Waters

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Developments in Waters Column Chemistries : BEH Technology

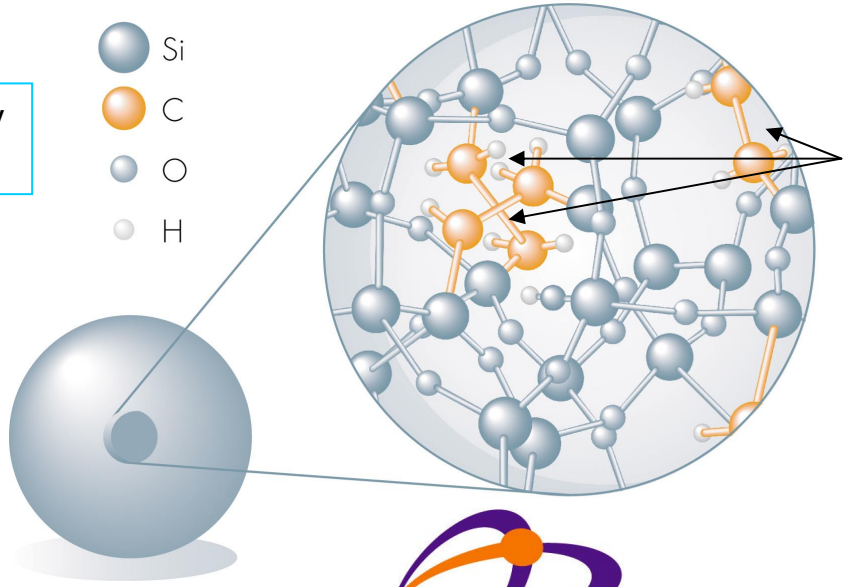
XTerra® Columns: First Generation Hybrid Particles

- **Initial Launch 1999**
- **Advantages of XTerra® Columns**:
  - Decreased peak tailing factors for basic analytes *vs*. silica columns.
  - Improved high-pH stability *vs*. silica columns
  - Comparable pore properties to conventional silica columns.
  - Similar bonded phases (C_{18}, C_8, Embedded Polar, Phenyl) as silica column, with comparable selectivities.
  - Similar particle sizes as Silica (2.5, 3.5, 5, 10 µm).
  - Improved chemical stability without major changes in separation conditions & without the drawbacks of many alternative packing materials.

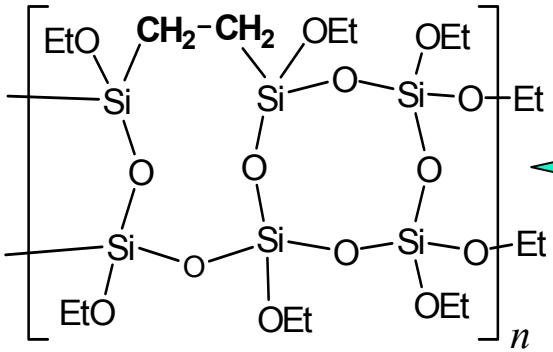
Neue, U. D.; *et. al.* *Am. Lab*., **1999**, *31*, 36
Cheng, Y.-F.; *et. al.* *LCGC*, **2000**, *18*, 1162

Introducing 2nd Generation Hybrid: Bridged Ethane-Silicon Hybrid Particles

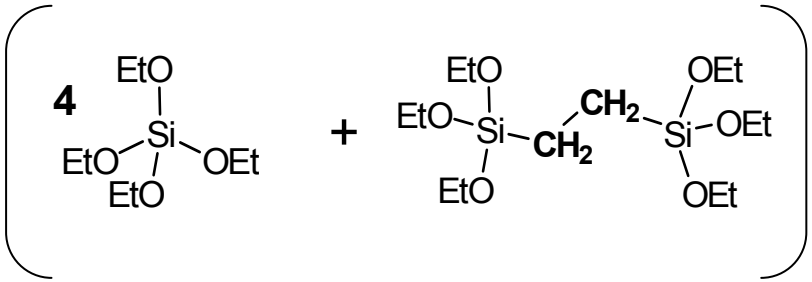
Waters Patented Technology
No. 6,686,035 B2



BEH Technology™



Polyethoxysilane
(BPEOS)



Tetraethoxysilane
(TEOS)

Bis(triethoxysilyl)ethane
(BTEE)

