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

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Abstract

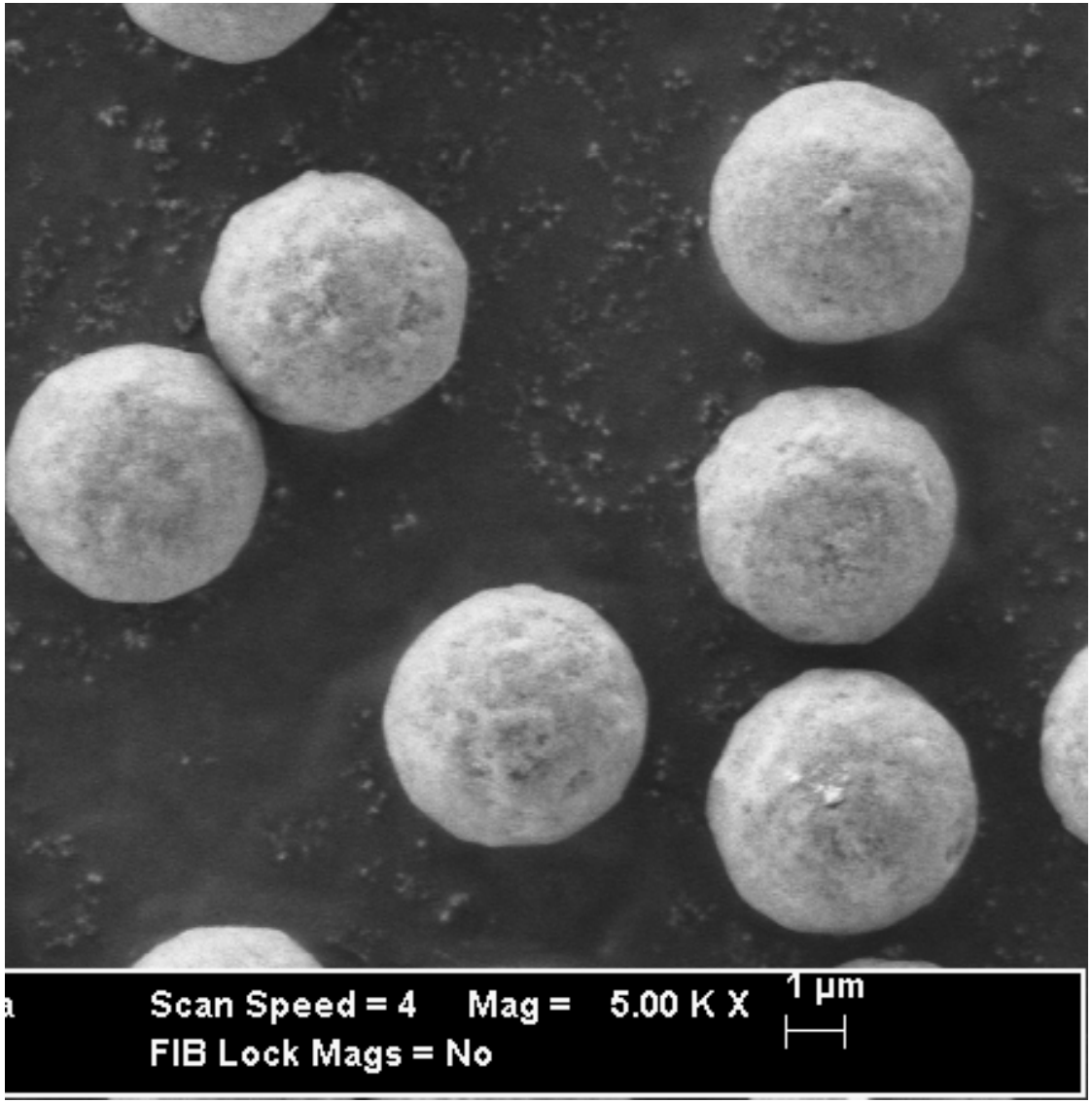
Since 2006, modern sub-3-µm superficially porous particles have been shown to deliver high speed separations comparable to sub-2-µm particles, in addition to high resolution separations for challenging samples. In 2012 larger particle size SPP particles for small molecule analyses were introduced. These 5-µm particles can deliver performance comparable to or better than 3-µm totally porous particles—at the backpressure of 5-µm 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-µm 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 mL/min) at normal HPLC pressures (< 400 bar/6000 psi). Such high-speed analyses are not achievable with sub-2-µm columns unless UHPLC/UPLC® instrumentation is used. Moreover, some users have found that peaks produced by the sub-2-µm columns were, in fact, too narrow for full fidelity with their fastest LC-MS scan rates. The slightly broader peaks obtained with 5-µm SPP columns did not have such a problem.

In addition to high throughput applications, 5-µm 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.

