

# **Larger Superficially Porous Particles: The “Long and Short of It” for HPLC Separations**

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## **Abstract**

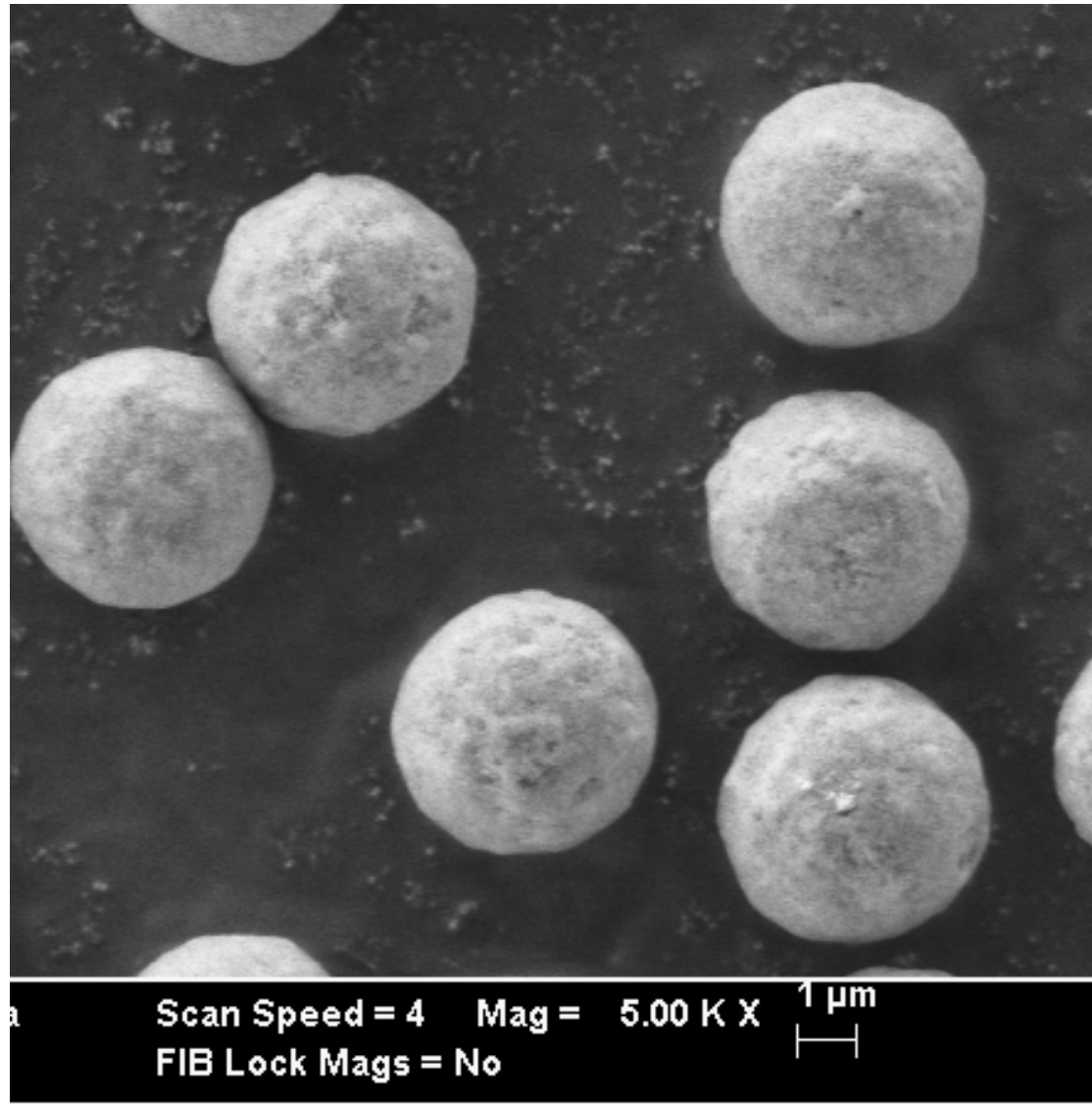
Since 2006, modern sub-3- $\mu\text{m}$  superficially porous particles have been shown to deliver high speed separations comparable to sub-2- $\mu\text{m}$  particles, in addition to high resolution separations for challenging samples. In 2012 larger particle size SPP particles for small molecule analyses were introduced. These 5- $\mu\text{m}$  particles can deliver performance comparable to or better than 3- $\mu\text{m}$  totally porous particles—at the backpressure of 5- $\mu\text{m}$  particles. Such features and benefits now bring the advantages of superficially porous particles to all applications of the HPLC instrument user.

Notably, these new 5- $\mu\text{m}$  SPP columns have initially fit a very useful niche for LC-MS users, in that they can be used for high throughput analyses with narrow-bore columns operated at high flow rates ( $> 1 \text{ mL/min}$ ) at normal HPLC pressures ( $< 400 \text{ bar}/6000 \text{ psi}$ ). Such high-speed analyses are not achievable with sub-2- $\mu\text{m}$  columns unless UHPLC/UPLC® instrumentation is used. Moreover, some users have found that peaks produced by the sub-2- $\mu\text{m}$  columns were, in fact, too narrow for full fidelity with their fastest LC-MS scan rates. The slightly broader peaks obtained with 5- $\mu\text{m}$  SPP columns did not have such a problem.

In addition to high throughput applications, 5- $\mu\text{m}$  SPP columns also bring high resolution separations to the realm of HPLC users. The much lower pressure of the larger particle size, coupled with high efficiency, permits longer columns or tandem columns to be applied for challenging HPLC separations.

