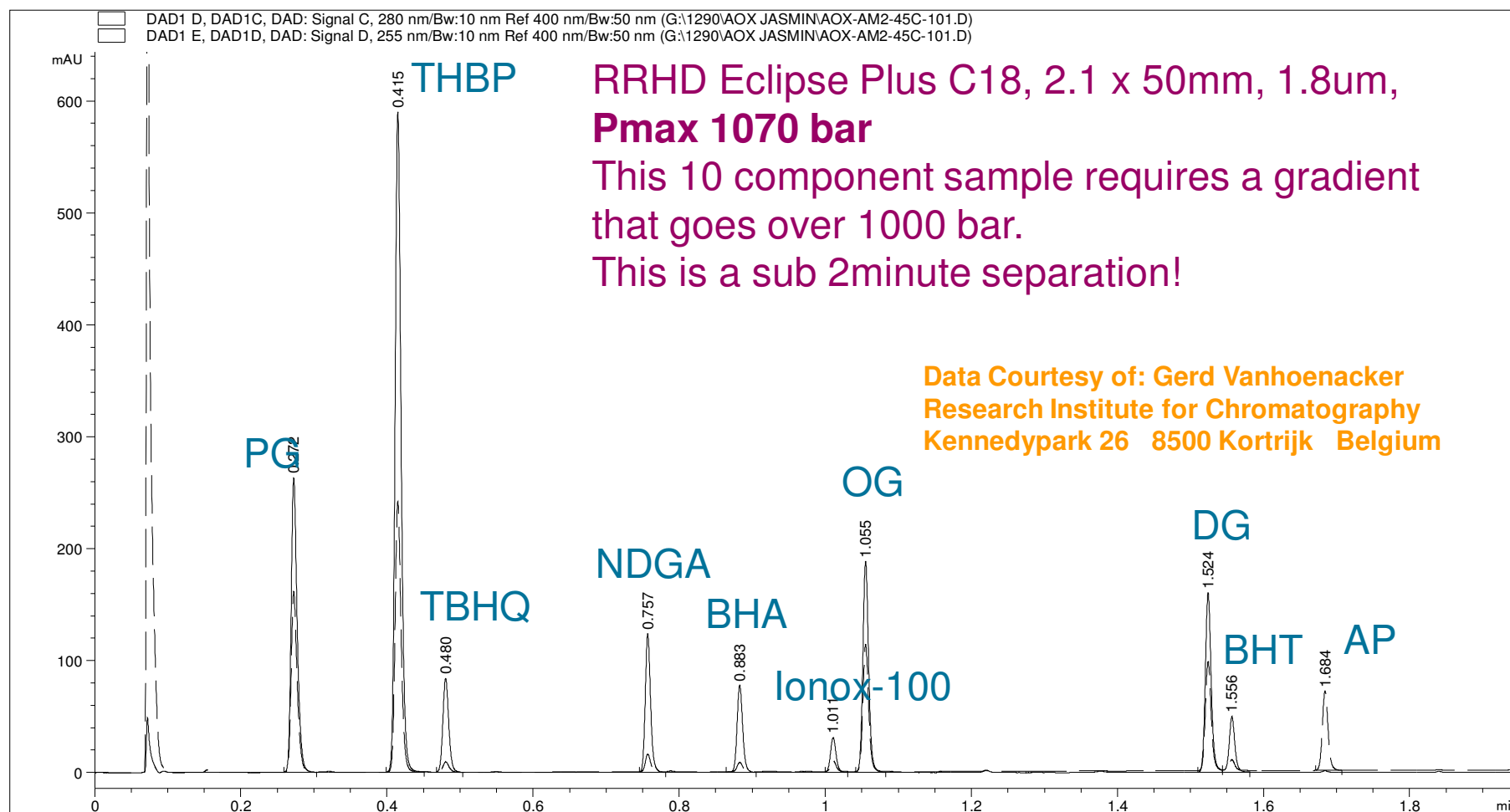
1. Fast analysis – especially with somewhat complex samples
 1. Gradient analysis with 4-10 components
2. More resolution in a limited amount of time
 1. Choosing different column lengths for the resolution desired
 2. Maximizing peak capacity in gradient separations of complex samples



RRHD – Rapid Resolution High Definition



Fast Analysis on ZORBAX Rapid Resolution High Definition Eclipse Plus C18



Fast Analysis on Agilent Zorbax Rapid Resolution High Definition Eclipse Plus C18

Method

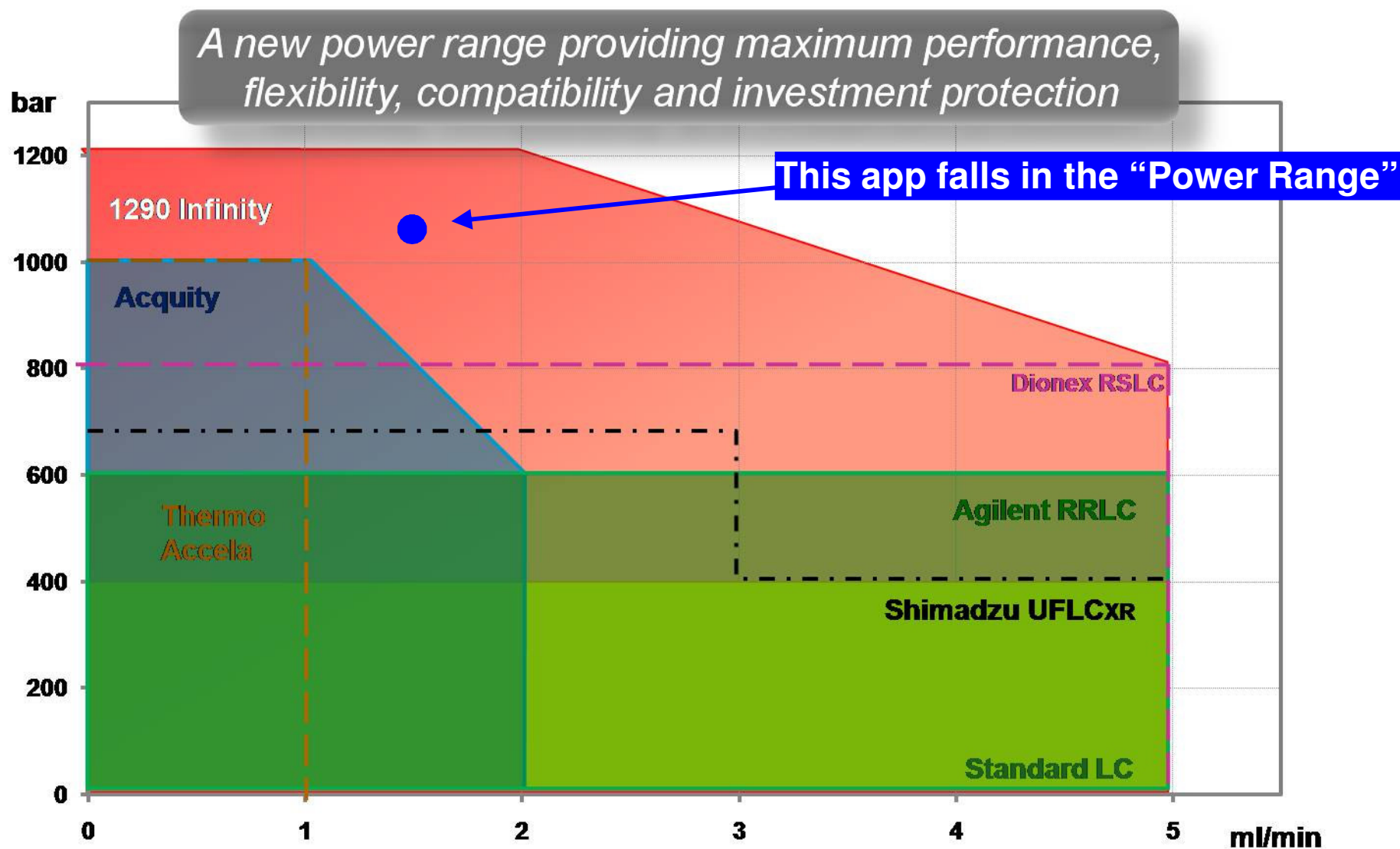
Column	ZORBAX RRHD Eclipse Plus C18 2.1 x 50 mm, 1.8 µm (SN USDAY01001)	
Mobile phase	A=0.02% H ₃ PO ₄ , B=acetonitrile/methanol 3/1	
Flow rate	1.6 mL/min	
Gradient	0-1.85 min	30-100% B
Injection volume	1 µL	
Detector	Sig=280/10 nm, Ref=400/50, 80 Hz Sig=255/10 nm, Ref=400/50, 80 Hz	
Temperature	45°C	
Sample	Antioxidant mixture, ca. 100 ppm	

A 1.6 mL/min flow rate is very fast, and allows a fast separation.

