HALO®

THE IMPORTANCE OF SUPERFICIALLY POROUS PARTICLES IN MODERNIZING HPLC METHODS

EAS 2019

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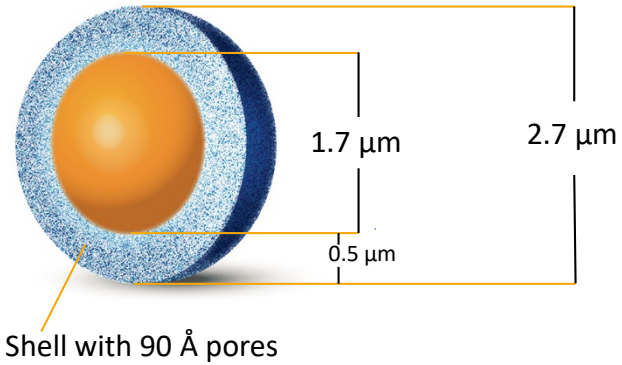
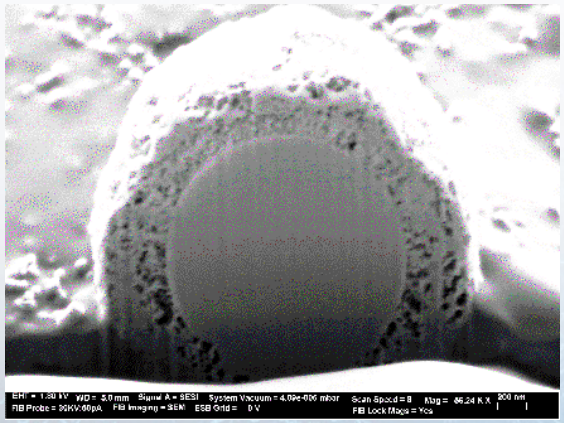
advancedmaterialstechnology

The Unique Superficially Porous Particle (SPP)

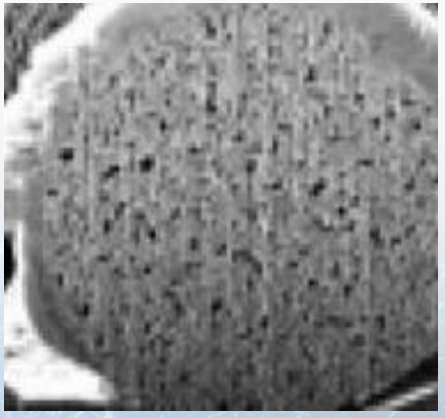


- Highest purity Type B silica
- Nonporous silica core
- Porous silica shell
- Shell thickness and pore size tightly controlled
- Particle size highly uniform

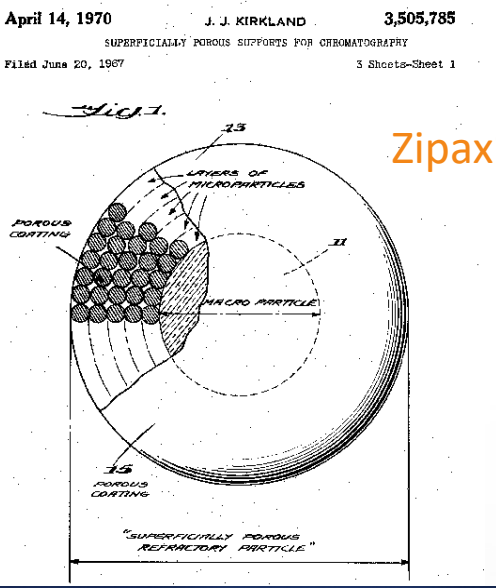
HALO® Particle (SPP)



Fully Porous Particle (FPP)



Milestones in Fused-Core® History


April 14, 1970 J. J. KIRKLAND 3,505,785
SUPERFICIALLY POROUS SUPPORTS FOR CHROMATOGRAPHY
Filed June 20, 1967 3 Sheets-Sheet 1
Fig. 1.
15
LAYERS OF MICROPARTICLES
POROUS COATING
11
MACRO PARTICLE
15
POROUS COATING
"SUPERFICIALLY POROUS REFRACTORY PARTICLE"

Zipax®

- **1960**- Golay first proposed superficially porous particles (SPPs) for GC.
- **1970**- Jack Kirkland (DuPont Company), inspired by a nucleotide separation of Horvath and coworkers using ~50 µm cores with thin layer of anion exchange resin, develops Zipax® particles with layers of silica sol on 30 µm glass beads.
- **1990**- Kirkland continues to advance fully porous particle technology by developing high purity Type B silica and creates a clear performance distinction between Type A and Type B.
- **2006**- Progressively smaller fully porous Type B silica particles develop rapidly in the HPLC column market. Kirkland meets the demand for higher speed and resolution and creates modern superficially porous particles that delivers higher performance at lower pressures.