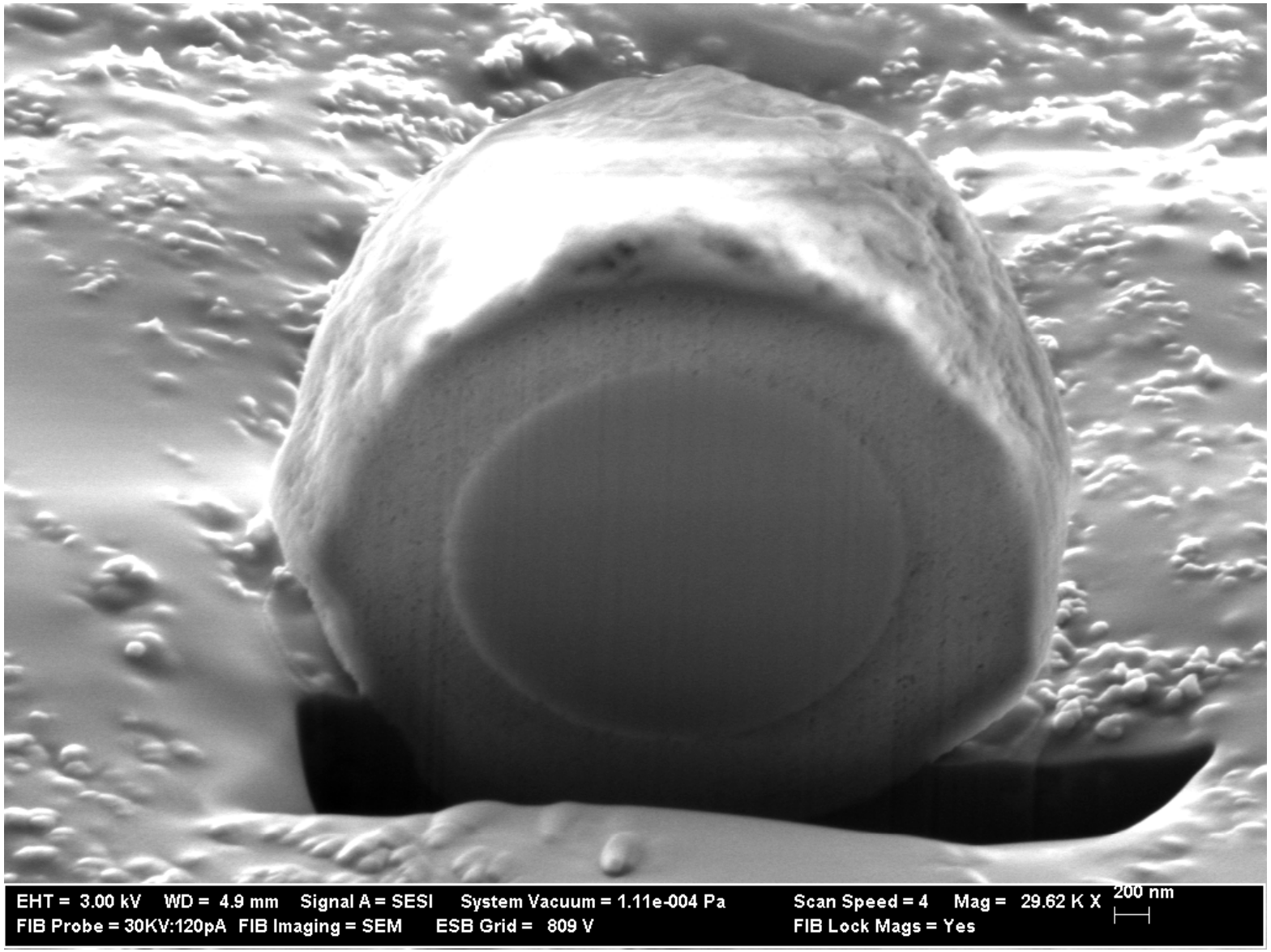
In this paper, we will demonstrate some of the advantages of these new particles and columns using several high throughput and high resolution examples versus comparable sub-2-µm separations.

SEM Photo of 5-micron Fused-Core Particles

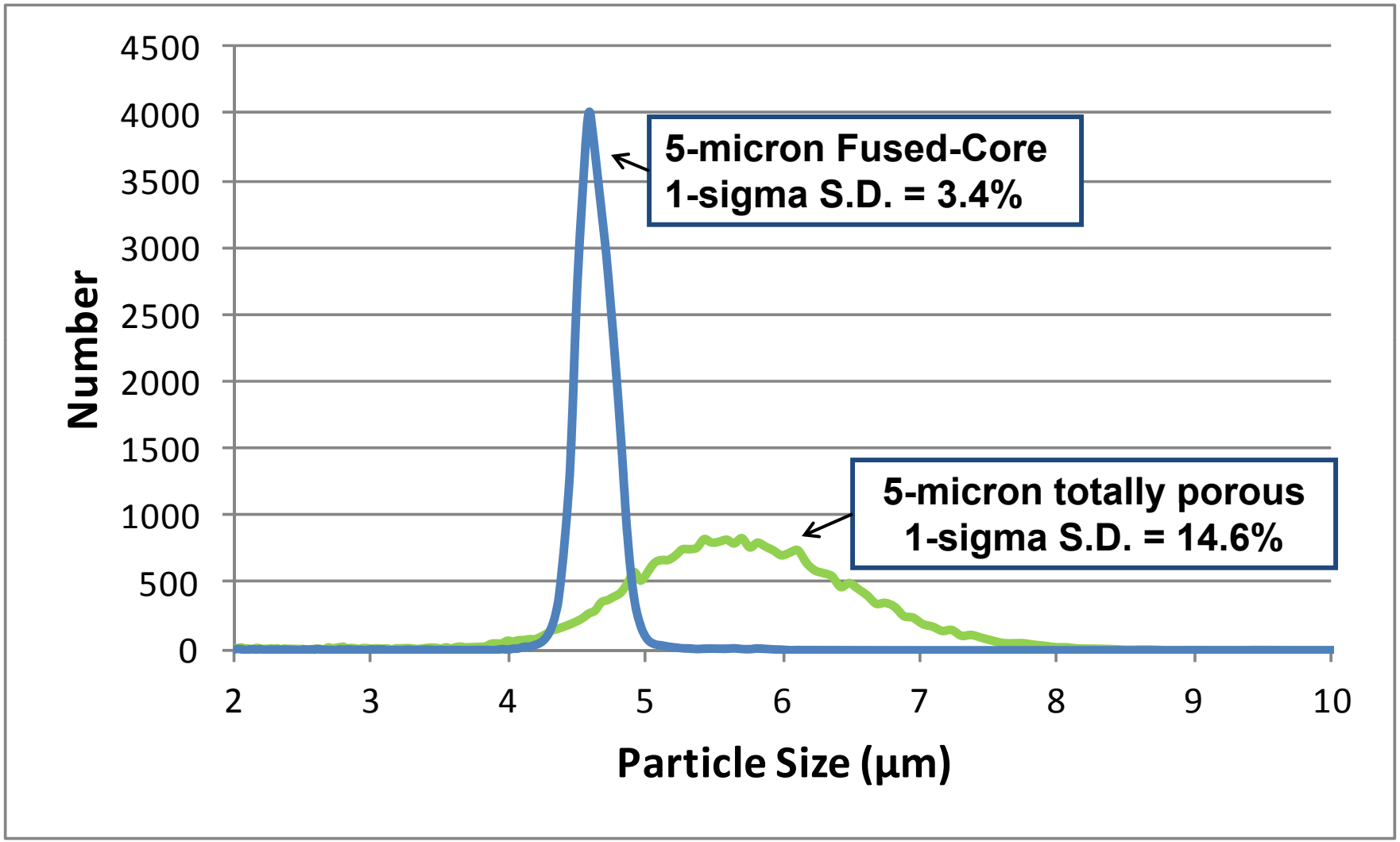


SEM Photo of FIB Sliced 5-micron Fused-Core Particle

(FIB = Focused Ion Beam)