Data Courtesy of: Gerd Vanhoenacker Research Institute for Chromatography
Kennedypark 26 8500 Kortrijk Belgium

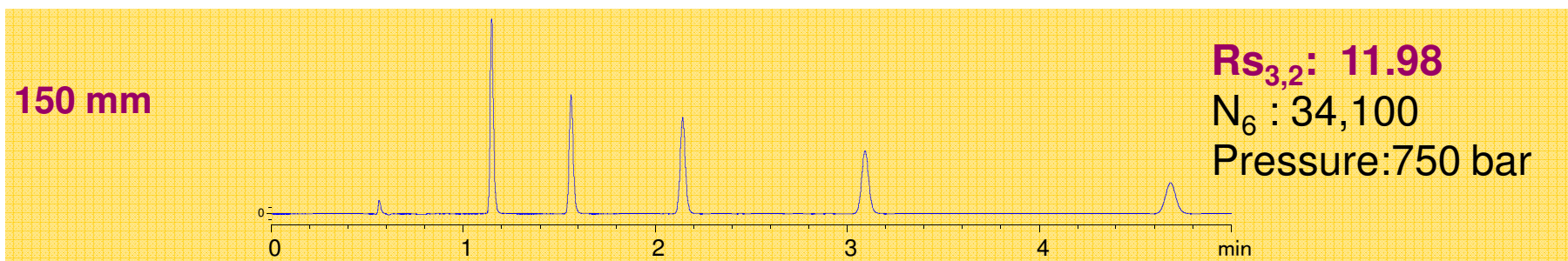
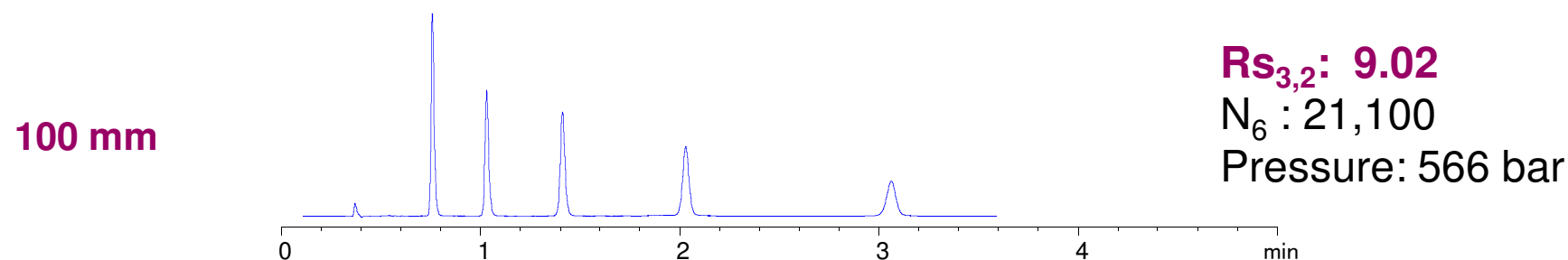
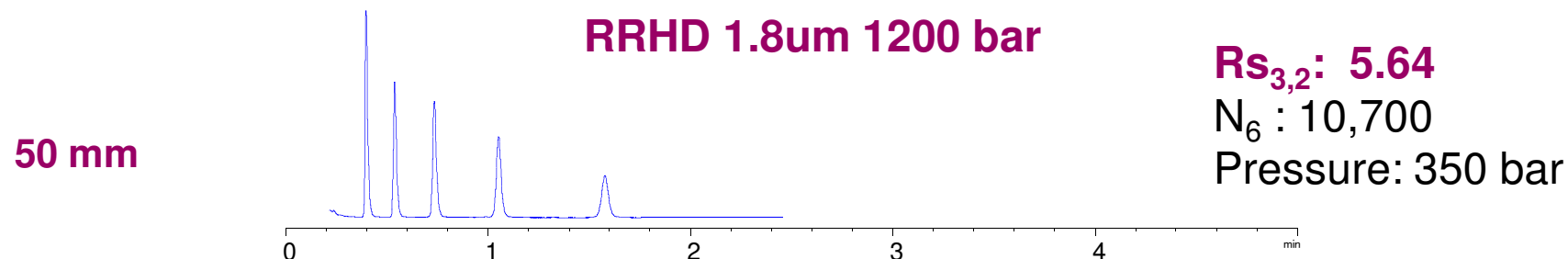


Where Does this Example Fall in the 1290 Infinity Range



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

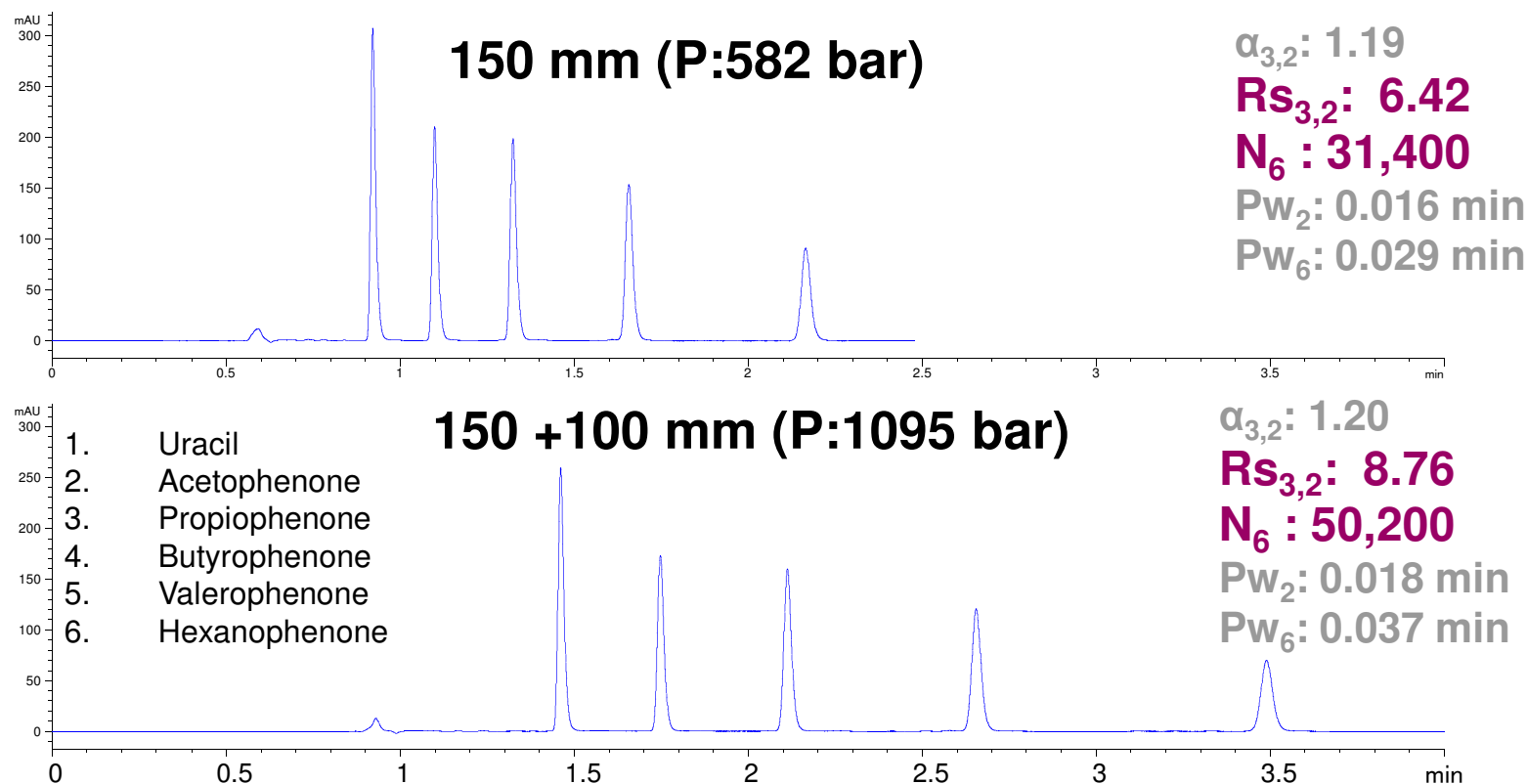


The 150mm length RRHD column becomes a good option for high resolution with the 1290 Infinity LC.