Anal. Chem. **2003**, *75*, 6781-6788

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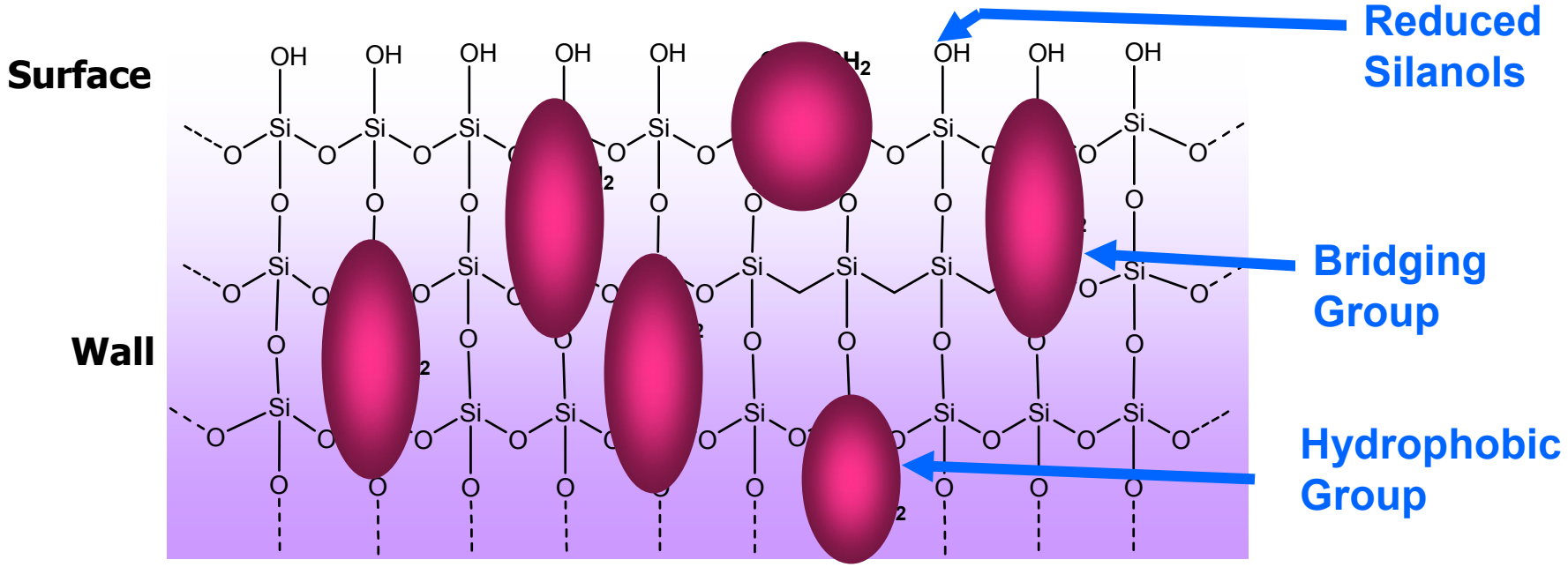
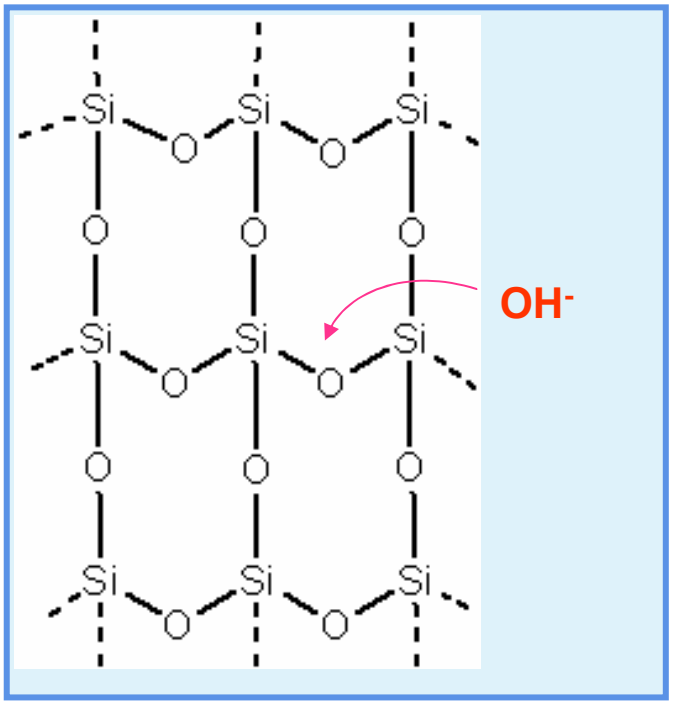
BEH Technology™ Particle Attributes



Hybrid Particle Attribute	RP-HPLC Consequence
Surface hybrid groups reduce surface silanol concentration	Reduced USP peak tailing factors for bases
Internal bridging groups provide high interconnectivity	Increased chemical & mechanical stability
Internal hybrid groups provide hydrophobicity	Increased high pH stability of column

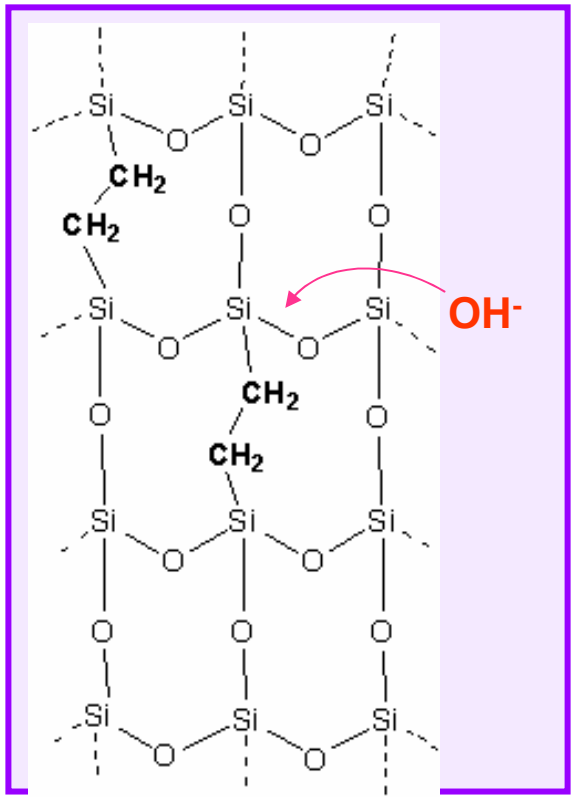
Particle Dissolution at High pH

Silica Particles



- Only 4 bonds need to be hydrolyzed
- $Si(OH)_4$ has high solubility in water
- **Easy to dissolve silica at pH >7**

BEH Technology™



- Up to 6 bonds need to be hydrolyzed
- Hydrophobic, lower reactivity than silica
- Si-O-Si bonds form as others break
- **Difficult to remove Bridge group!**