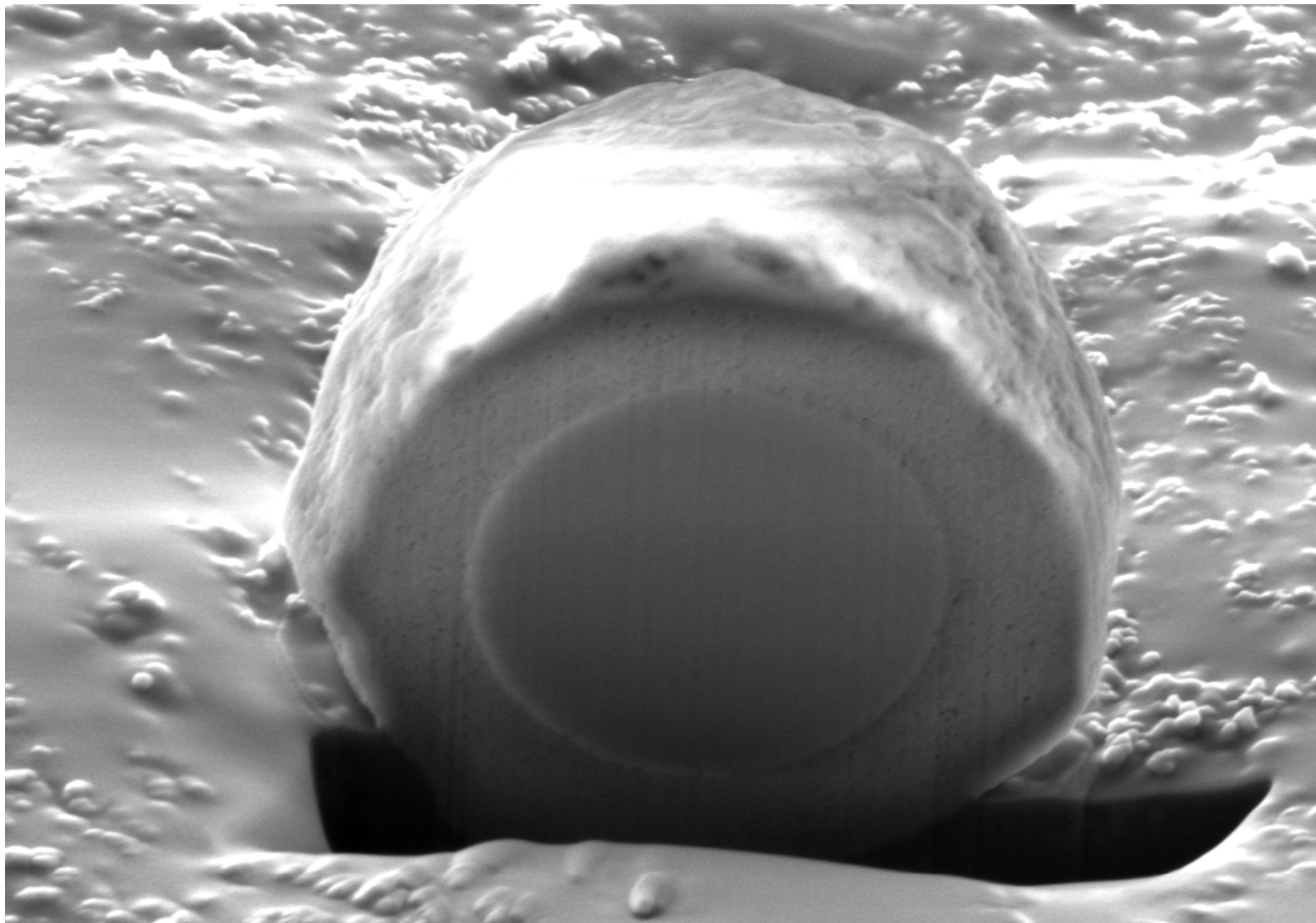
In this paper, we will demonstrate some of the advantages of these new particles and columns using several high throughput and high resolution examples versus comparable sub-2- $\mu\text{m}$  separations.

## SEM Photo of 5-micron Fused-Core Particles



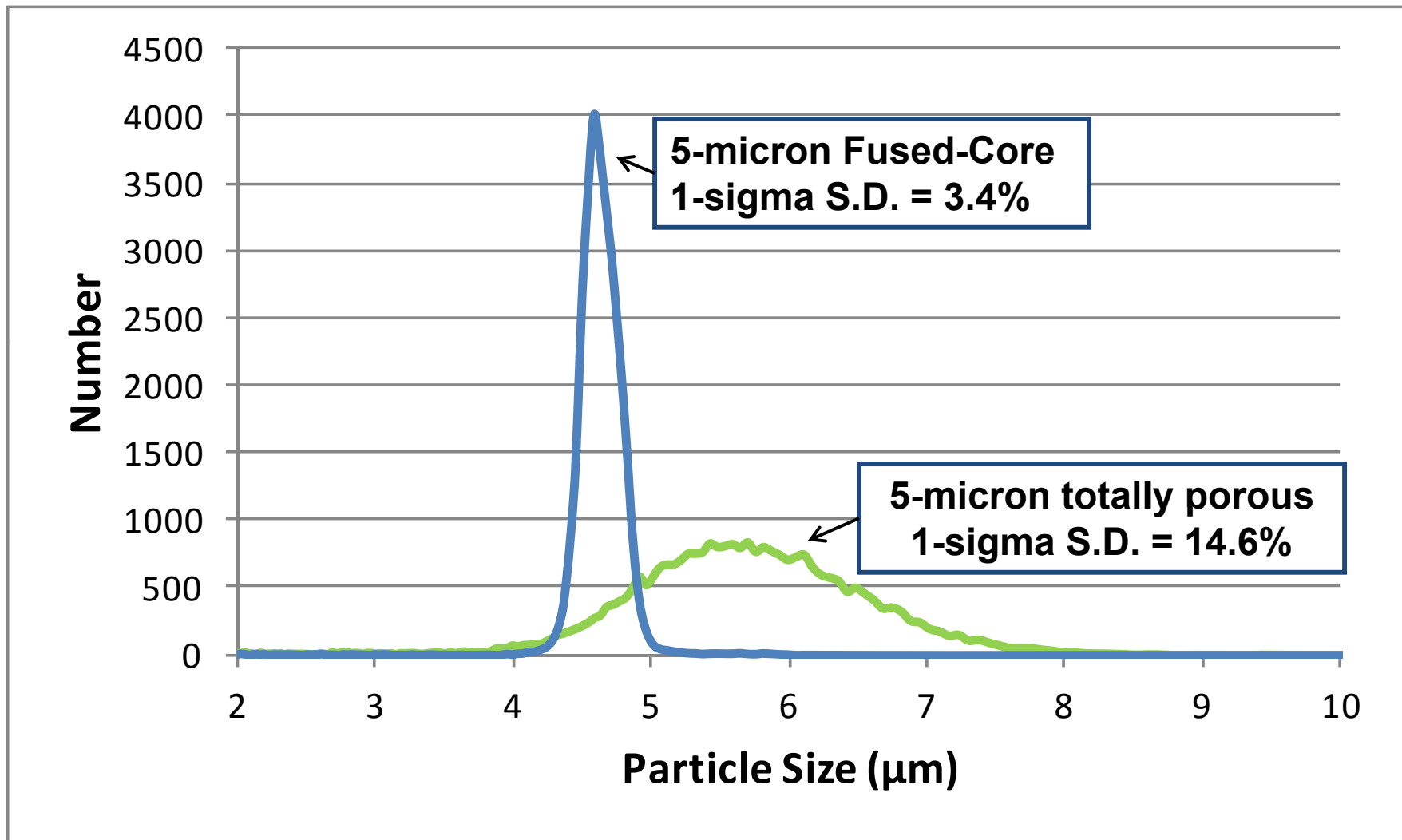
# SEM Photo of FIB Sliced 5-micron Fused-Core Particle

(FIB = Focused Ion Beam)



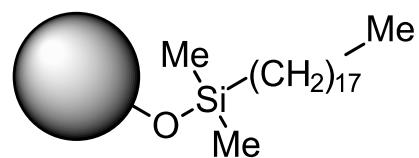
EHT = 3.00 kV WD = 4.9 mm Signal A = SEI System Vacuum = 1.11e-004 Pa Scan Speed = 4 Mag = 29.62 K X 200 nm  
FIB Probe = 30KV:120pA FIB Imaging = SEM ESB Grid = 809 V FIB Lock Mags = Yes