Gain Efficiency and Resolution with Long Length

150 + 100 mm = 250 mm 1.8um @ 1100 bar

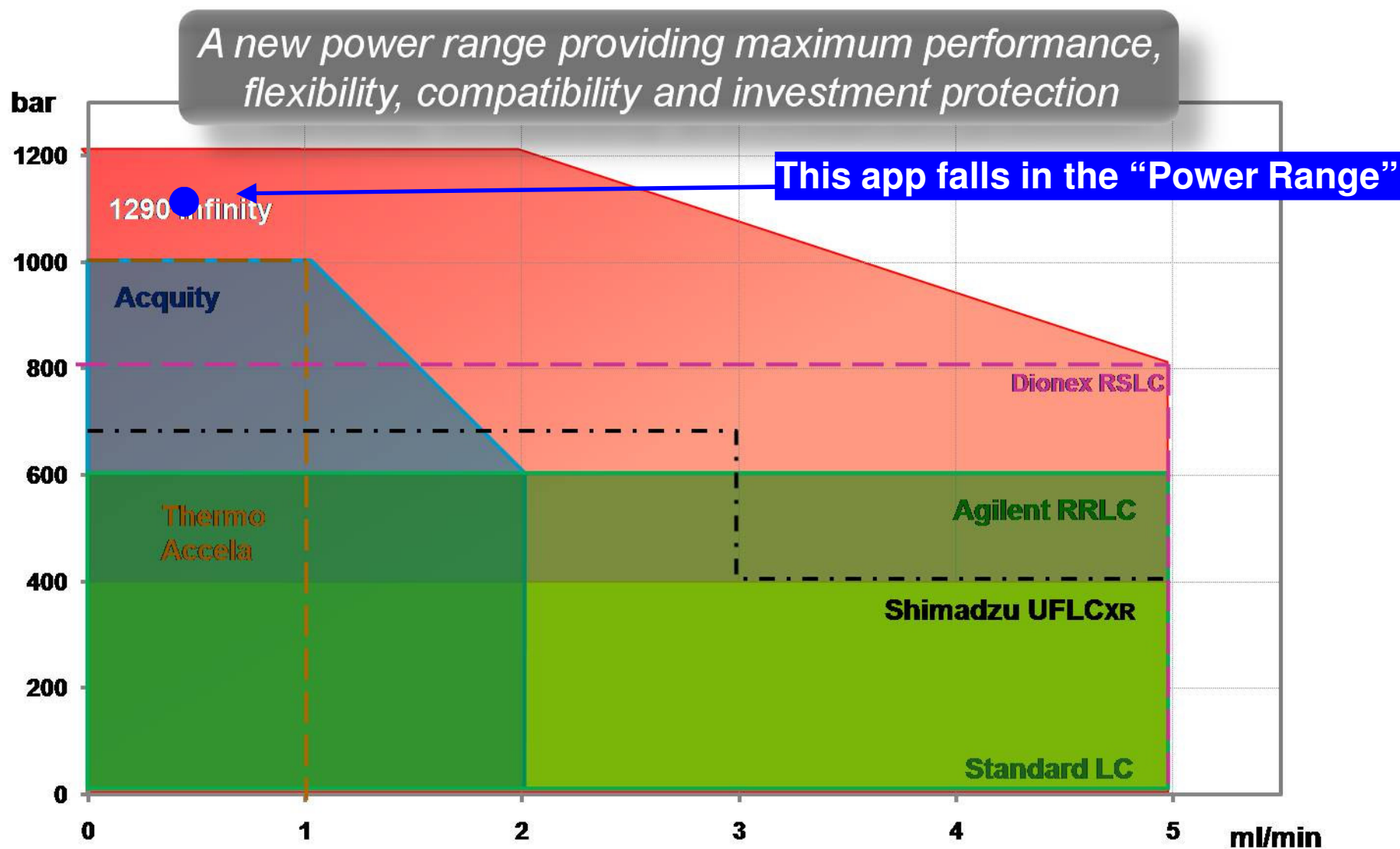
Mobile Phase 25:75 water:acetonitrile, Flow Rate: 0.5 mL/min, ambient temperature,
ZORBAX RRHD SB-C18, 1.8um columns



High Rs in 3.5 minutes, 250mm, 1.8um at 1100 bar
Resolution is fine on 150mm column, but for even more Rs longer column is used.

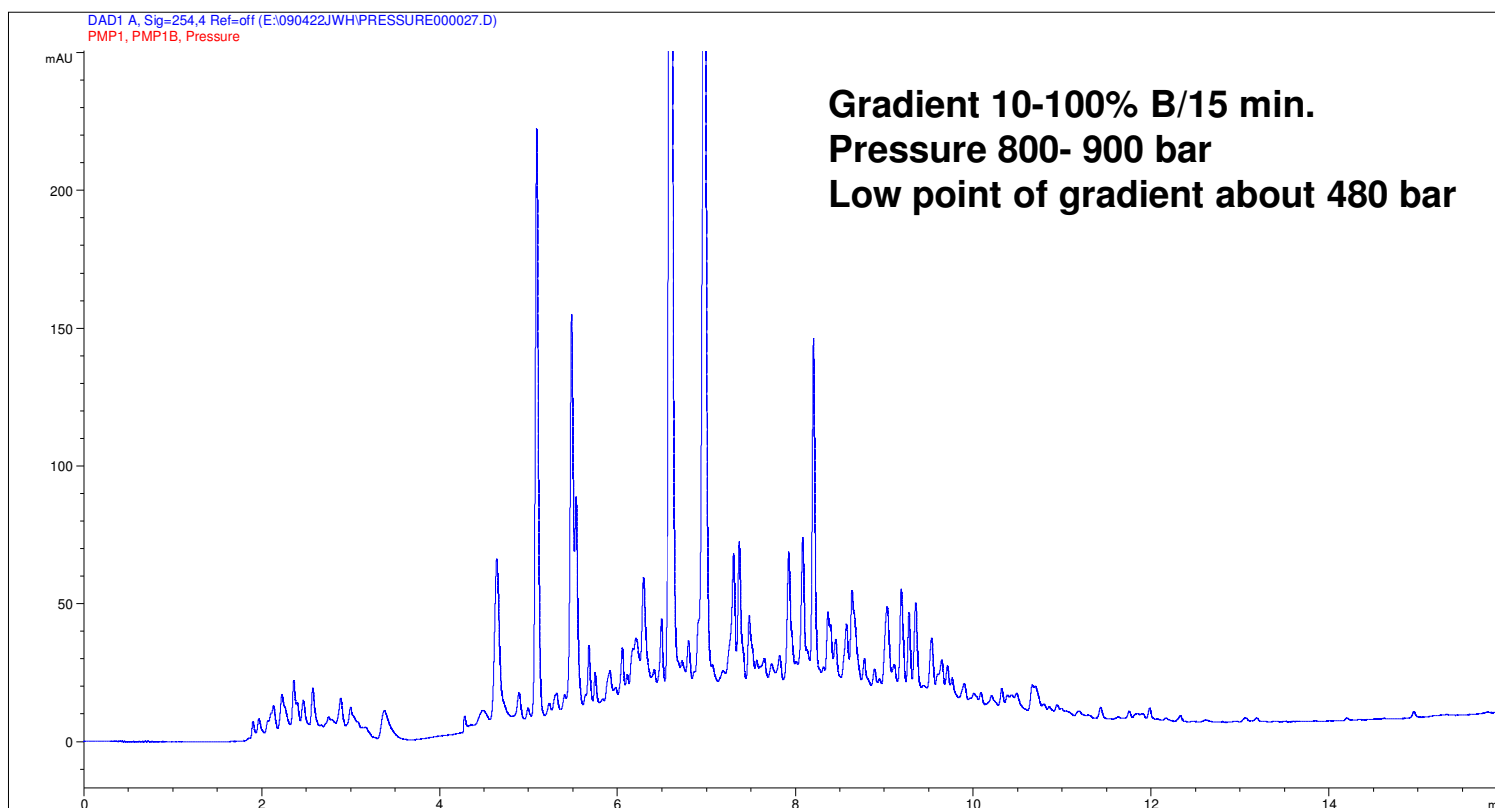


Where Does this Example Fall in the 1290 Infinity Range



High Resolution Separation with Long 1.8um Columns

Mobile Phase A: water B:acetonitrile, F=0.25 mL/min, ambient temperature,
Columns: ZORBAX RRHD, SB-C18, **2.1 x 150+100 mm**, 1.8um



Long column or columns in series provide max Rs with this complex sample – smoked food flavoring.