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XBridge™ HPLC Columns

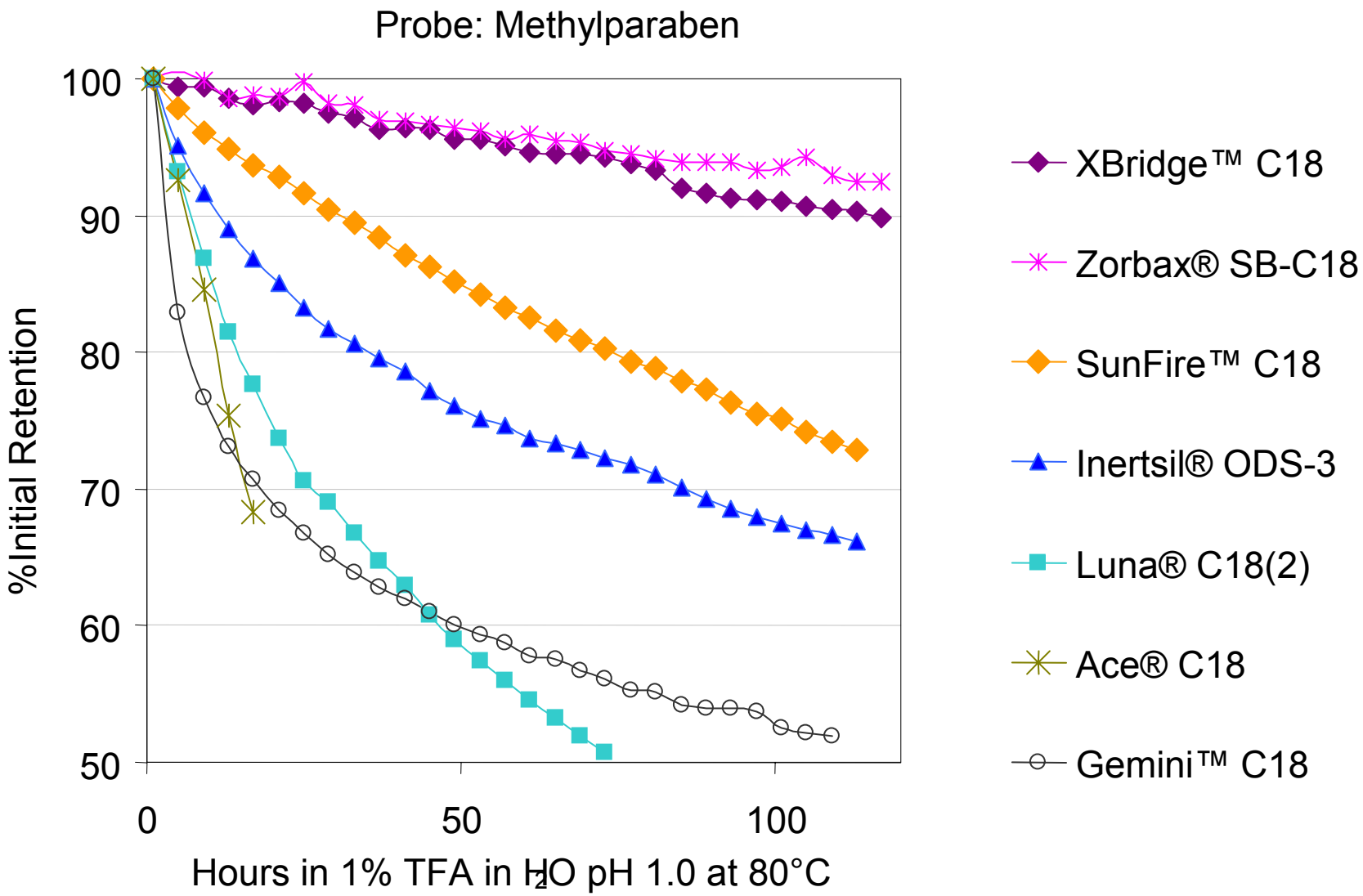
Particle Property Comparisons

	BEH Technology™	XTerra®
Particle Size	1.7 µm→ **ACQUITY UPLC™ BEH** 2.5, 3.5, 5 µm→ **XBridge™**	2.5, 3.5, 5, 10 µm
% Carbon, Unbonded	6.6 %C	7.0 %C
Surface Area	185 m^2/g	179 m^2/g
Pore Volume	0.7 cm^3/g	0.7 cm^3/g
Pore Diameter	135 Å	120 Å
Metal Content (Na, Fe, Al)	≤ 10 ppm	≤ 20 ppm
Phase Ratio	92 m^2/cm^3	91 m^2/cm^3

$$\text{Phase Ratio} = (1 - \varepsilon_i)\ \frac{A_S}{V_p + 1/\rho_S}$$

A_S = Surface Area
V_p = Pore Volume
Interstitial Column Porosity (ε_i) = 0.4
Skeletal Density (ρ_s) = 2.02 g/cm^3