## Improved Size Distributions for 5-Micron Particles

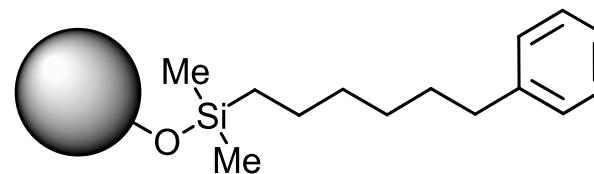


Measured on a Multisizer 3 Coulter Counter

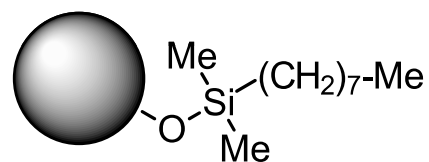
# Different Stationary Phases for Modifying Selectivity



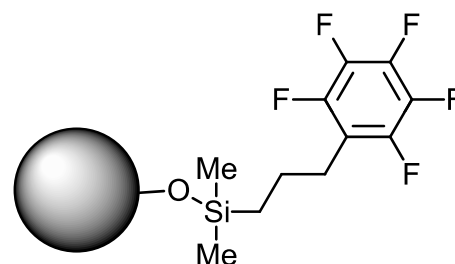
**C18 (octadecyl)**



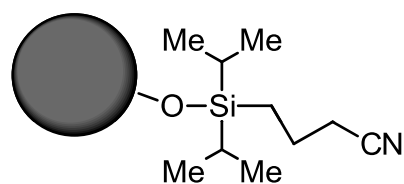
**Phenyl-Hexyl**



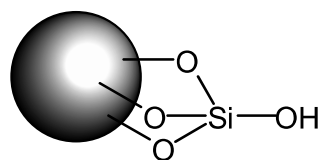
**C8 (octyl)**



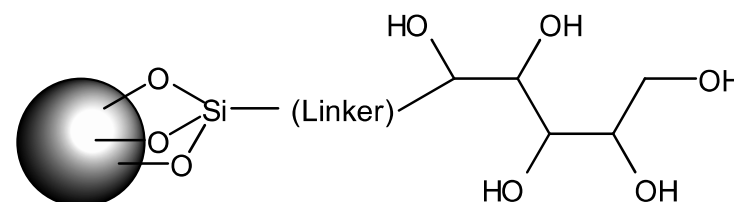
**PFP (pentafluorophenylpropyl)**



**ES-CN**



**HILIC**



**Penta-HILIC**

# 3 $\mu\text{m}$ Performance with 5 $\mu\text{m}$ Pressure

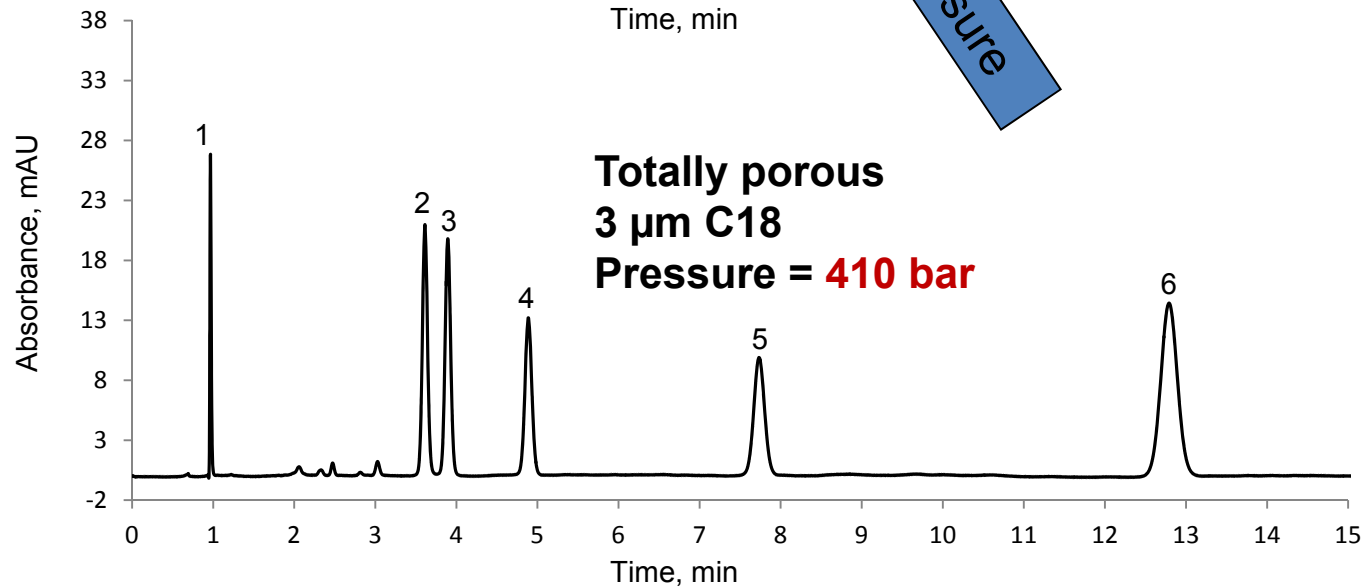
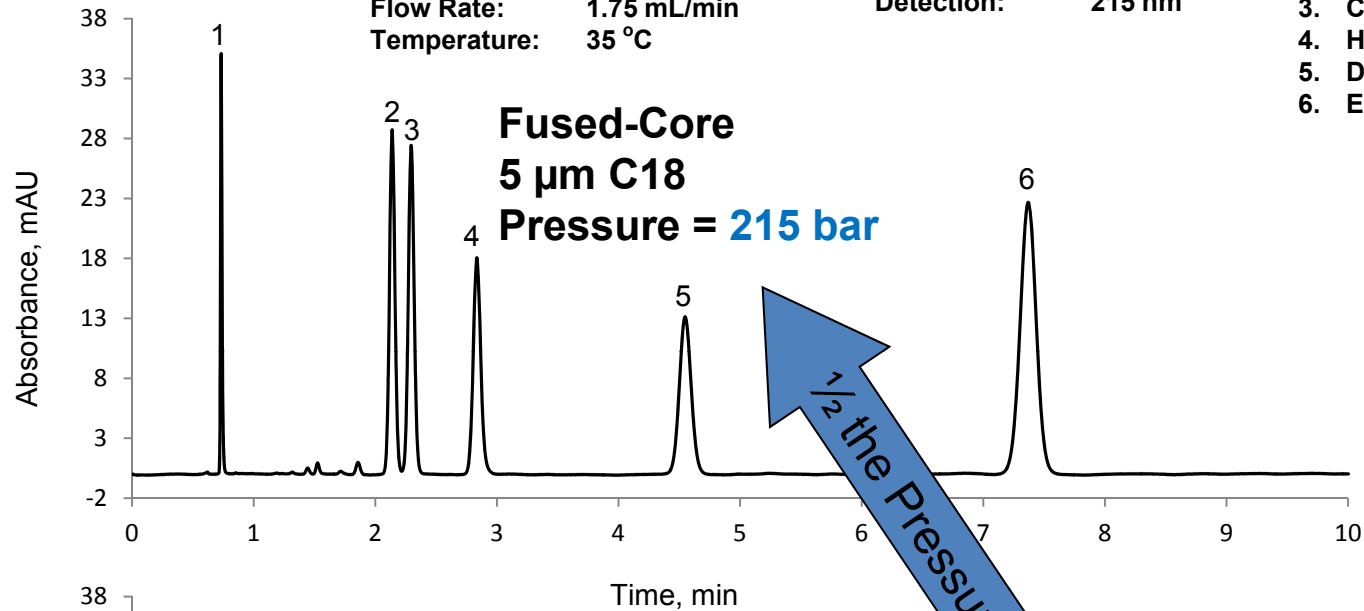
## Conditions:

Columns: 4.6 x 150 mm  
Mobile Phase: 50/50 water/methanol  
Flow Rate: 1.75 mL/min  
Temperature: 35 °C

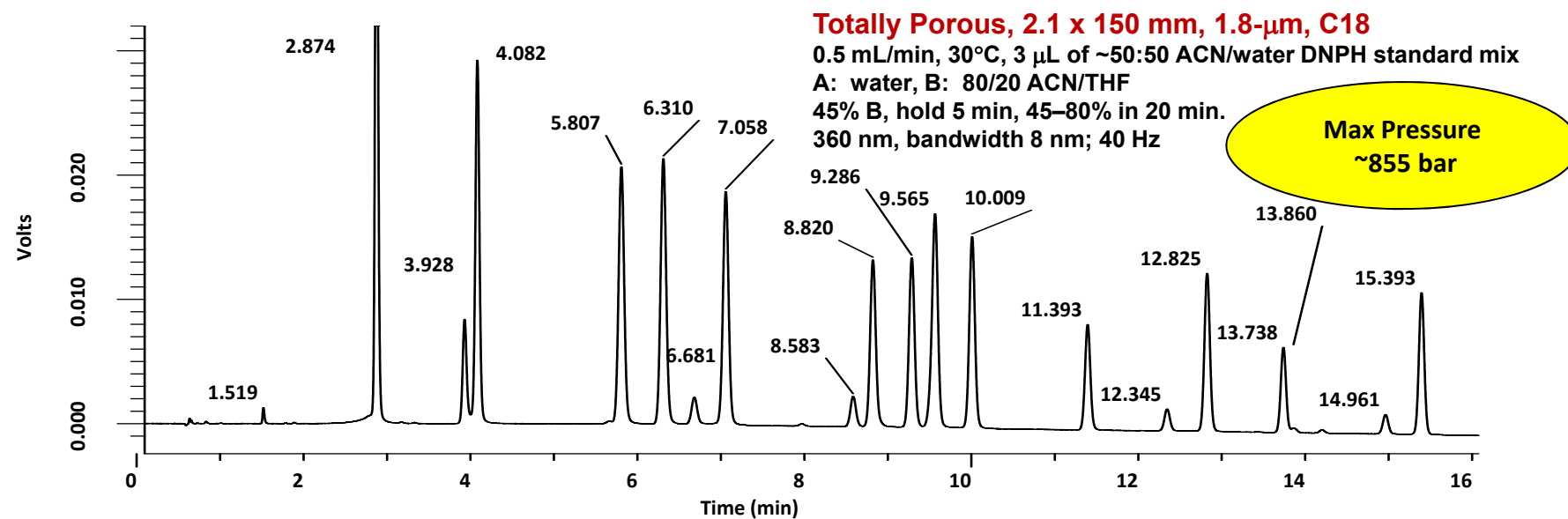
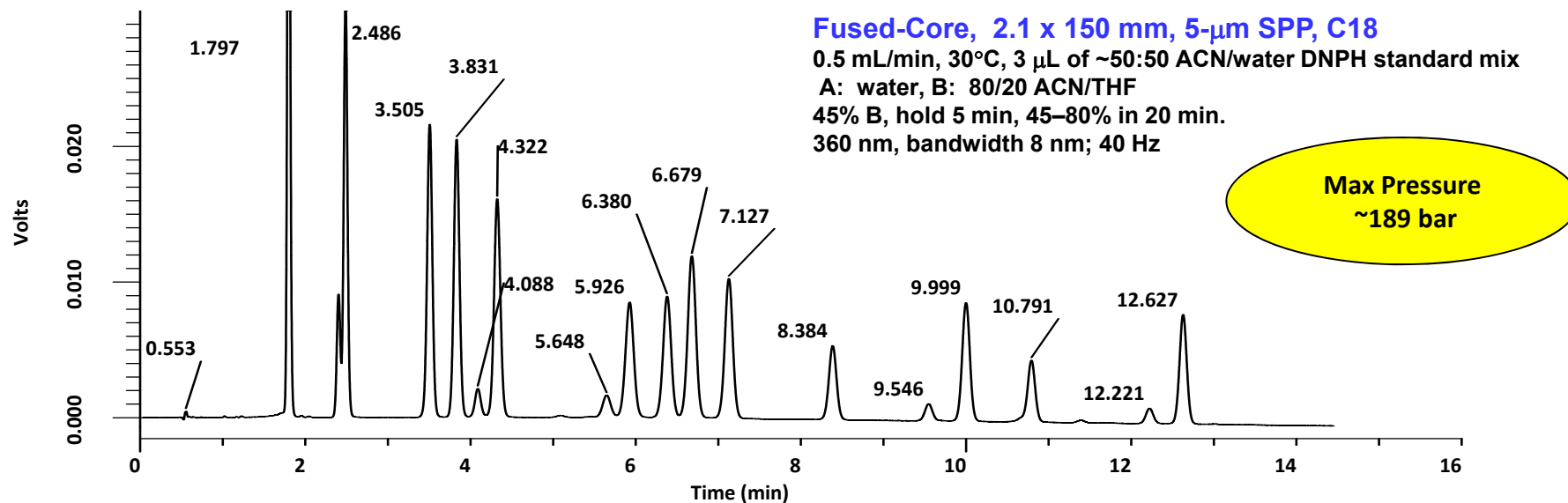
Injection: 5  $\mu\text{L}$   
Instrument: Agilent 1200  
Detection: 215 nm

## Peak Identities in order:

1. Uracil
2. Prednisone
3. Cortisone
4. Hydrocortisone
5. Dexamethasone
6. Estrone



# Use 5 $\mu\text{m}$ SPP Columns to Achieve High Resolution at Low Pressure



**PEAK IDENTITIES (in order):** Formaldehyde-2,4-DNPH, Acetaldehyde-2,4-DNPH, Acetone-2,4-DNPH, Acrolein-2,4-DNPH, Propionaldehyde-2,4-DNPH, Crotonaldehyde-2,4-DNPH, 2-Butanone-2,4-DNPH, Methacrolein-2,4-DNPH, Butyraldehyde-2,4-DNPH, Benzaldehyde-2,4-DNPH, Valeraldehyde-2,4-DNPH, m-Tolualdehyde-2,4-DNPH, and Hexaldehyde-2,4-DNPH  
 Note: Small peaks preceding labeled aliphatic aldehyde peaks are minor geometric isomers (syn/anti).