Achieving Optimal Resolution for a Gradient with RRHD Column Choices

Columns range in length from 50 – 150mm.

Column length and gradient time can be changed to optimize the resolution and retention of the analytes.

$$k^* = \frac{t_G \bullet F}{S \bullet \Delta\Phi \bullet V_m}$$

These 3 parameters can be easily changed.

t_G = gradient time

F = flow rate

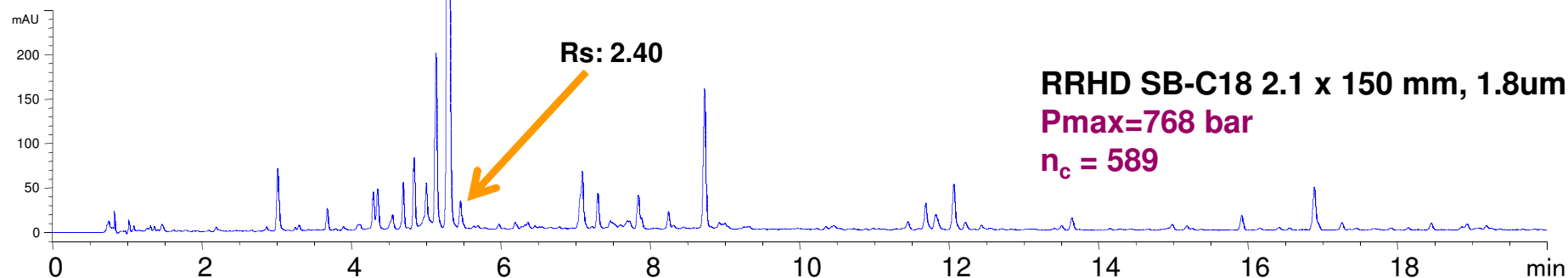
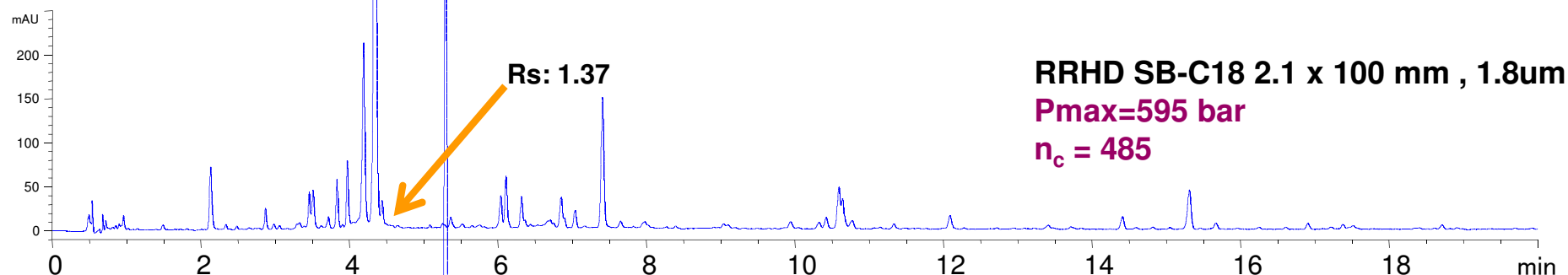
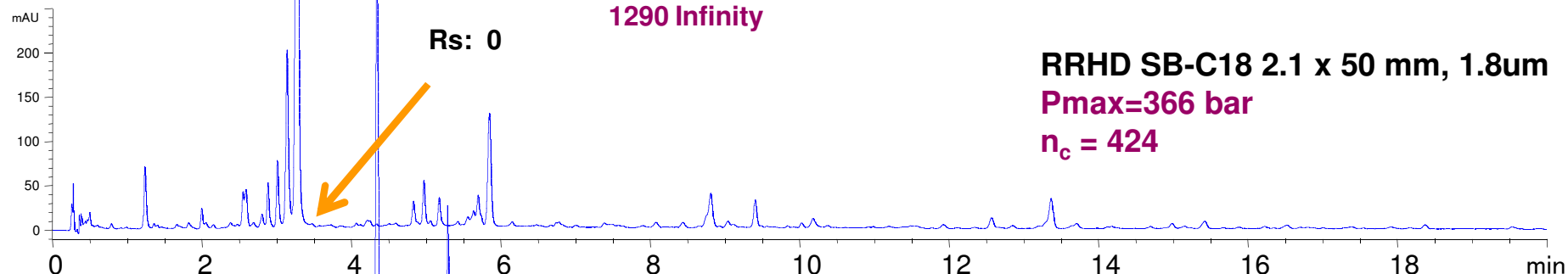
**V_m = column volume
(change by changing column length)**



Separation of Licorice Root on RRHD Columns – 30 minute gradient on each length

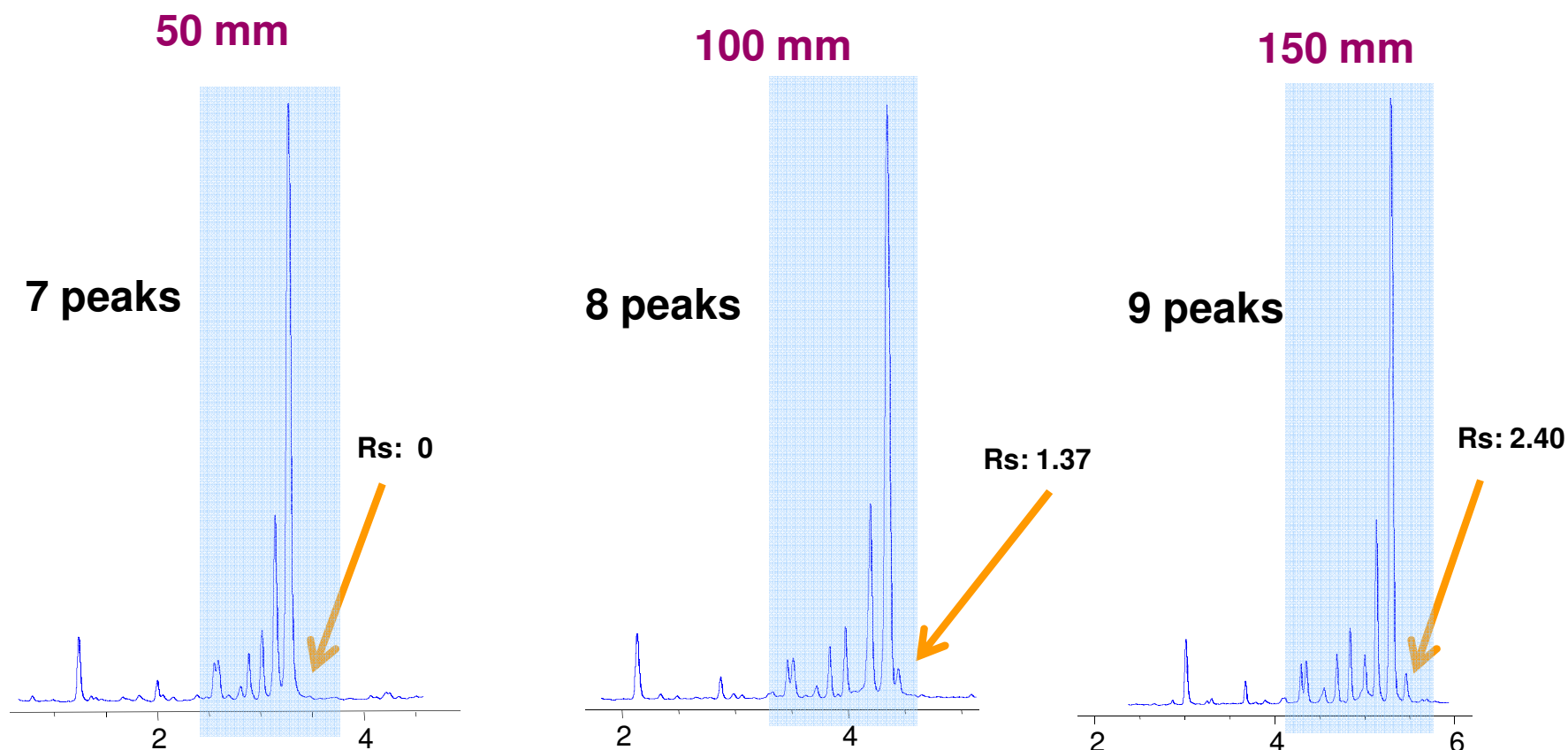
Mobile Phase: 10-100% B /30 min A: 0.1% formic acid (fa) B: acetonitrile with 0.1% fa
F=0.4 mL/min Ambient temperature 280nm UV