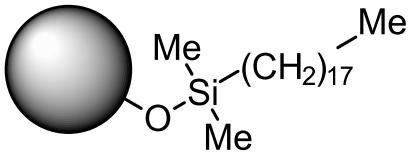


Improved Size Distributions for 5-Micron Particles

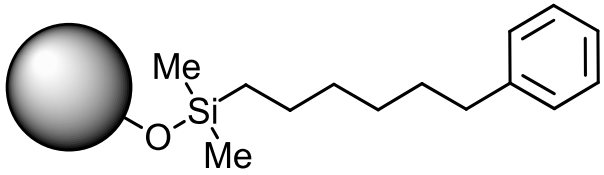


Measured on a Multisizer 3 Coulter Counter

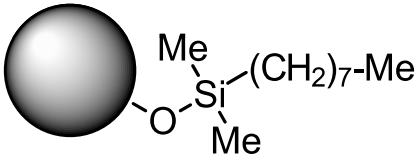
Different Stationary Phases for Modifying Selectivity



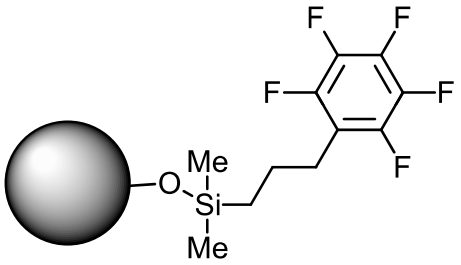
C18 (octadecyl)



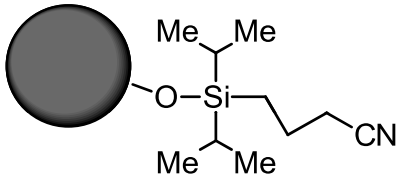
Phenyl-Hexyl



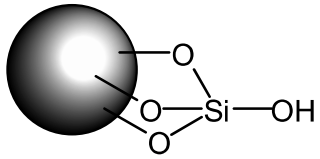
C8 (octyl)



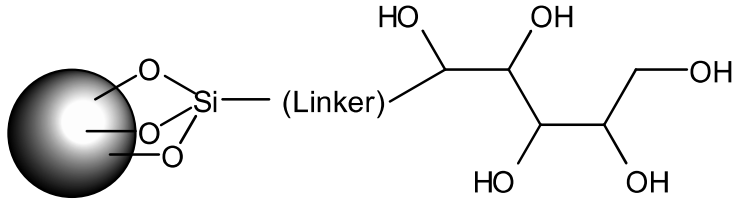
PFP (pentafluorophenylpropyl)