Accelerated Low-pH Stability Test



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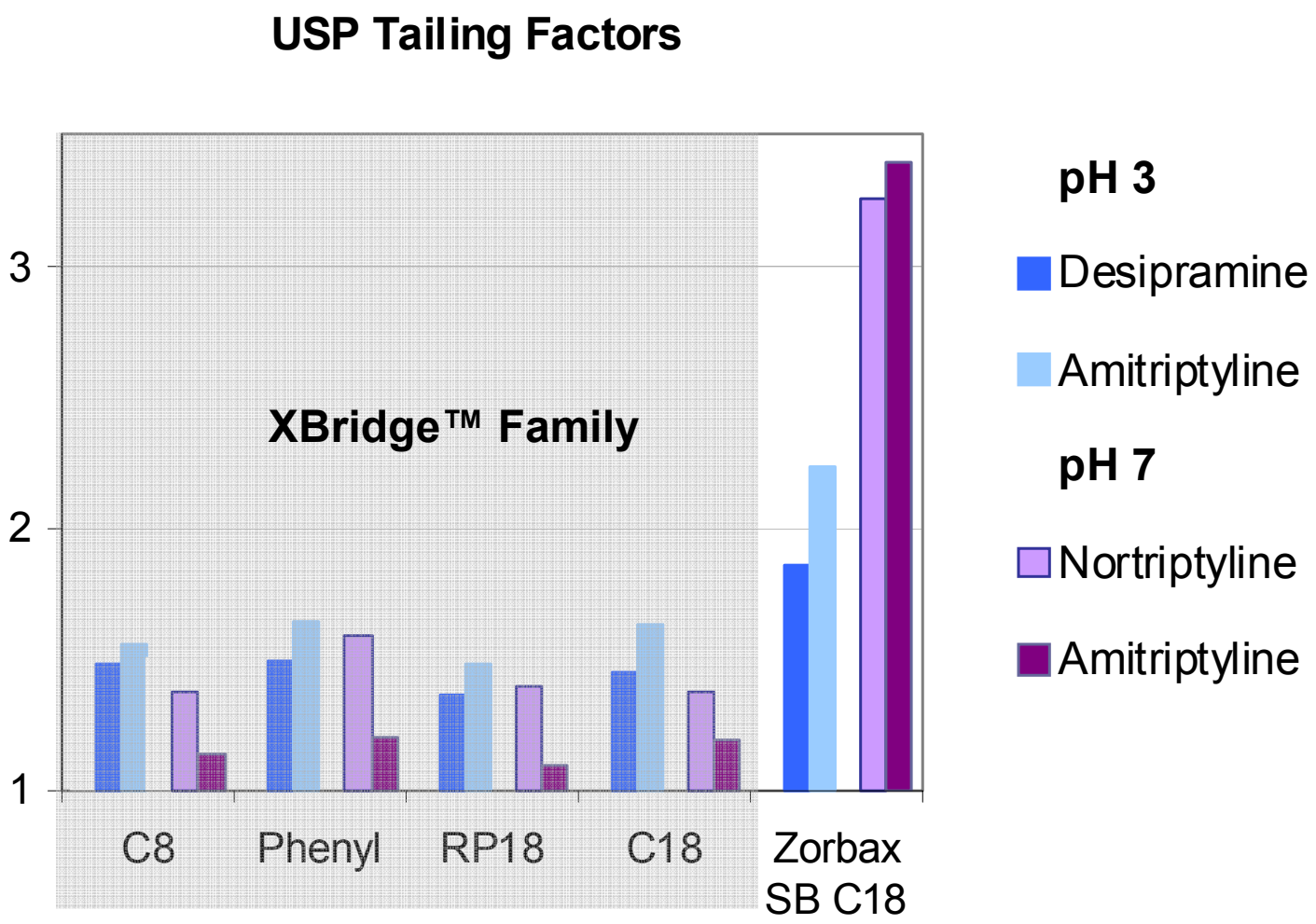
pH 3 and pH 7 Peak Shape Comparisons