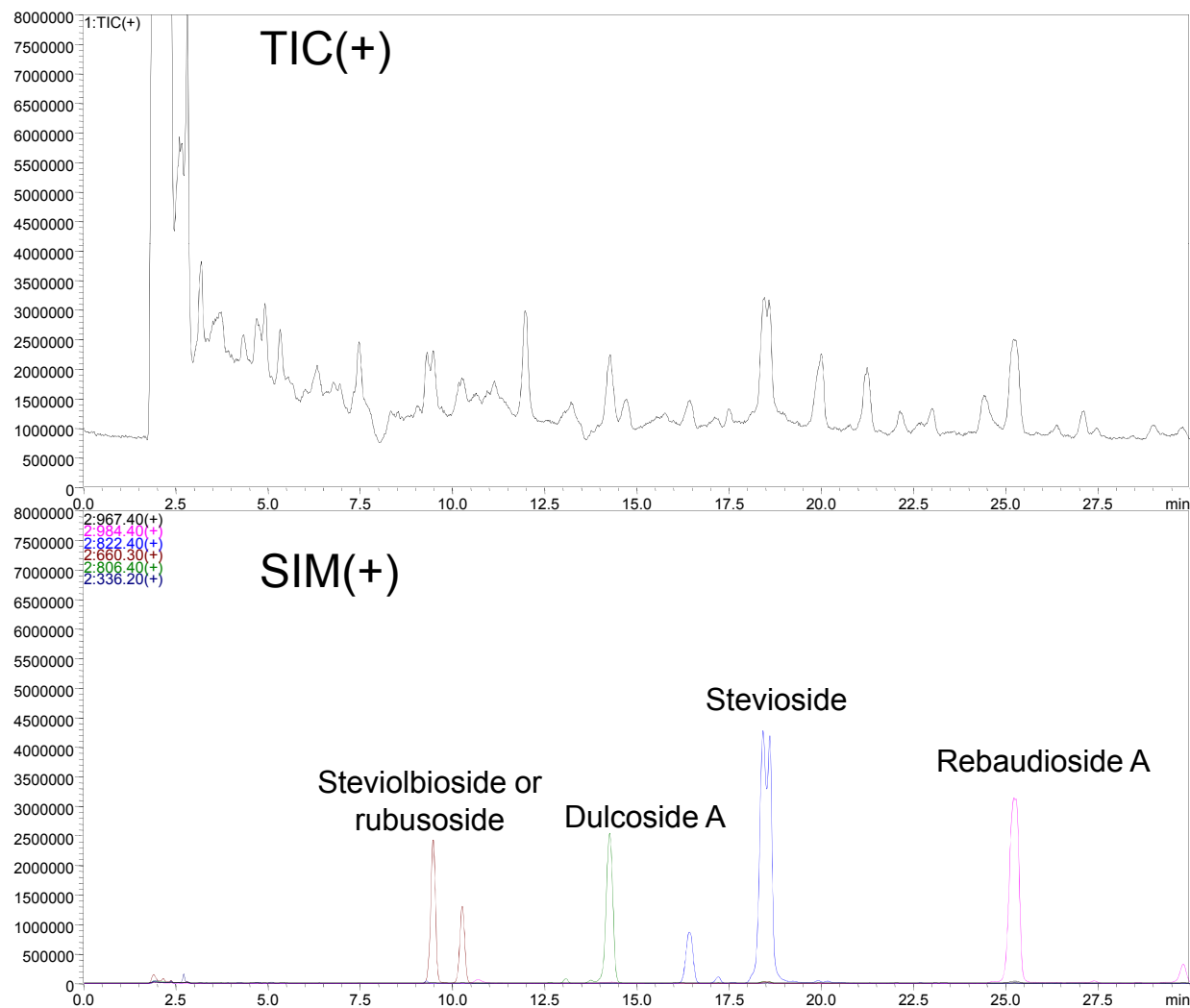


# High Resolution Separation of Stevia Extract using 5 $\mu$ m Fused-core Particles: Positive Ion Mode

Fused-Core, 3.0 x 250 mm, HALO-5 Penta-HILIC; 0.5 mL/min, ambient, 5  $\mu$ L of stevia extract; A: 50/50 water/ACN with 5 mM Ammonium Formate, pH 3; B: 5/95 water/ACN with 5 mM Ammonium Formate, pH 3; Gradient: 91-80% ACN in 30 min; ESI + 4.5 kV; 200-1200 m/z; Capillary: 250  $^{\circ}$ C; Nebulizing gas flow: 1.5 L/min; Heat block: 350  $^{\circ}$ C; Drying gas: 15 L/min