ES-CN



HILIC



Penta-HILIC

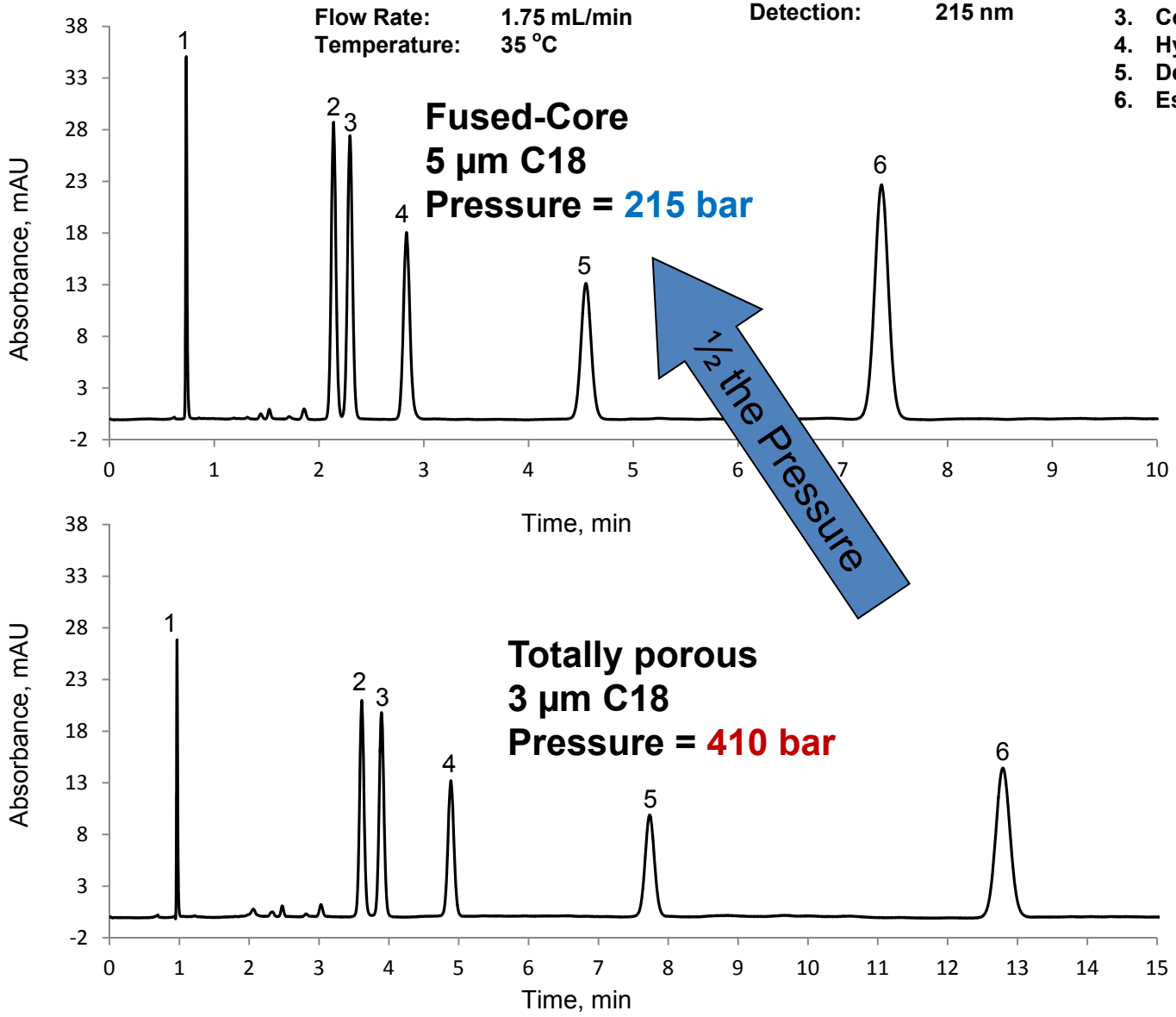
3 µm Performance with 5 µm Pressure

Conditions:

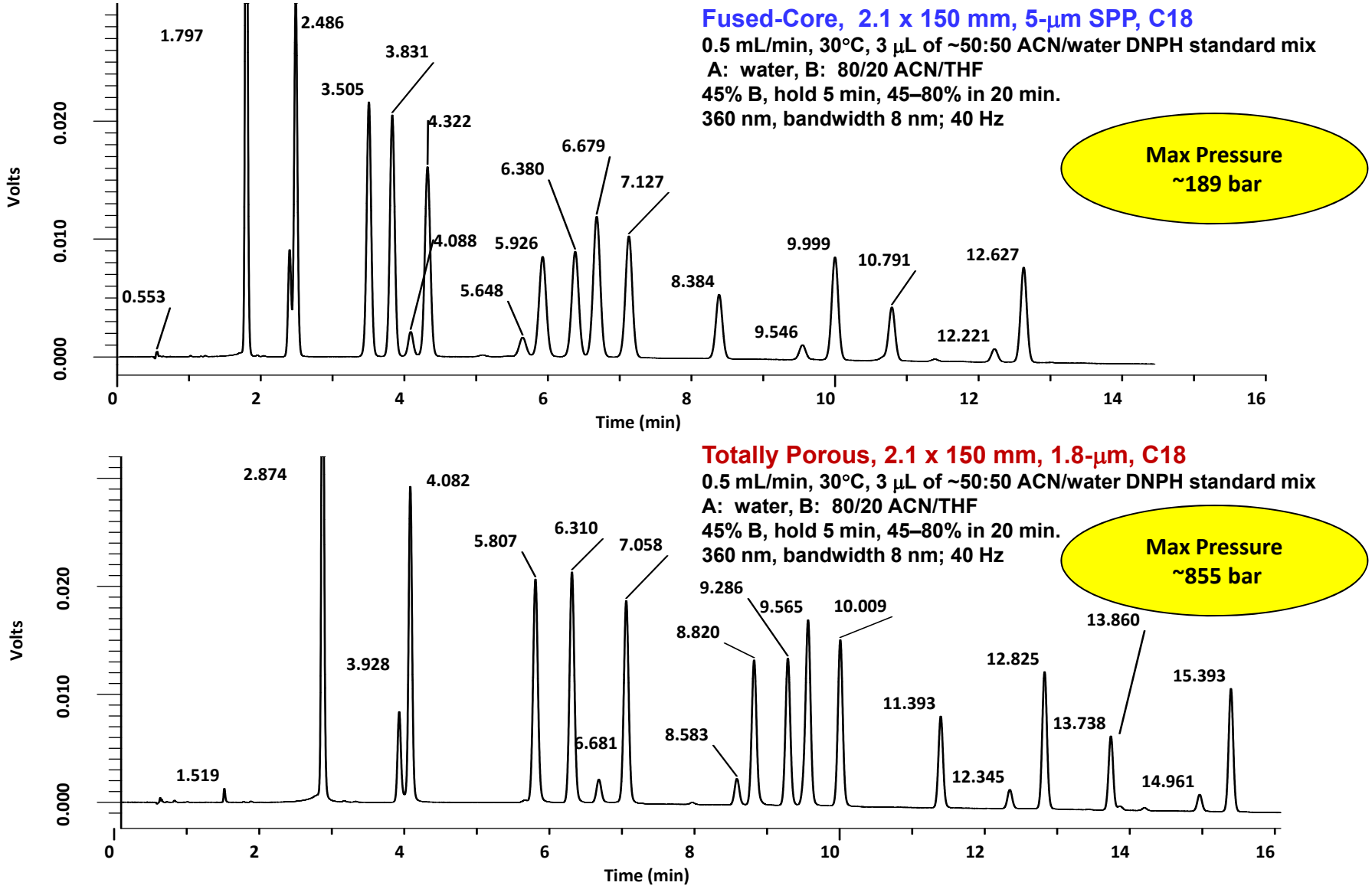
Columns:	**4.6 x 150 mm**	**Injection:**	**5 µL**
Mobile Phase:	**50/50 water/methanol**	**Instrument:**	**Agilent 1200**
Flow Rate:	**1.75 mL/min**	**Detection:**	**215 nm**
Temperature:	**35 °C**		

Peak Identities in order:

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



Use 5 µm SPP Columns to Achieve High Resolution at Low Pressure



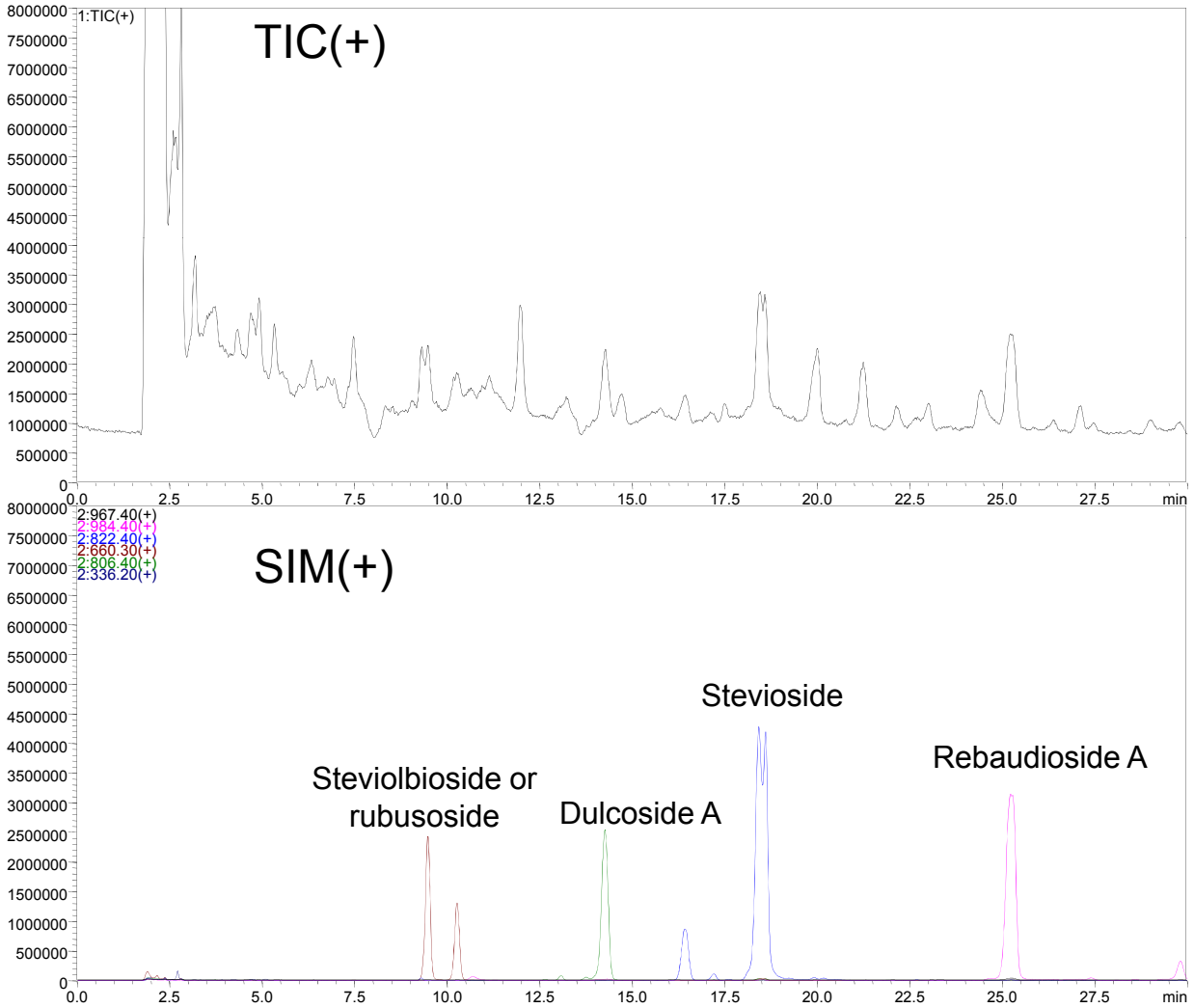
PEAK IDENTITIES (in order): Formaldehyde-2,4-DNPH, Acetaldehyde-2,4-DNPH ,Acetone-2,4-DNPH,Acrolein-2,4-DNPH,Propionaldehyde-2,4-DNPH, Crotonaldehyde-2,4-DNPH, 2-Butanone-2,4-DNPH, Methacrolein-2,4-DNPH, Butyraldehyde-2,4-DNPH, Benzaldehyde-2,4-DNPH,Valeraldehyde-2,4-DNPH,m-Tolualdehyde-2,4-DNPH, and 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 5µm Fused-core Particles: Positive 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

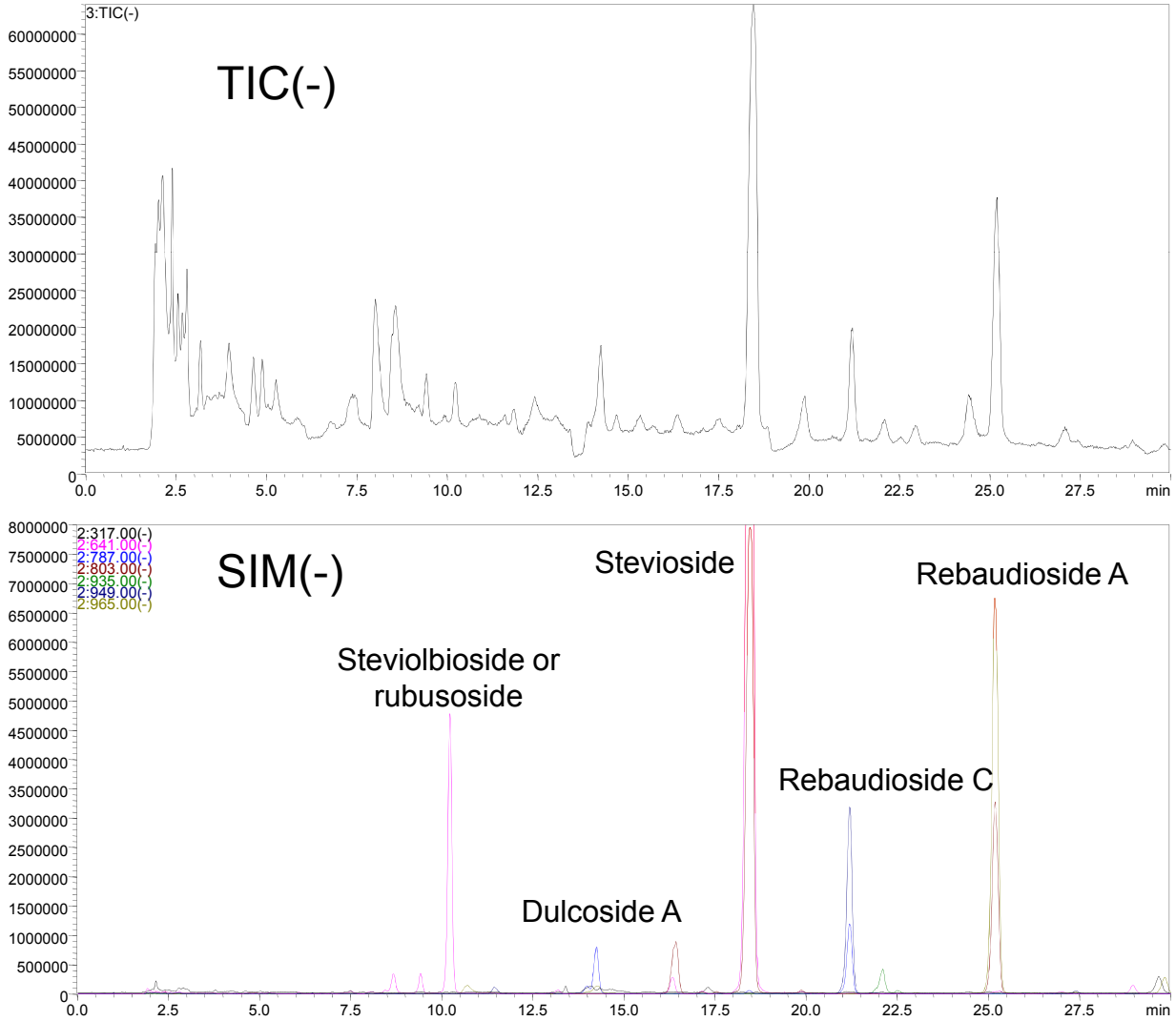
Extraction Procedure:

1. Weigh 400 mg of Stevia Leaves (Sigma S5381)
2. Crush with mortar and pestle
3. Add 8 mL of 50/50 ACN/H_2O
4. Sonicate for 15 min
5. Filtered sample using 25 mm syringe filter with 0.2 µm PTFE membrane (VWR 28145-495)
6. Centrifuge at 10k rpm and collect supernatant
7. Dilute 400 µL sample in 600 µL ACN for an overall sample solvent concentration of 80/20 ACN/H_2O.
8. Centrifuge diluted sample at 10 k rpm and inject the supernatant.

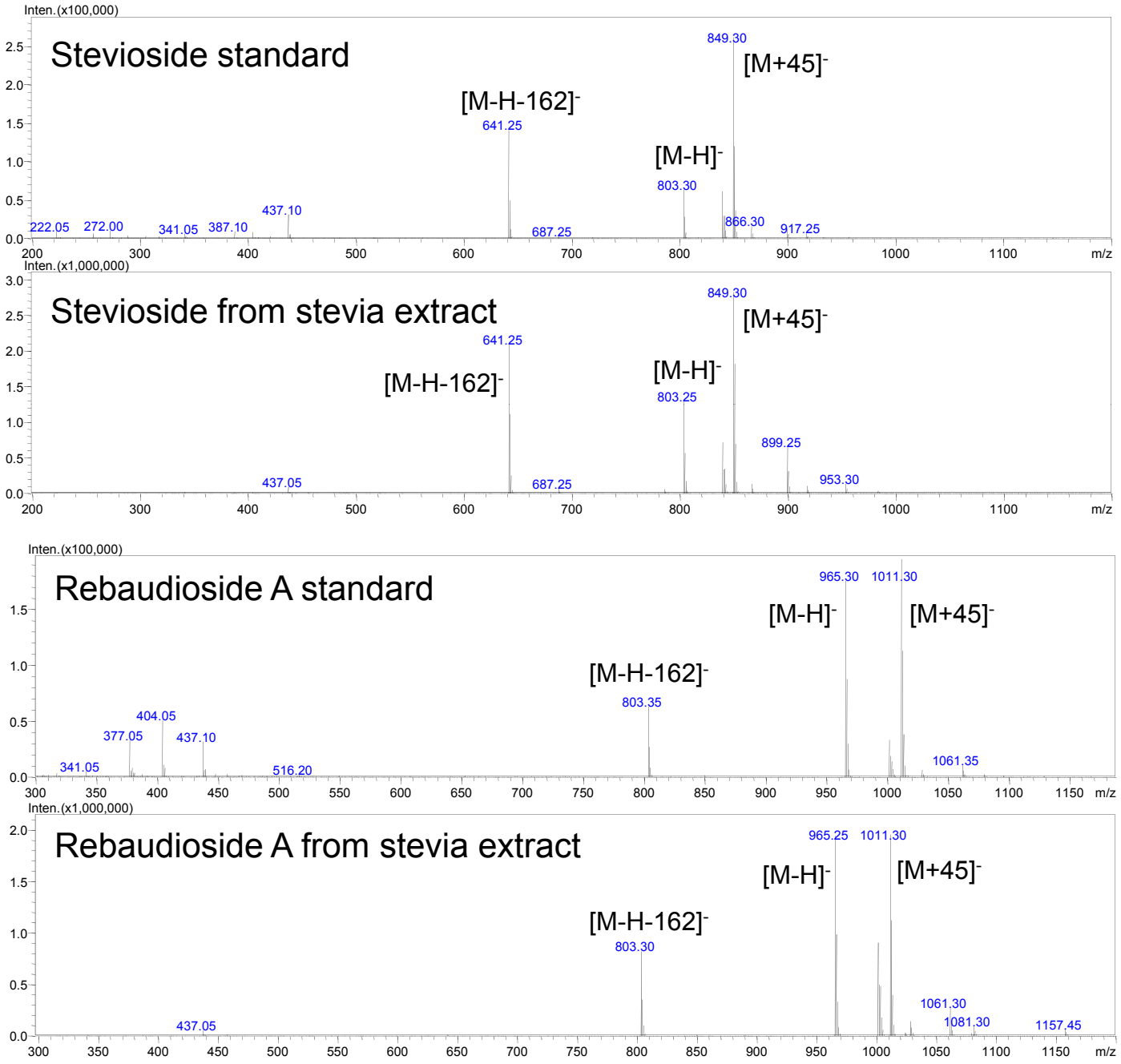


High Resolution Separation of Stevia Extract using 5µm Fused-core Particles: Negative Ion Mode