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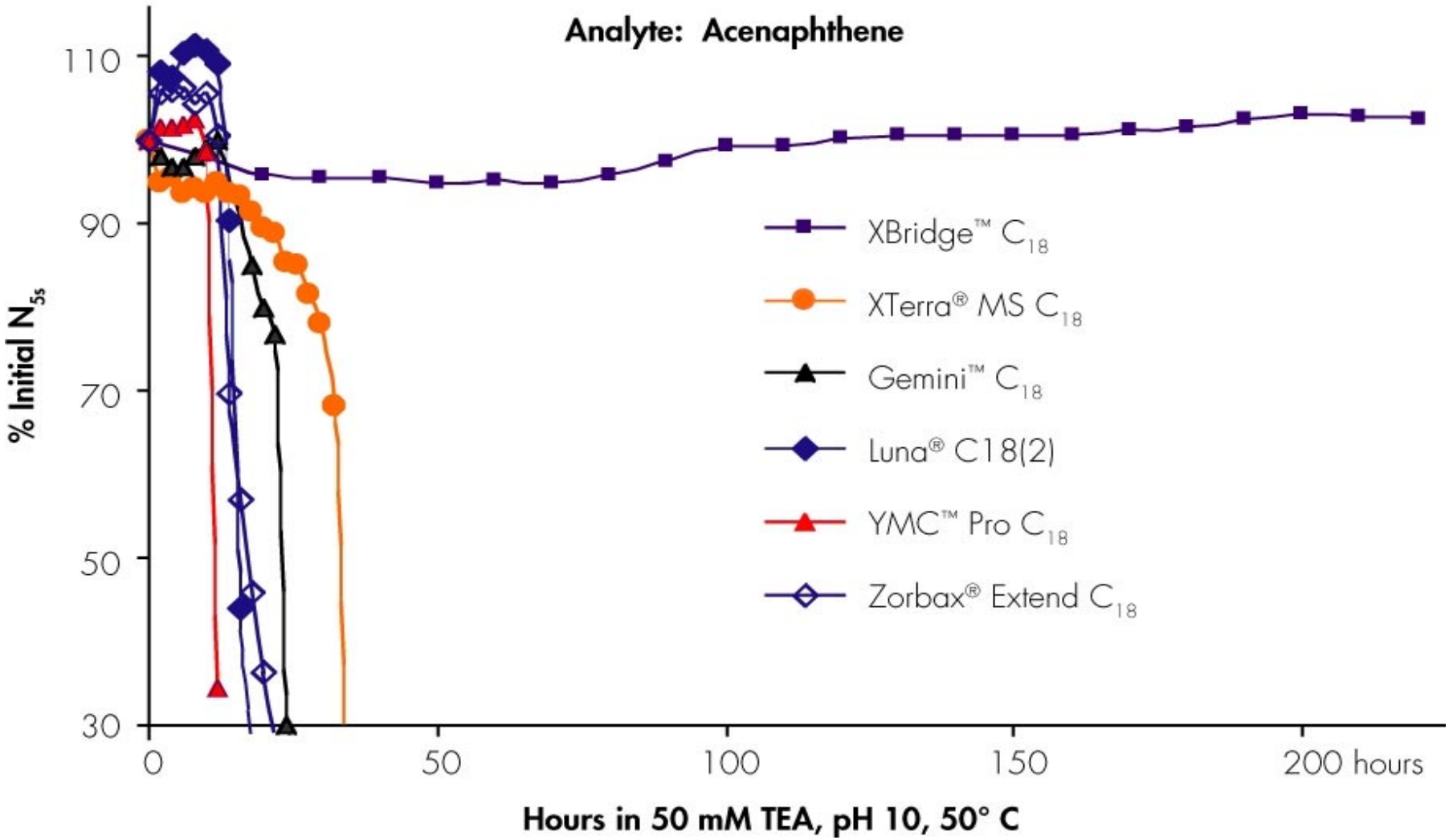
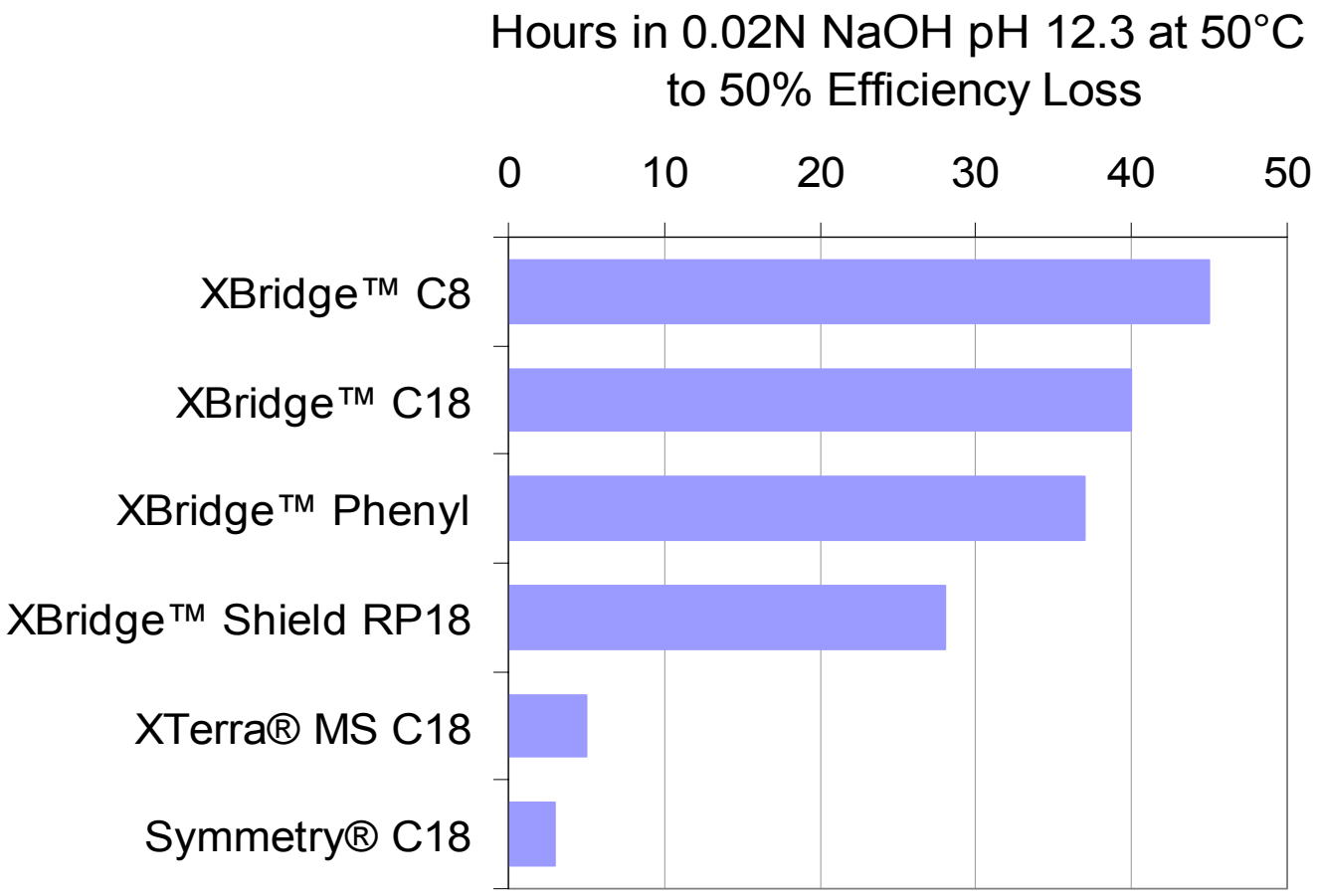
Accelerated High pH Stability Test