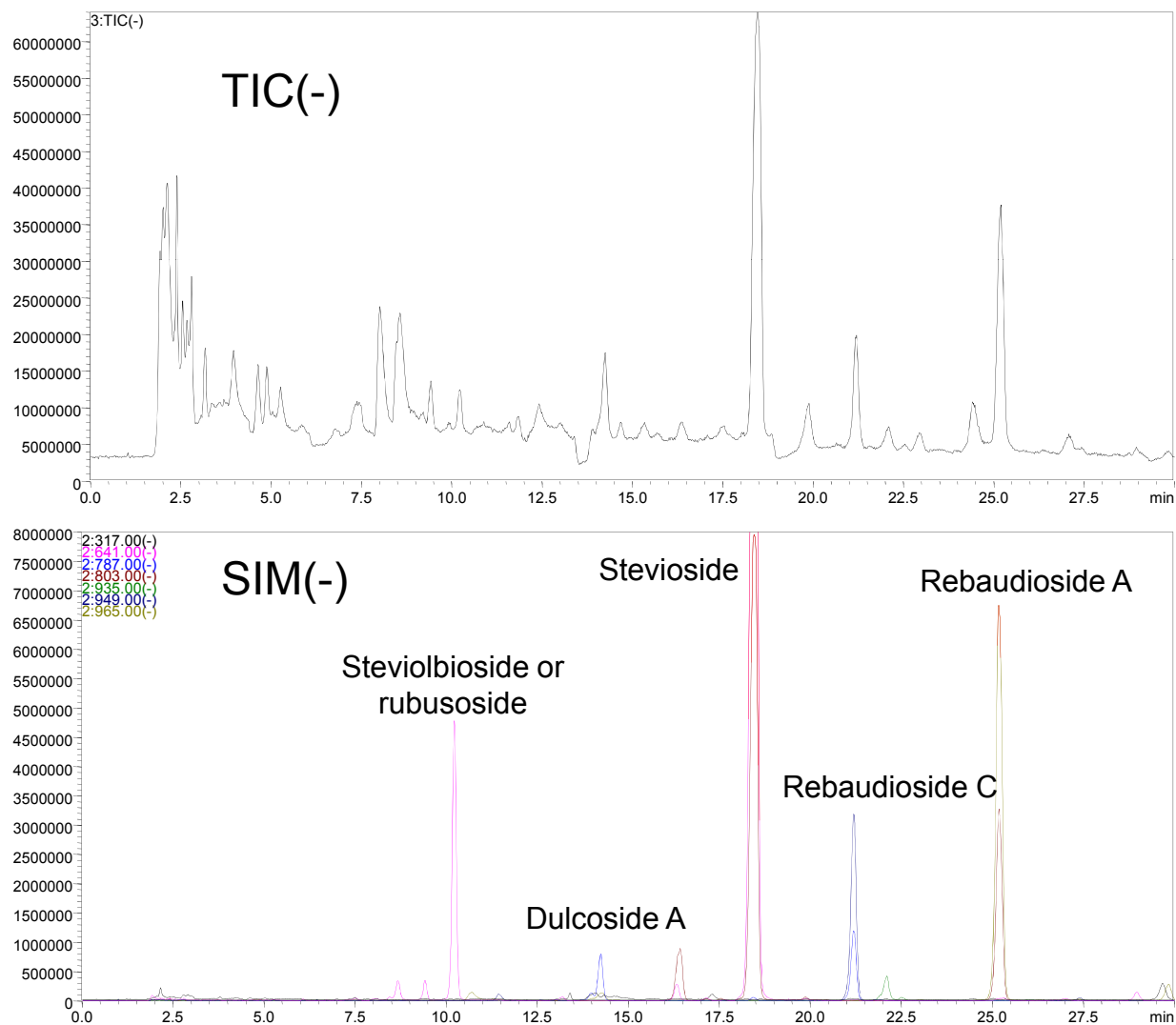
## Extraction Procedure:

1. Weigh 400 mg of Stevia Leaves (Sigma S5381)
2. Crush with mortar and pestle
3. Add 8 mL of 50/50 ACN/H<sub>2</sub>O
4. Sonicate for 15 min
5. Filtered sample using 25 mm syringe filter with 0.2  $\mu$ m PTFE membrane (VWR 28145-495)
6. Centrifuge at 10k rpm and collect supernatant
7. Dilute 400  $\mu$ L sample in 600  $\mu$ L ACN for an overall sample solvent concentration of 80/20 ACN/H<sub>2</sub>O.
8. Centrifuge diluted sample at 10 k rpm and inject the supernatant.

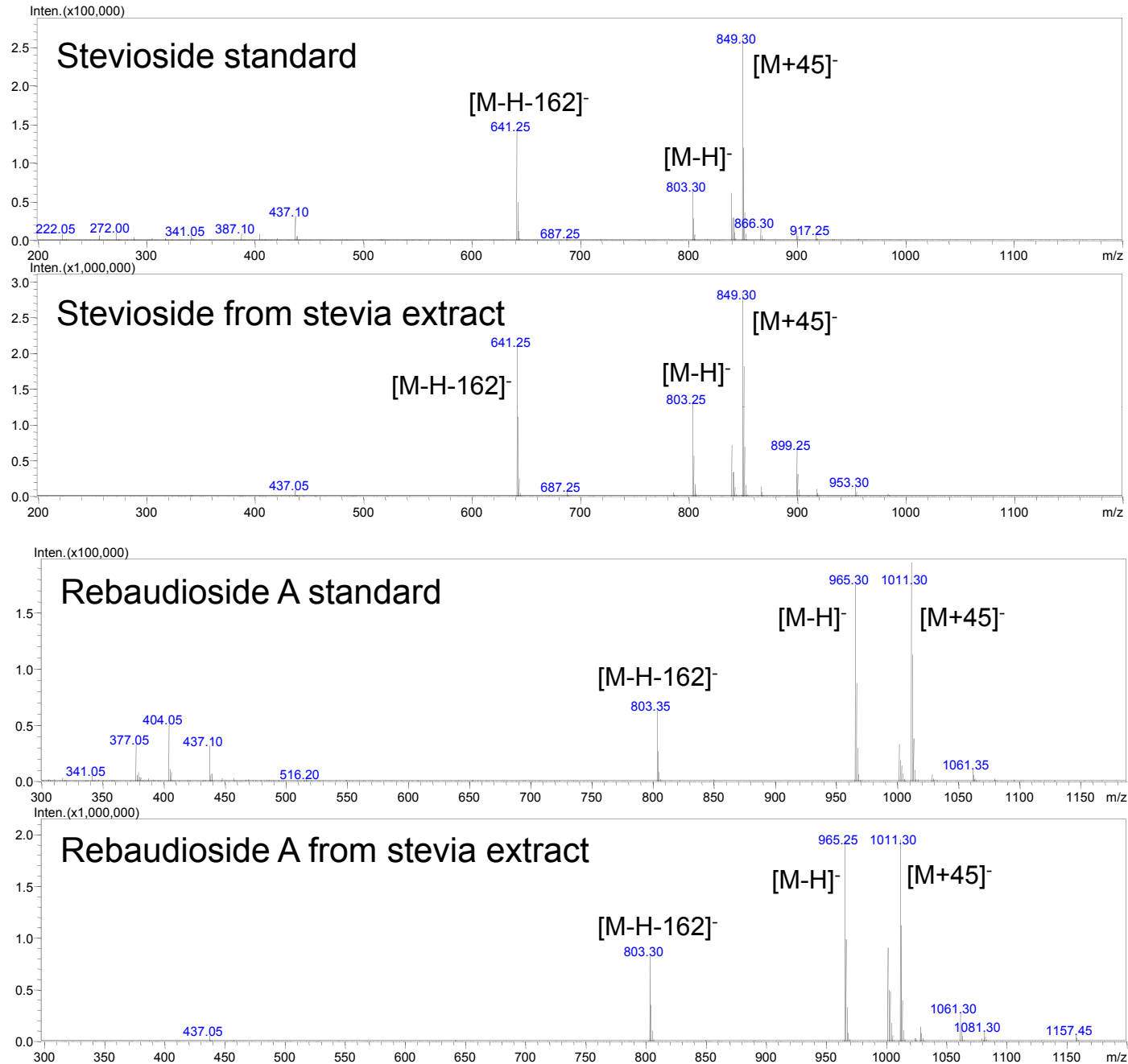


# High Resolution Separation of Stevia Extract using 5µm Fused-core Particles: Negative Ion Mode

Fused-Core, 3.0 x 250 mm, HALO-5 Penta-HILIC; 0.5 mL/min, ambient, 5 µL of stevia extract; A: 50/50 water/ACN with 5 mM Ammonium Formate, pH 3; B: 5/95 water/ACN with 5 mM Ammonium Formate, pH 3; Gradient: 91-80% ACN in 30 min; ESI - 4.5 kV; 200-1200 m/z; Capillary: 250 °C; Nebulizing gas flow: 1.5 L/min; Heat block: 350 °C; Drying gas: 15 L/min