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

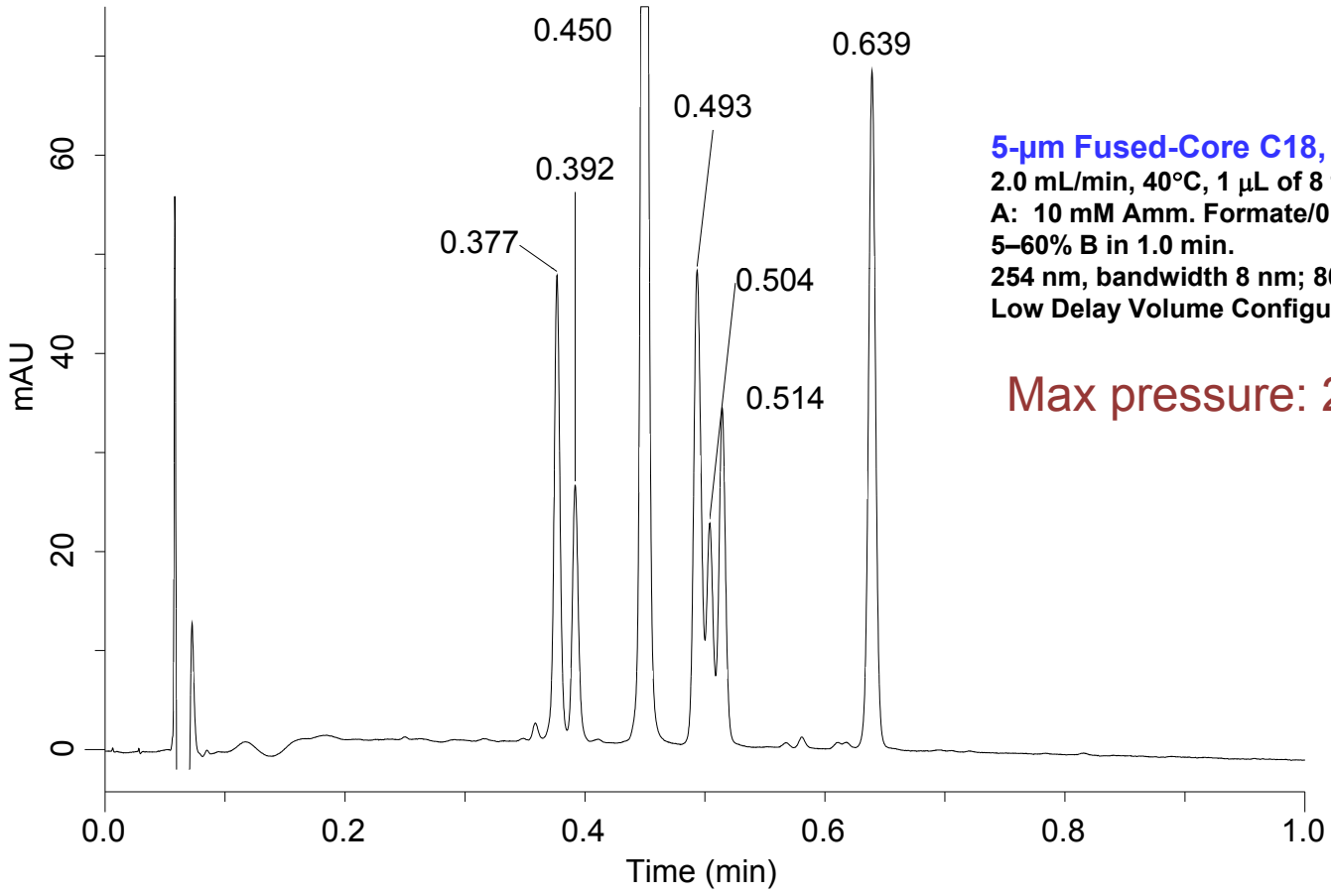


Go Ballistic with 5-µm HPLC Columns

- 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-µm Fused-Core Column



5-µm Fused-Core C18, 2.1 x 50 mm
2.0 mL/min, 40°C, 1 µL of 8 flavonoids in MeOH
A: 10 mM Amm. Formate/0.1% formic acid, B: ACN
5–60% B in 1.0 min.
254 nm, bandwidth 8 nm; 80 Hz; Agilent 1200 SL;
Low Delay Volume Configuration ~ 120 µL; ADVR ON