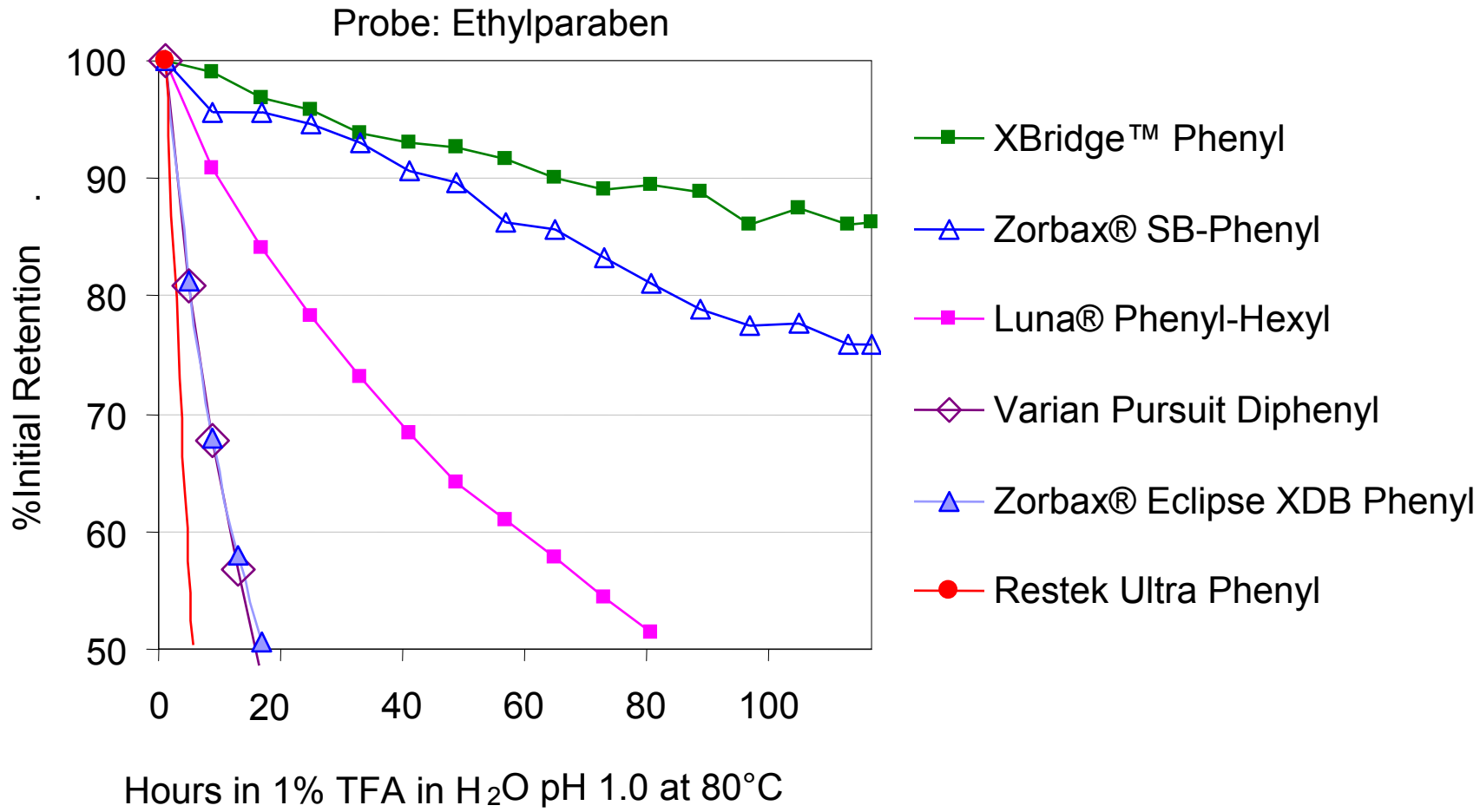


Taking pH Stability to the Extremes



Challenge mobile phase: 0.02N NaOH pH 12.3 at 50°C
Test mobile phase: 50/50 (v/v) acetonitrile/water at 50°C
Test probe: Decanophenone

Phenyl Column Ligand Stability at Low pH



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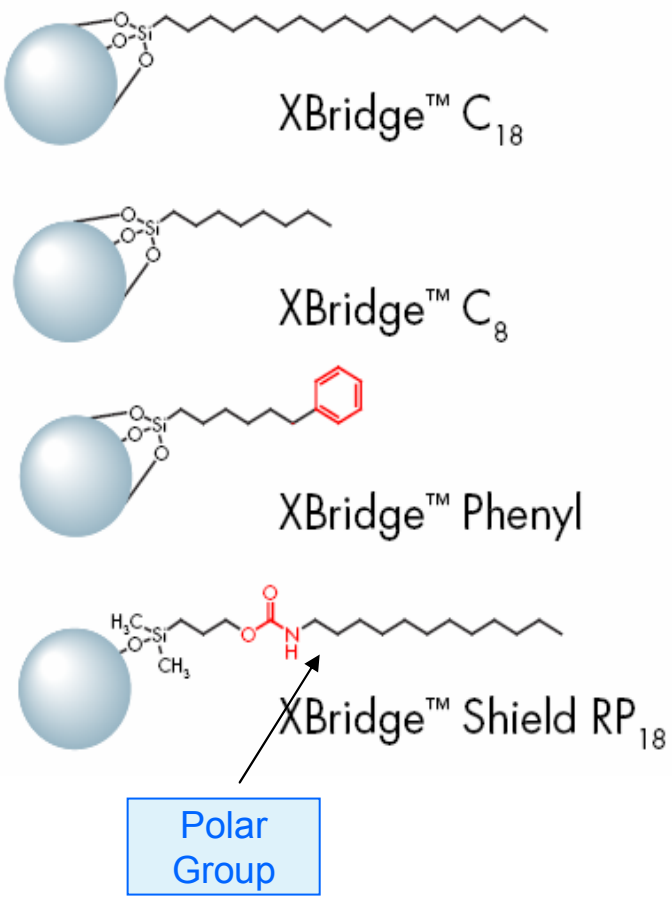
XBridge™ Columns

Bonded Phase



Step 1 %C	Step 1 Coverage	Endcap	Final %C
16.1 %C	3.2 µmol/m²	Proprietary	17.5 %C
11.1 %C	3.1 µmol/m²	Proprietary	12.8 %C
13.1 %C	3.2 µmol/m²	Proprietary	14.6 %C
16.6 %C	3.3 µmol/m²	TMS	16.7 %C

Cycle Time Optimization: Building on BEH Technology™

BEH Technology™

First Introduced at Pittcon 2004 as ACQUITY UPLC™ BEH Columns
Chemistry Offerings Expanded at Pittcon 2005