1290 Infinity



Separation of Licorice Root on RRHD Columns – 30 minute gradient on each length

Mobile Phase: 10-100% B /30 min A: 0.1% formic acid (fa) B: acetonitrile with 0.1% fa F=0.4 mL/min Ambient temperature 280nm UV
1290 Infinity



Increasing peak capacity led to increased Rs for this sample.

Peak Capacity Comparisons – Length and Gradient Time

nc= tg/wbaseline of last eluting peak¹

Licorice Root sample

Gradient Time \ Column Length	10 minutes	20 minutes	30 minutes
50	313	358	424
100	343	418	485
150	386	483	589

Shaded boxes indicate comparable peak capacities

1. Neue, U.D., et. al, Advances in Chromatography, 41, 93 (2001)



RRHD 1.8um Columns

The part numbers!

- These new columns will have new part numbers (table below)
- The column shipping with the 1290 Infinity LC will be the **ZORBAX Rapid Resolution High Definition (RRHD) Eclipse Plus C18, 2.1 x 50mm, 1.8um**

PN	Description
959759-902	RRHD Eclipse Plus C18, 2.1 x 150mm, 1.8um
959758-902	RRHD Eclipse Plus C18, 2.1 x 100mm, 1.8um
959757-902	RRHD Eclipse Plus C18, 2.1 x 50mm, 1.8um
859700-902	RRHD SB-C18, 2.1 x 150mm, 1.8um
858700-902	RRHD SB-C18, 2.1 x 100mm, 1.8um
857700-902	RRHD SB-C18, 2.1 x 50mm, 1.8um
859700-906	RRHD SB-C8, 2.1 x 150mm, 1.8um
858700-906	RRHD SB-C8, 2.1 x 100mm, 1.8um
857700-906	RRHD SB-C8, 2.1 x 50mm, 1.8um



Why Two Different C18 Columns?

Eclipse Plus C18 and StableBond C18

Different selectivity

- Eclipse Plus C18 vs. StableBond C18

Optimum peak shape

- Achieved with endcapped C18 – Eclipse Plus C18

Optimum stability at low pH

- Achieved with non-endcapped C18 – StableBond C18

Additional bonded phases will be converted to the RRHD format

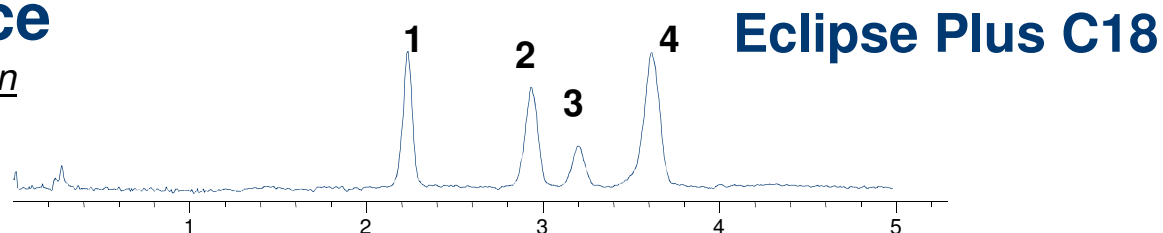


Different ZORBAX RRHT/RRHD C18 Bonded Phases for Maximum Selectivity

Mobile phase: (69:31) ACN: water Flow 1.5 mL/min. Temp: 30 °C Detector: Single Quad ESI
positive mode scan **Columns: RRHT 4.6 x 50 mm 1.8 µm**
Sample: 1. anandamide (AEA) 2. Palmitoylethanolamide (PEA) 3. 2-arachinoylglycerol (2-AG)
4. Oleoylethanolamide (OEA)

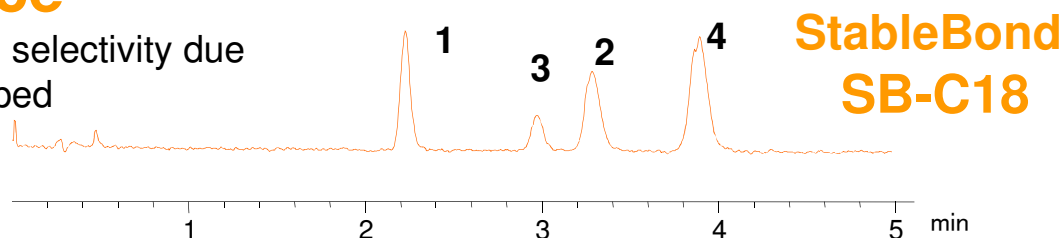
1st choice

Best Resolution
& Peak Shape



2nd choice

Good alternate selectivity due
to non-endcapped



Multiple bonded phases for most effective method development.



Peak Shape of Comparison of sub 2 um columns

Eclipse Plus is Superior to Acquity BEH C18

Mobile Phase = 80% 25 mM phosphate (pH 3): 20% ACN, Flow Rate = 0.3 mL/min Detection: 205nm
Analyte: Dextromethorphan