2006 — **Original HALO® 2.7 µm SPP**
changed the perception of what is required for high efficiency separations

2012 — **HALO® 5 µm SPP**
robust replacement to conventional 5 µm particle columns with SPP benefits

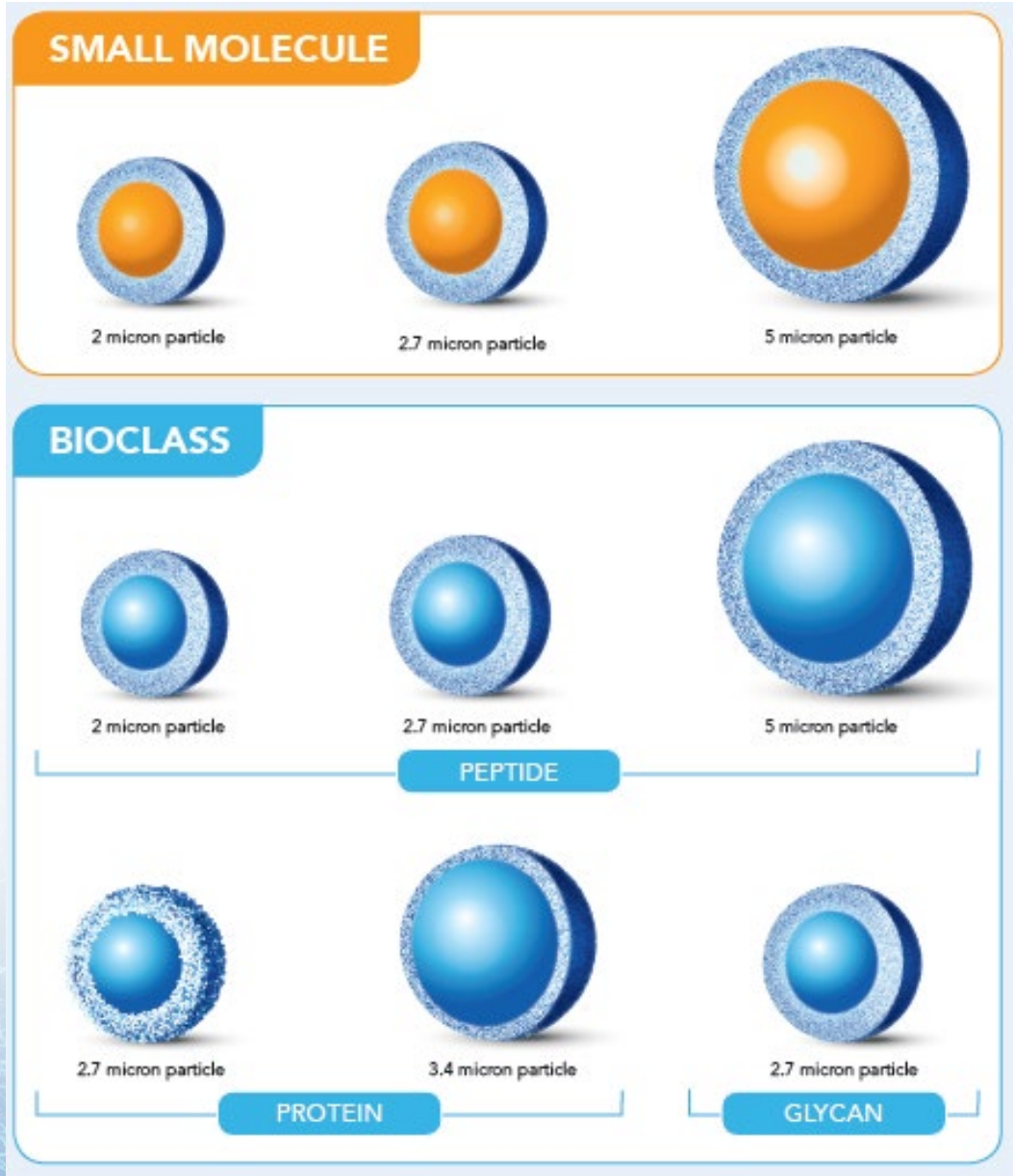
2013 — **HALO® BioClass Line Introduced**
Protein, Peptide and Glycan solutions to meet the challenges of biomolecule separations