Acquity
Ultra Performance LC™

First Introduced at HPLC 2005

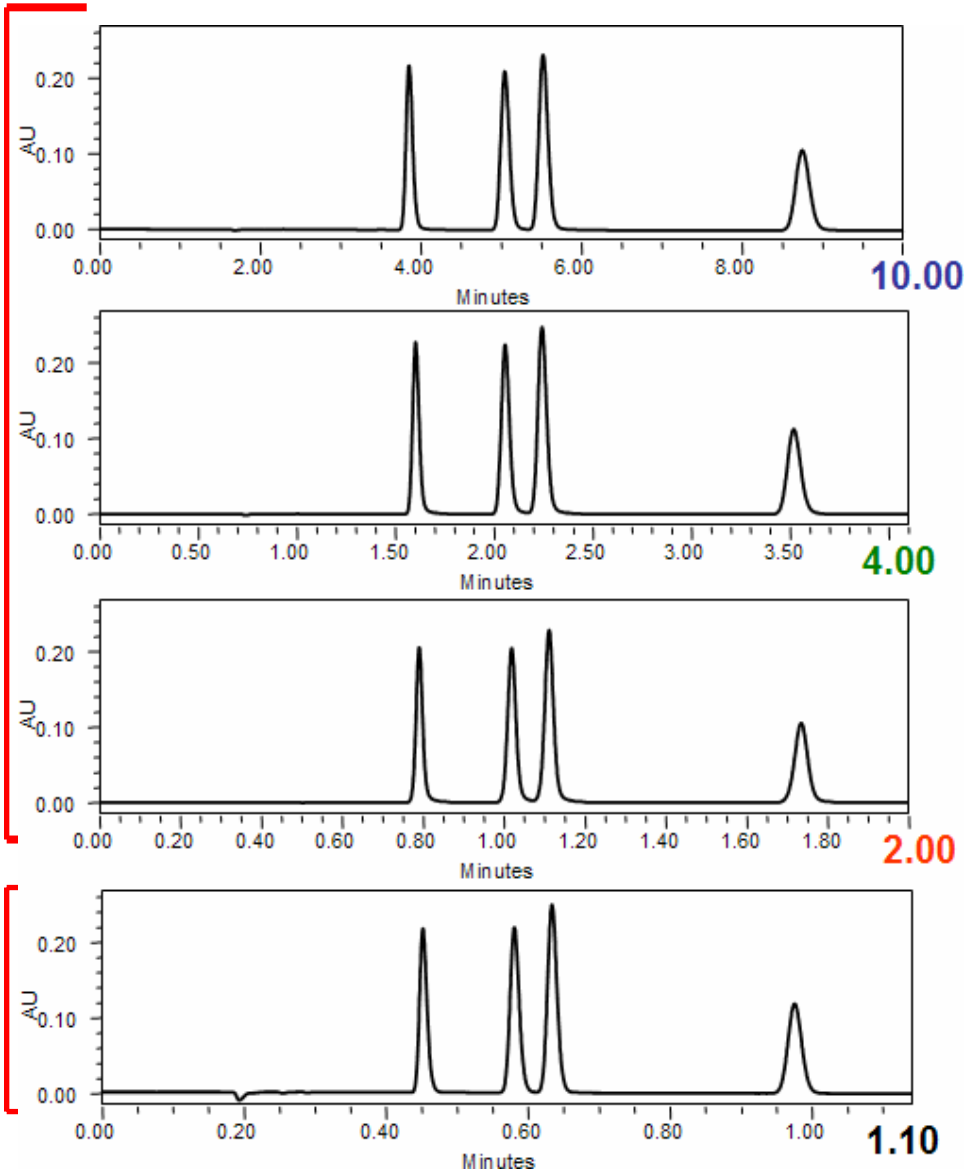
XBridge™ COLUMNS

Ease of Migration from HPLC to UPLC™

Simplified Purification and Isolation

Scaling from HPLC to UPLC™ - Constant L/dp

HPLC



UPLC™

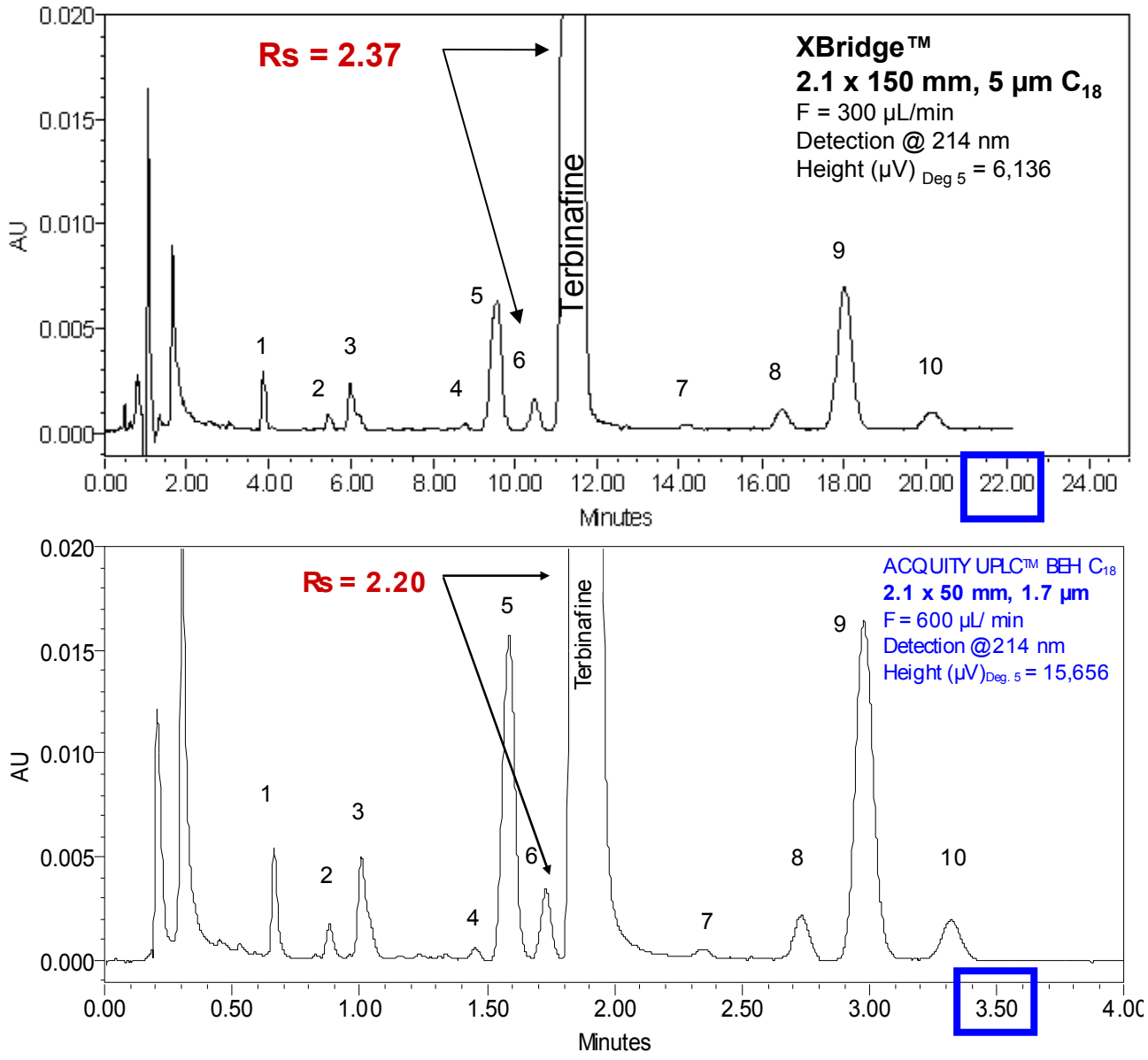
5 µm – 150 mm
Injection = 5.0 µL
Flow rate = 0.2 mL/min
$Rs_{(2,3)}$ = 2.28