Max pressure: 210 bar

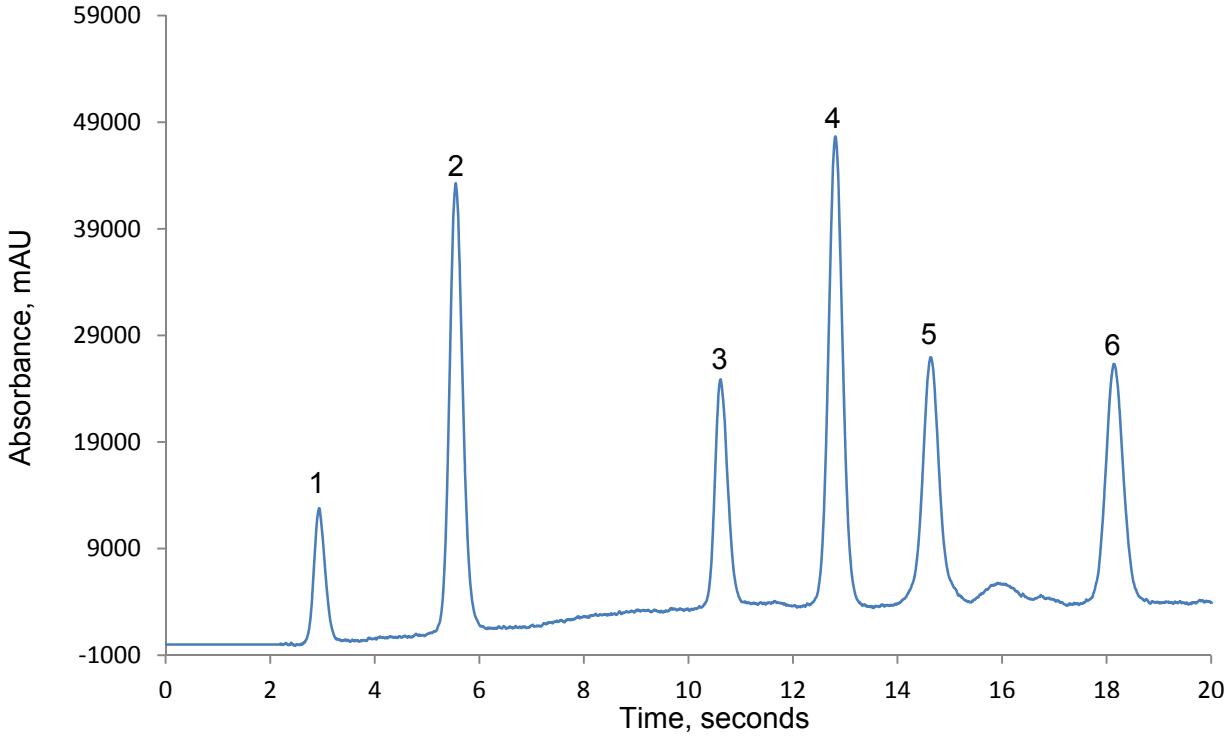
Analytes, in elution order: hesperidin, myricetin , quercetin, naringenin & apigenin (coeluted), hesperetin, kaempferol, biochanin

20 Second Gradient Using 5-µm 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 µL
Flow Cell: 1 µL micro
LC System: Shimadzu Nexera

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

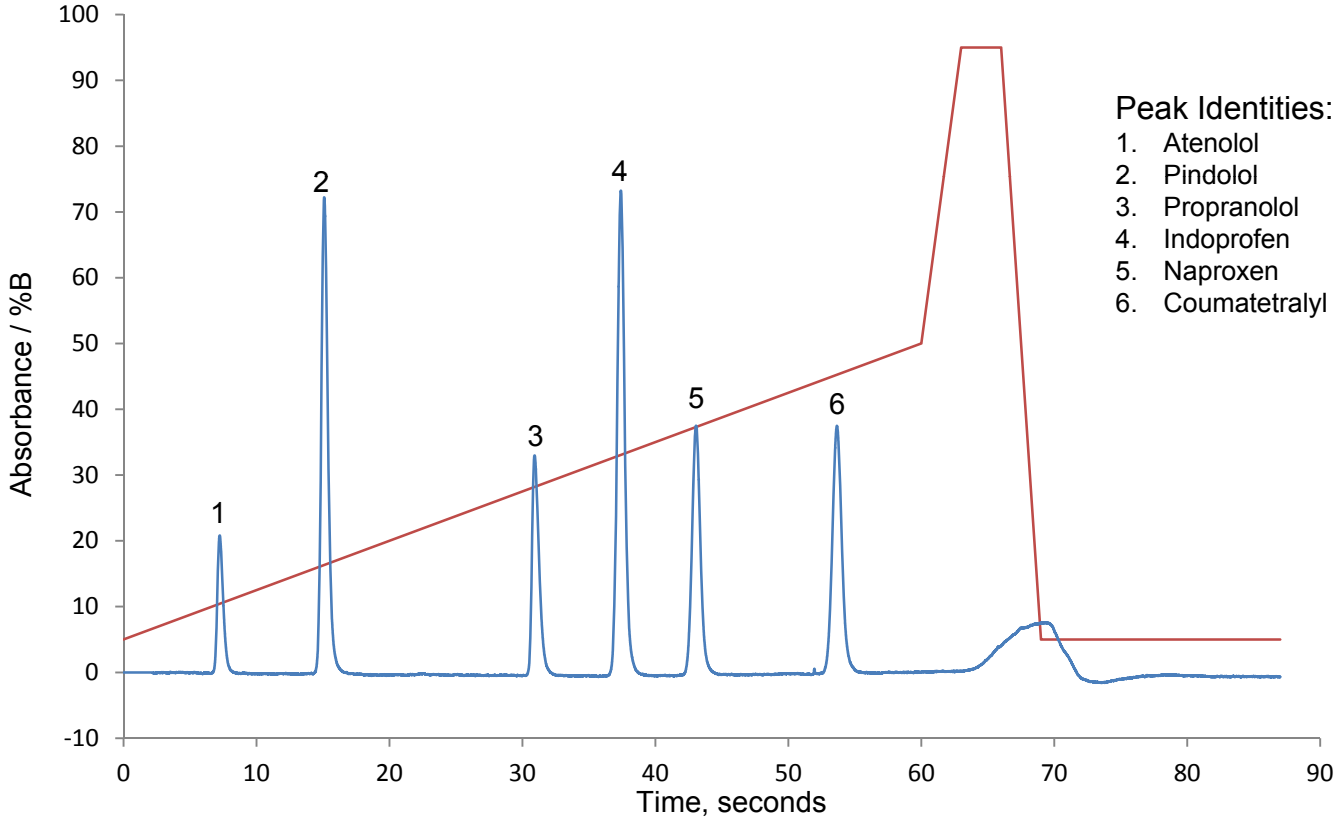


Ballistic Gradient and Fast Re-equilibration with 5-µm Fused-Core Column

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 µL
Flow Cell: 1 µL 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 µm SPP are comparable to columns packed with 3 µm totally porous particles with about half the operating pressures.
- High resolution separations can be achieved using 5 µm SPP columns at significantly lower back pressures than columns packed with sub-2-µm totally porous particles.
- Narrow-bore 5 µm SPP columns are well-suited for high speed “ballistic gradient” separations.

Acknowledgment

Special thanks to Robert Moran for assistance with chromatographic measurements.