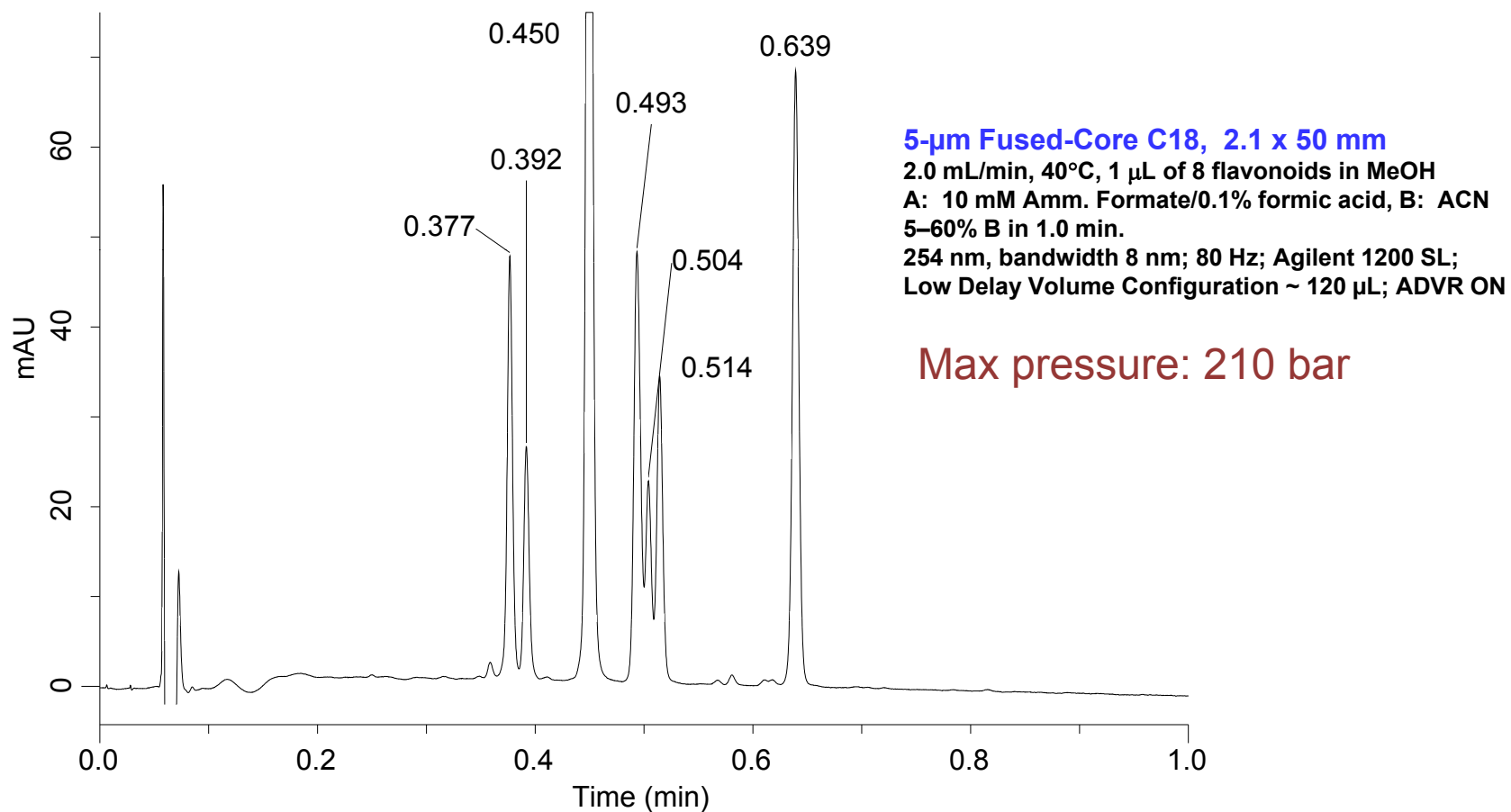
# Confirmation of Peak Identities Using Standards



## Go Ballistic with 5- $\mu$ m HPLC Columns

- Ballistic gradients
  - Utilize narrow-bore columns (1.0, 2.1 mm ID)
  - High mobile phase linear velocities (fast flow rates for column diameter)
  - Fast gradient times (1 – 5 minutes) over a wide range of organic modifier (e.g., 5 – 95% or 0 – 100%)
- Useful when high throughput and ruggedness is required
  - Analyzing biological sample extracts (urine, plasma, liposomes, etc.)
  - Assessing identity and purity of compound libraries
  - Screening new product candidates
  - Following reactions
  - Monitoring dissolution experiments

## Example Ballistic Gradient Run on 5- $\mu$ m Fused-Core Column



Analytes, in elution order: hesperidin, myricetin, quercetin, naringenin & apigenin (coeluted), hesperetin, kaempferol, biochanin

# 20 Second Gradient Using 5- $\mu$ m Fused-Core Column

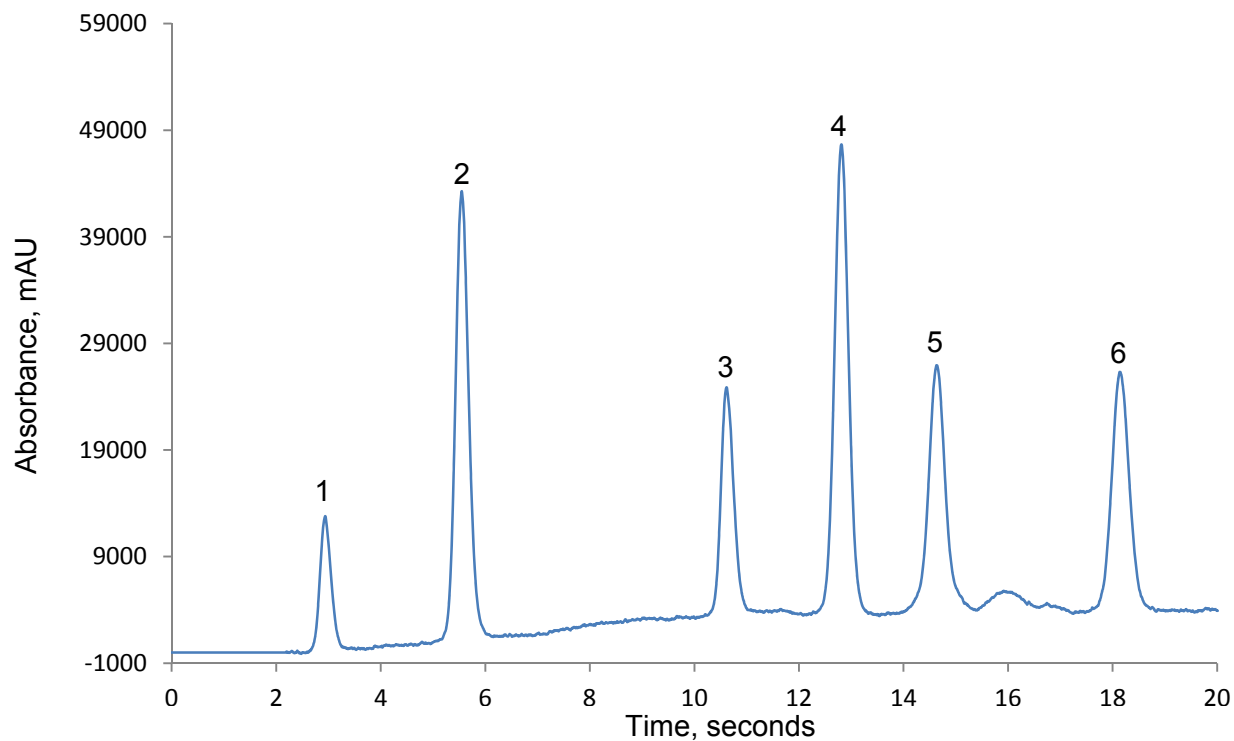
## TEST CONDITIONS:

Column: 2.1 x 20 mm, HALO-5 C18  
A = Water/0.1% TFA  
B = Acetonitrile/0.1% TFA  
Gradient: 5-50% B in 20 seconds  
Flow Rate: 6 mL/min  
Pressure: 549 bar

Temperature: 40°C  
Detection: UV 254 nm, PDA  
Injection Volume: 0.5  $\mu$ L  
Flow Cell: 1  $\mu$ L micro  
LC System: Shimadzu Nexera

## Peak Identities:

1. Atenolol
2. Pindolol
3. Propranolol
4. Indoprofen
5. Naproxen
6. Coumatetralyl



# Ballistic Gradient and Fast Re-equilibration with 5- $\mu$ m Fused-Core Column

## TEST CONDITIONS:

Column: 2.1 x 20 mm, HALO-5 C18

Mobile Phase: 11/89: A/B

A = Water/0.1% TFA

B = Acetonitrile/0.1% TFA

Gradient: 5-50% B in 60 seconds

Flow Rate: 2 mL/min

Pressure: 172 bar

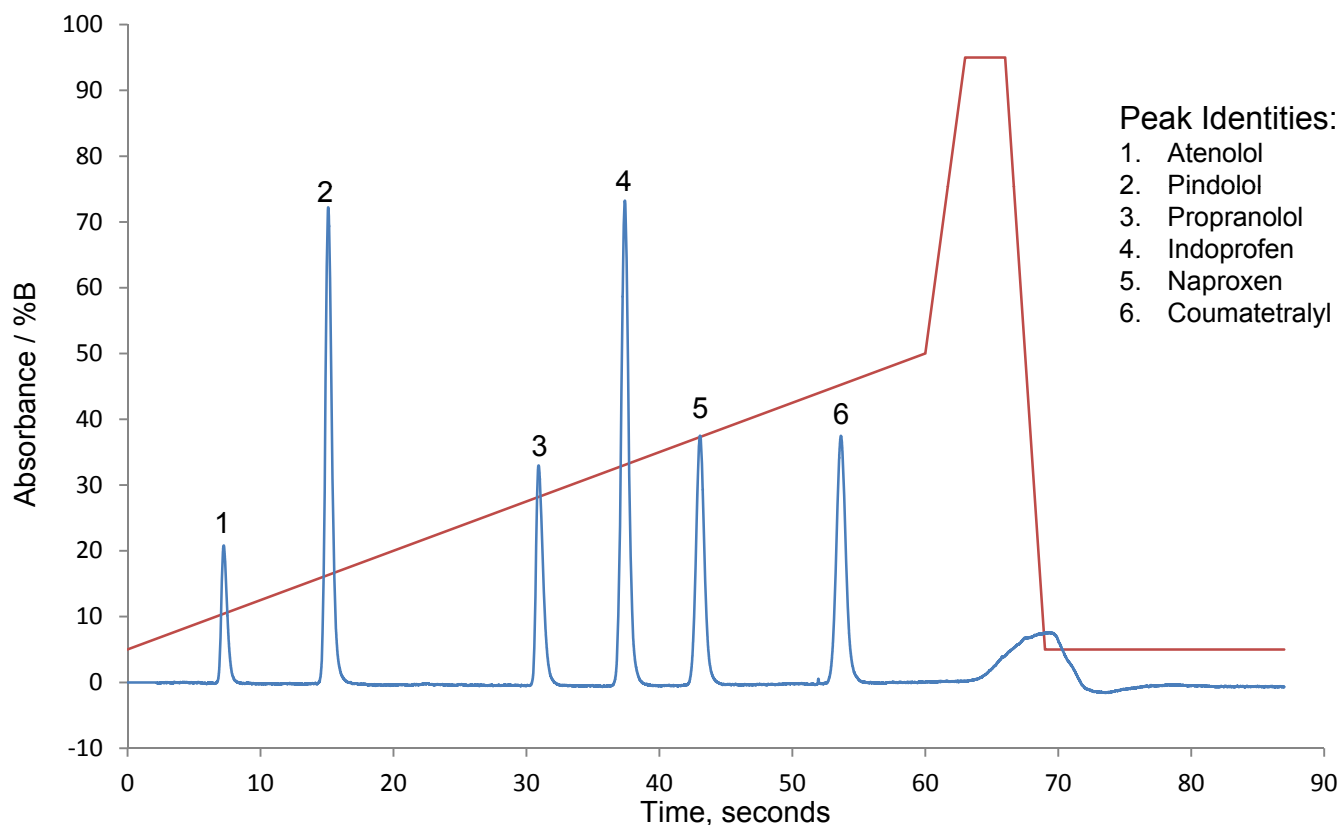
Temperature: 40°C

Detection: UV 254 nm, PDA

Injection Volume: 0.5  $\mu$ L

Flow Cell: 1  $\mu$ L micro

LC System: Shimadzu Nexera



## Peak Identities:

1. Atenolol
2. Pindolol
3. Propranolol
4. Indoprofen
5. Naproxen
6. Coumatetralyl

## Gradient Program

Time (s)	% ACN
0	5
60	50
63	95
66	95
69	5
87	5

Fast re-equilibration on Fused-core columns enables high throughput analysis. Total cycle time is < 90 seconds for injection and re-equilibration. The gradient program appears in red.

## Conclusions

- Column efficiencies of 5  $\mu\text{m}$  SPP are comparable to columns packed with 3  $\mu\text{m}$  totally porous particles with about half the operating pressures.
- High resolution separations can be achieved using 5  $\mu\text{m}$  SPP columns at significantly lower back pressures than columns packed with sub-2- $\mu\text{m}$  totally porous particles.
- Narrow-bore 5  $\mu\text{m}$  SPP columns are well-suited for high speed “ballistic gradient” separations.



# Acknowledgment

Special thanks to Robert Moran for assistance with chromatographic measurements.