3.5 µm – 100 mm
Injection = 3.3 µL
Flow rate = 0.3 mL/min
$Rs_{(2,3)}$ = 2.32

2.5 µm – 75 mm
Injection = 2.5 µL
Flow rate = 0.5 mL/min
$Rs_{(2,3)}$ = 2.34

1.7 µm – 50 mm
Injection = 1.7 µL
Flow rate = 0.6 mL/min
$Rs_{(2,3)}$ = 2.29

Scaling HPLC to UPLC™: Stability-Indicating Assay



Speed ↑ 6X

Sensitivity ↑ 2.5X (same inj. Volume)

Rs = 1X

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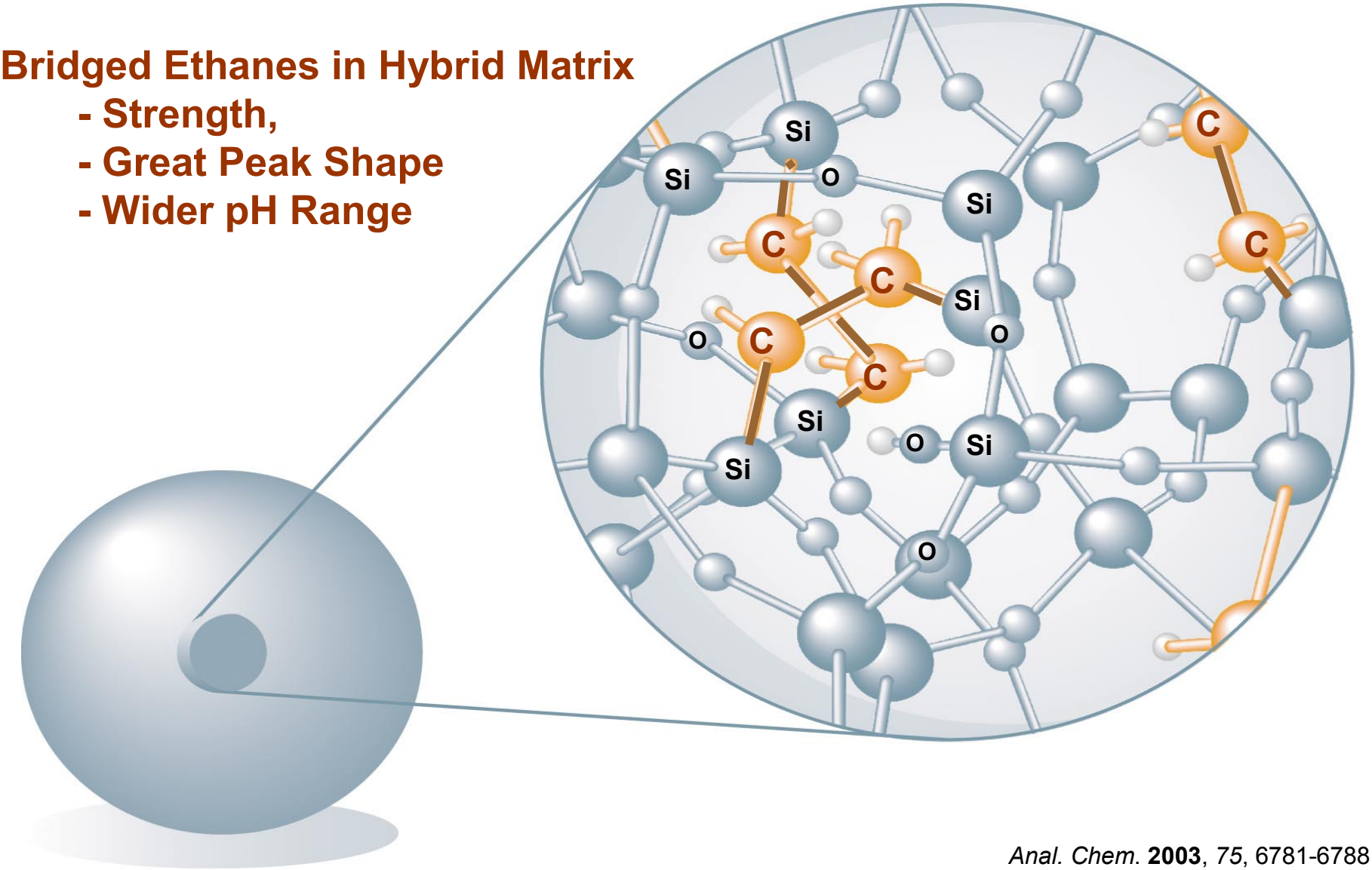
New 2nd Generation Hybrid Bridged Ethane-Silicon Hybrid Particles

Bridged Ethanes in Hybrid Matrix

- Strength,
- Great Peak Shape
- Wider pH Range



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Buffer 3 : Phosphate pH 12