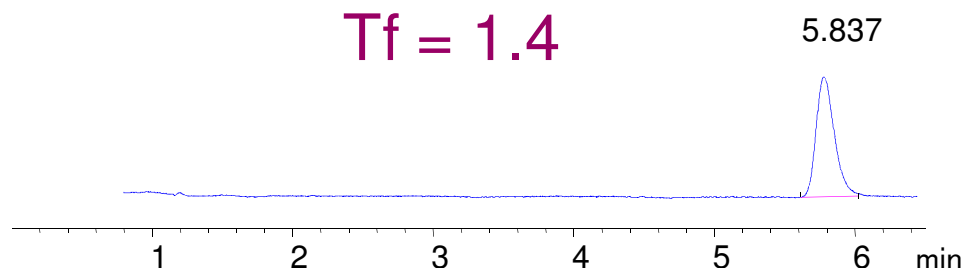
ACQUITY BEH C18

2.1 x 50 mm, 1.7 μ m

P = 228 Bar N = 10107,

Peak Width = 0.137 min,

Tf = 1.39



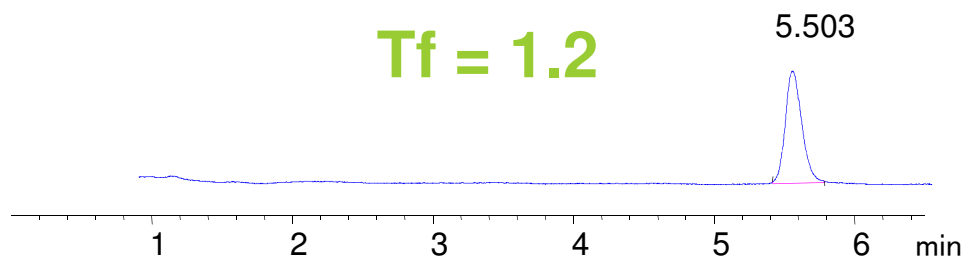
RRHT Eclipse Plus C18

2.1 x 50 mm, 1.8 μ m

P = 202 Bar N = 10881,

Peak Width = 0.124 min,

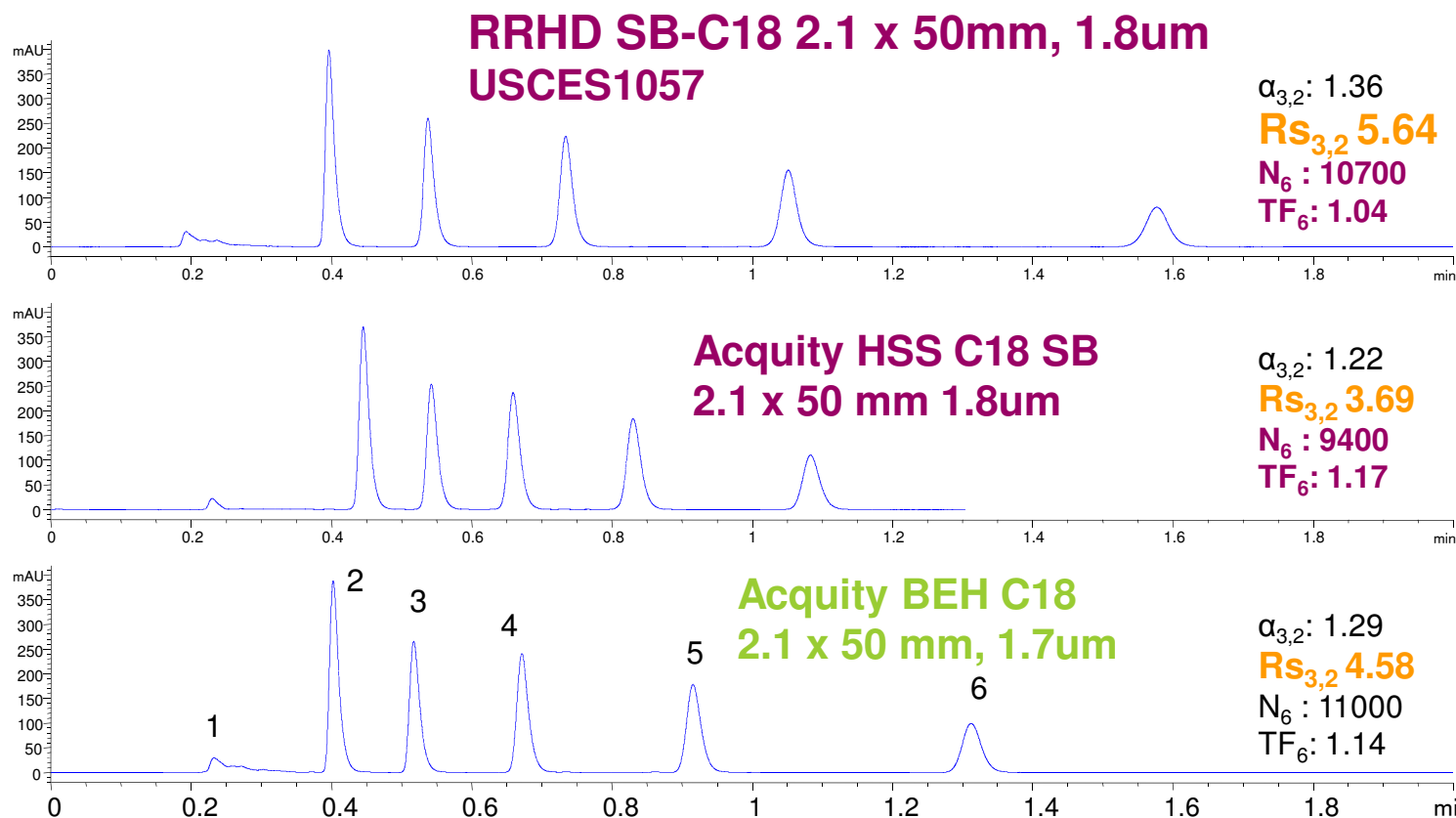
Tf = 1.23



Many columns are competitive and slight improvements in peak shape can be important to maximum sensitivity.

Resolution on RRHD vs. Other Sub 2um on 1290 Infinity

Mobile Phase: 40:60 water:acetonitrile, 0.1% Formic Acid Flow Rate: 0.5 mL/min, Temp: ambient, Det: UV 254 nm Columns: RRHD SB-C18, 2.1 x 50mm, 1.8um and competitive Peaks: 1: uracil, 2:acetophenone, 3 propiophenone, 4 butyrophenone, 5 valerophenone, 6 hexanophenone



StableBond offers greatest Rs and is still the best column for low pH lifetime on the market!

Are There Other Column Types for UHPLC?

Review of Van Deemter Curves for Efficiency

Column types for UHPLC – depending on speed, resolution, and throughput requirements

1. **RRHD columns** – 1.8um columns for high productivity up to 1200 bar

2. **RRHT columns** – 1.8um columns for fast LC and with a 600 bar pressure limit

- Ideal for fast LC and for working at the 600 bar pressure limit
- Available in lengths from 20mm to 150 mm, with 20, 30, 50 and 75mm lengths ideal for fast high resolution separations
- 4.6 mm ID columns ideal for use at this lower pressure limit, with 3.0 and 2.1 mm ID columns also available

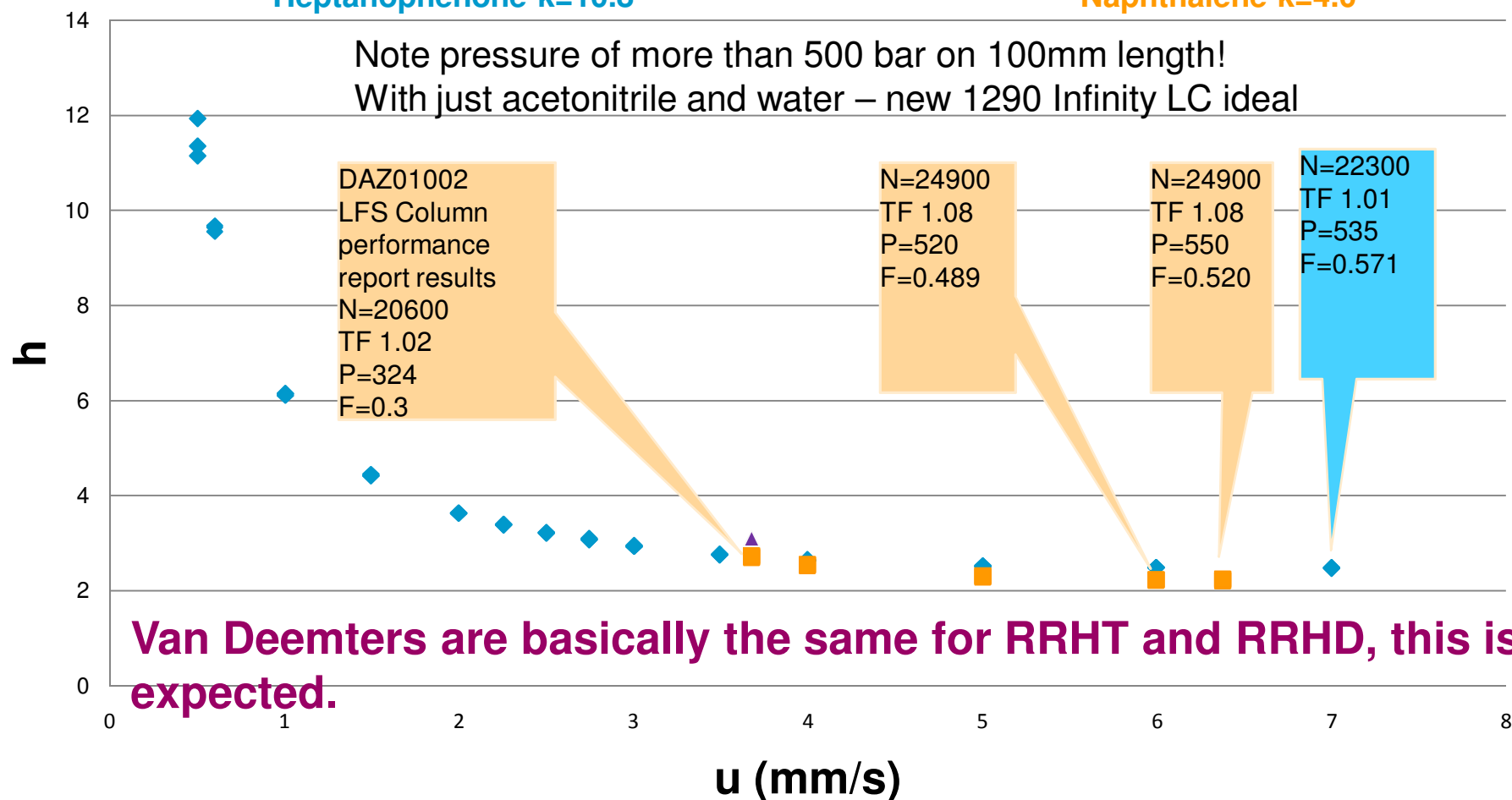
3. Upcoming **Poroshell 120 columns**



Van Deemters for RRHT and RRHD Columns

RRHT Eclipse Plus C18, 2.1 x 100 mm, 1.8 μ m;
PN 959764-902, SN USUXU01664, LN B08101
Heptanophenone $k=10.8$

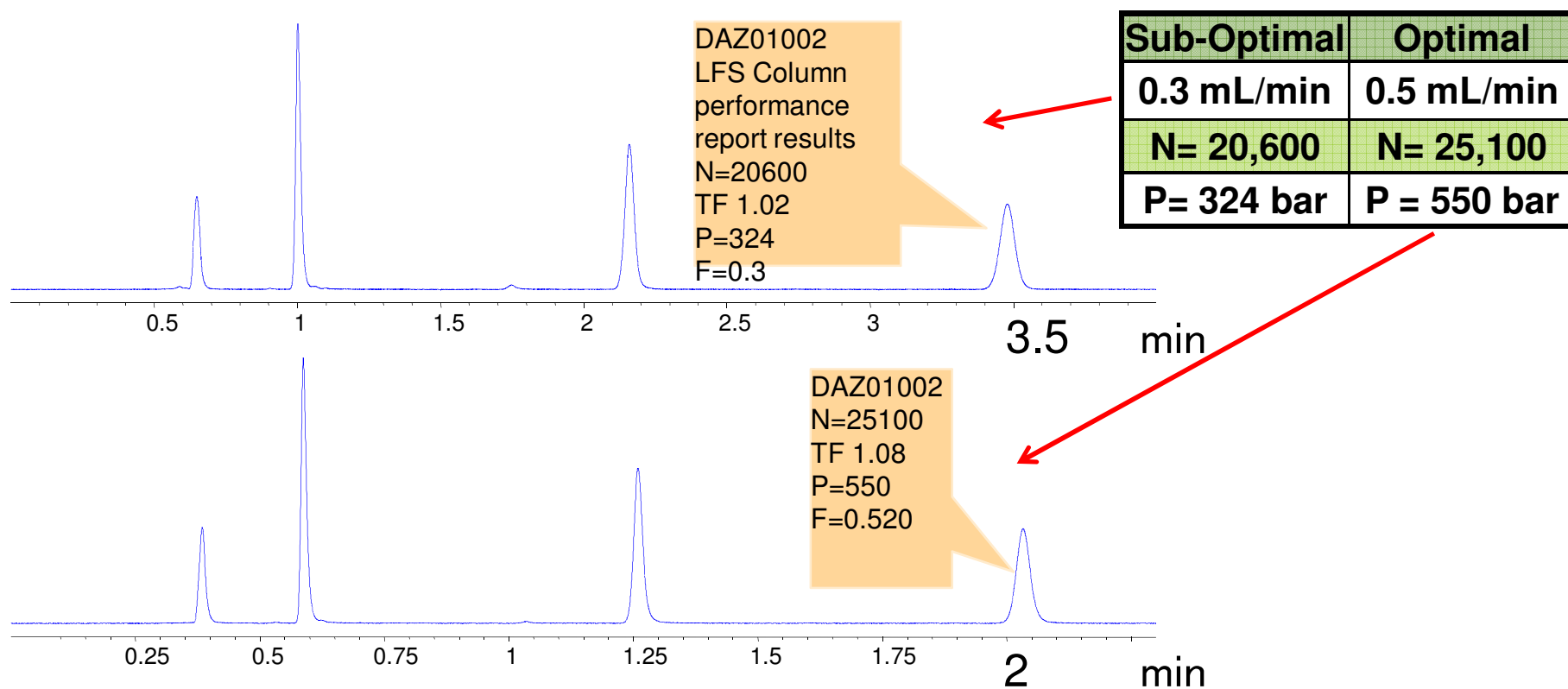
RRHD Eclipse Plus C18, 2.1 x 100 mm, 1.8 μ m;
PN 959758-902, SN DAZ01002, LN B009013
Naphthalene $k=4.0$



2.1 x 100 RRHD Optimum flow rate is about 0.5 mL/min. (60% MeCN, Themostatted 26°C, ~LC Performance Report Conditions)

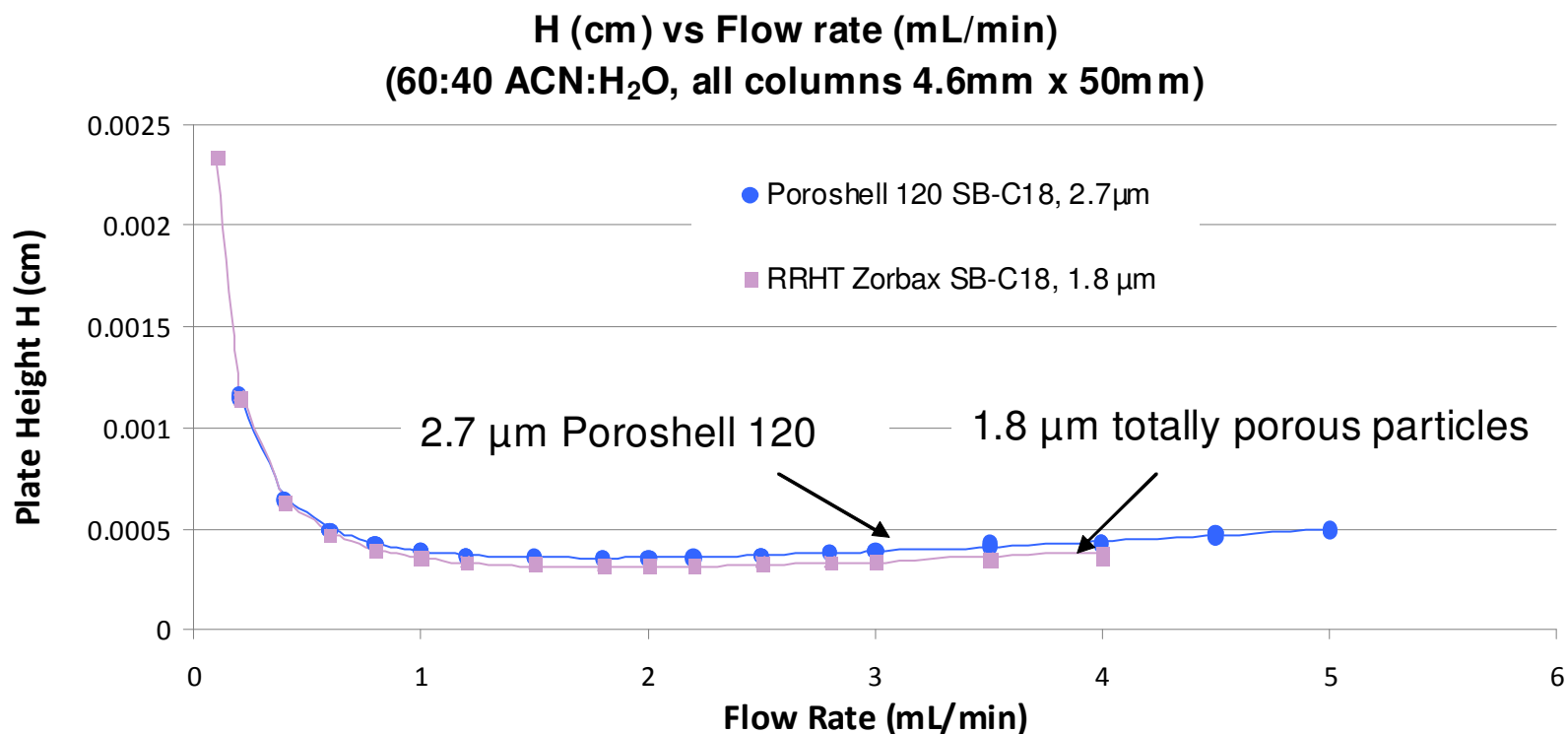
Why Is It Important to Know the Optimal Flow Rate?

Efficiency is Highest at Optimal Flow Rate



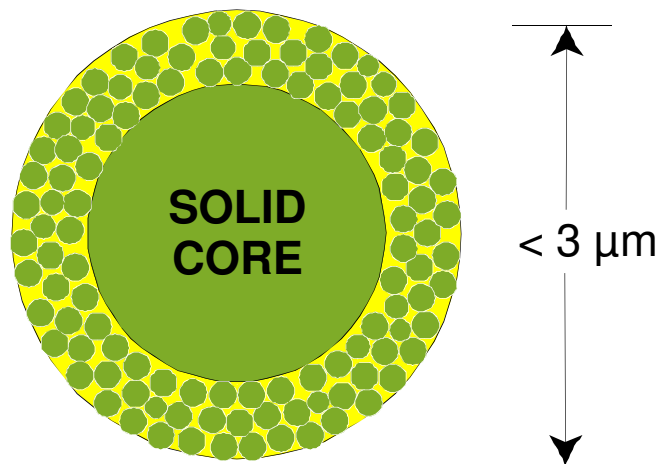
At sub optimal flow rates the efficiency is lower and all of the benefits of a 1.8 μ m columns or UHPLC technology may not be apparent.

Van Deemter for Poroshell 120 and RRHT

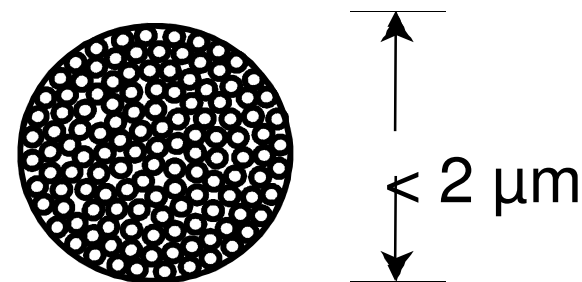


Superficially porous particles and 1.8μm particles have similar efficiencies and are both choices for UHPLC.

Poroshell 120 Particle technology



Poroshell 120
Superficially porous



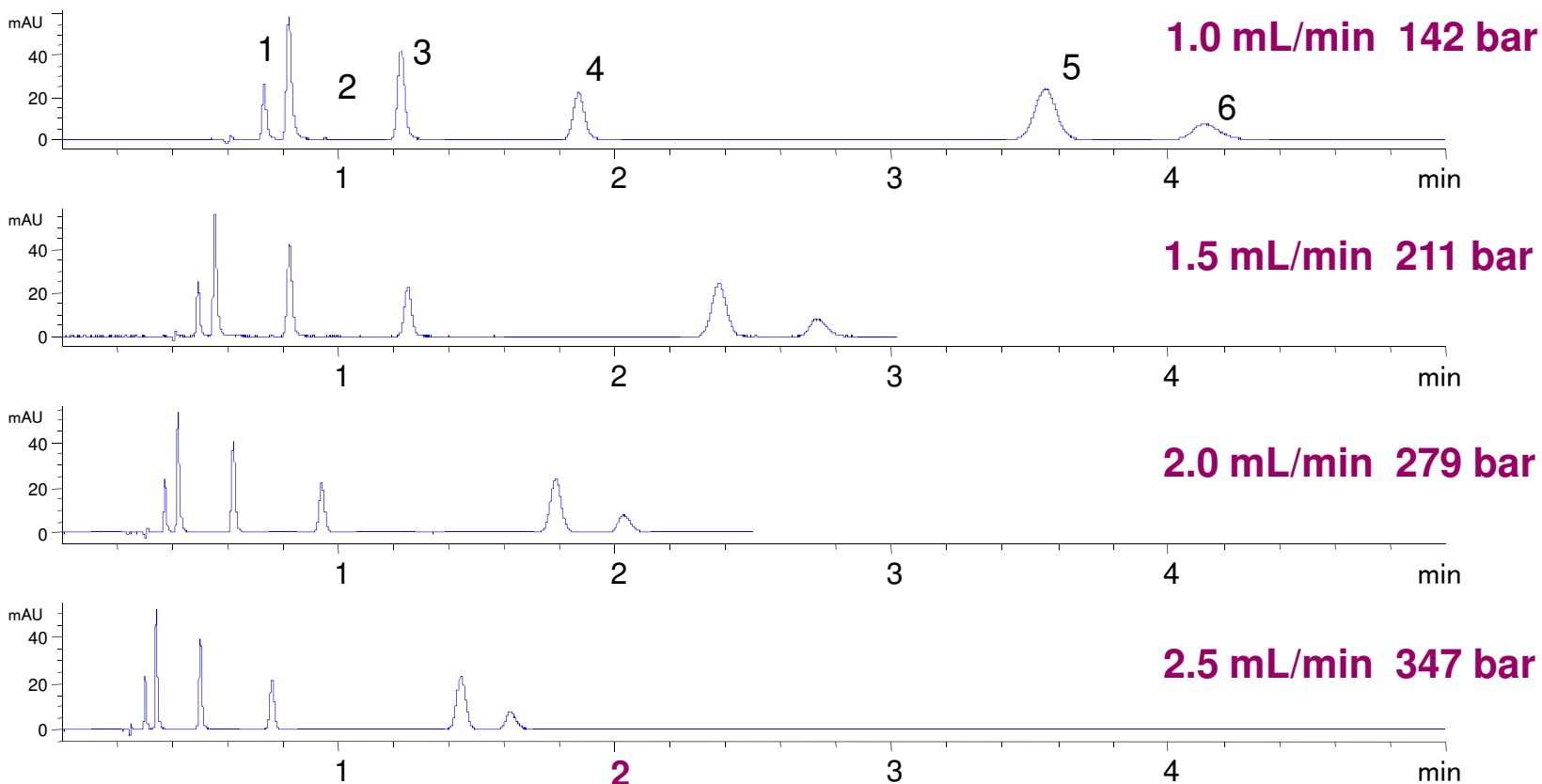
Totally porous
Fully porous
sub-two-
micron (STM)

T. Maloney, J. Cunliffe, J. Sep. Sci. 2007, 30, 3104 – 3109
G. Guiochon et al, Journal of Chromatography A, 1157 (2007) 289–303
G. Guiochon et al, Journal of Chromatography A, 1166 (2007) 30–46
Yu Zhang et al, Journal of Chromatography A, 1216 (2009) 4597–4605



Fast Analysis of Analgesics on Poroshell 120 – High Flow Rates, for Fast Analysis are Possible

1. Acetaminophen 2. Caffeine 3. 2-acetamidophenol 4. Acetanilide 5. Phenacetin 6. Salicylic acid



Conditions: Column: Poroshell 120 SB-C18, 4.6 x 50mm, 2.7 μ m Mobile Phase: 0.2% Formic Acid 80% water:20% ACN
Temperature: 25 °C, Detection: 275 nm, Sample Injection: 2 μ l



Conclusions

New Rapid Resolution High Definition (1.8um) columns are one solution for UHPLC systems and are unique in the marketplace because of their ability to be used over >1000 bar.

This makes them ideal for use with the 1290 Infinity LC.

Other UHPLC columns are also available including the RRHT, 600 bar columns with over 140 choices. The 4.6 mm ID columns represent a unique choice for easy to use UHPLC performance.

The upcoming release of the Poroshell 120 columns will provide a 3rd option for UHPLC type columns because a