2014 — **HALO® 2 µm SPP**
the go-to SPP for highest efficiency separations with UHPLC technology

2017 — **HALO® 1000 Å Protein**
First 1000 Å pore size providing the widest pore available in an SPP that delivered significant gains in resolution of large protein complexes

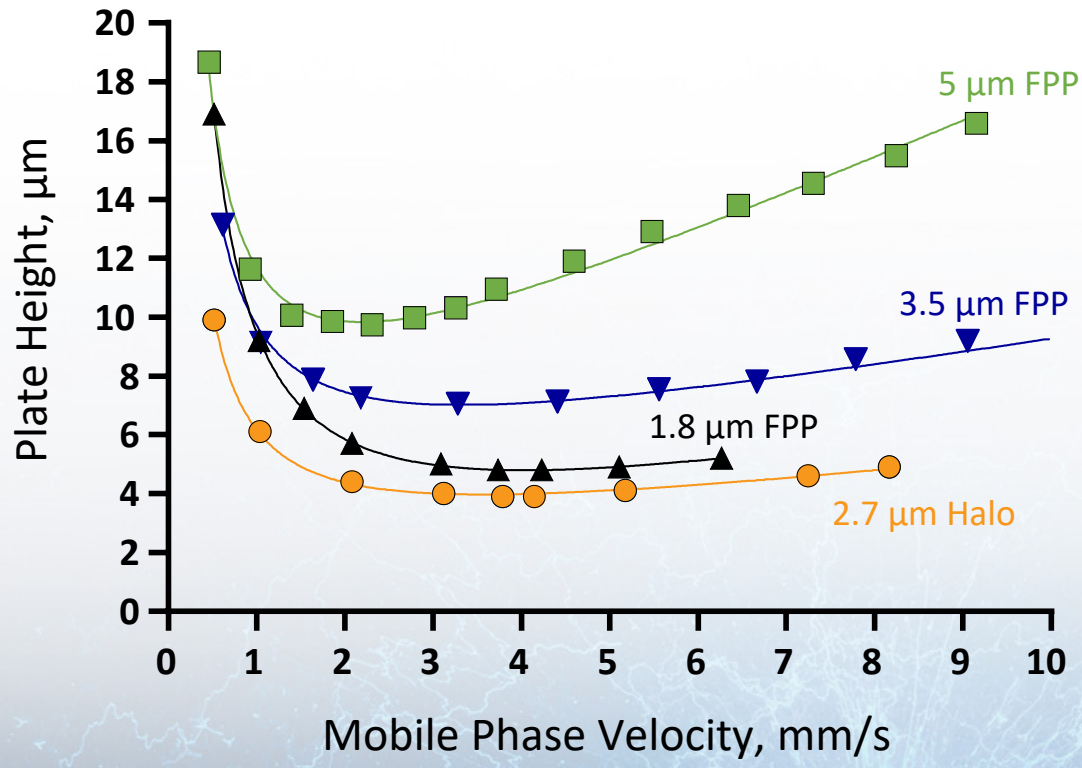
HALO® Fused-Core® Family

- Various stationary phases with particle and pore size morphologies
- 90 Å for small molecules
- 160 Å for intermediate size molecules
- 400 Å and 1000 Å for large molecules



How SPP Design Benefits HPLC Separation

Effect of Particle Size and Type (small-pore)
Columns: 4.6 x 50 mm
5 µm FPP C18
3.5 µm FPP C18
1.8 µm FPP C18
2.7 µm HALO C18
Solute: naphthalene
Mobile phase: 60% ACN/40% water Temperature: 24 °C



van Deemter Equation

$$H = A + \frac{B}{\mu} + C\mu$$

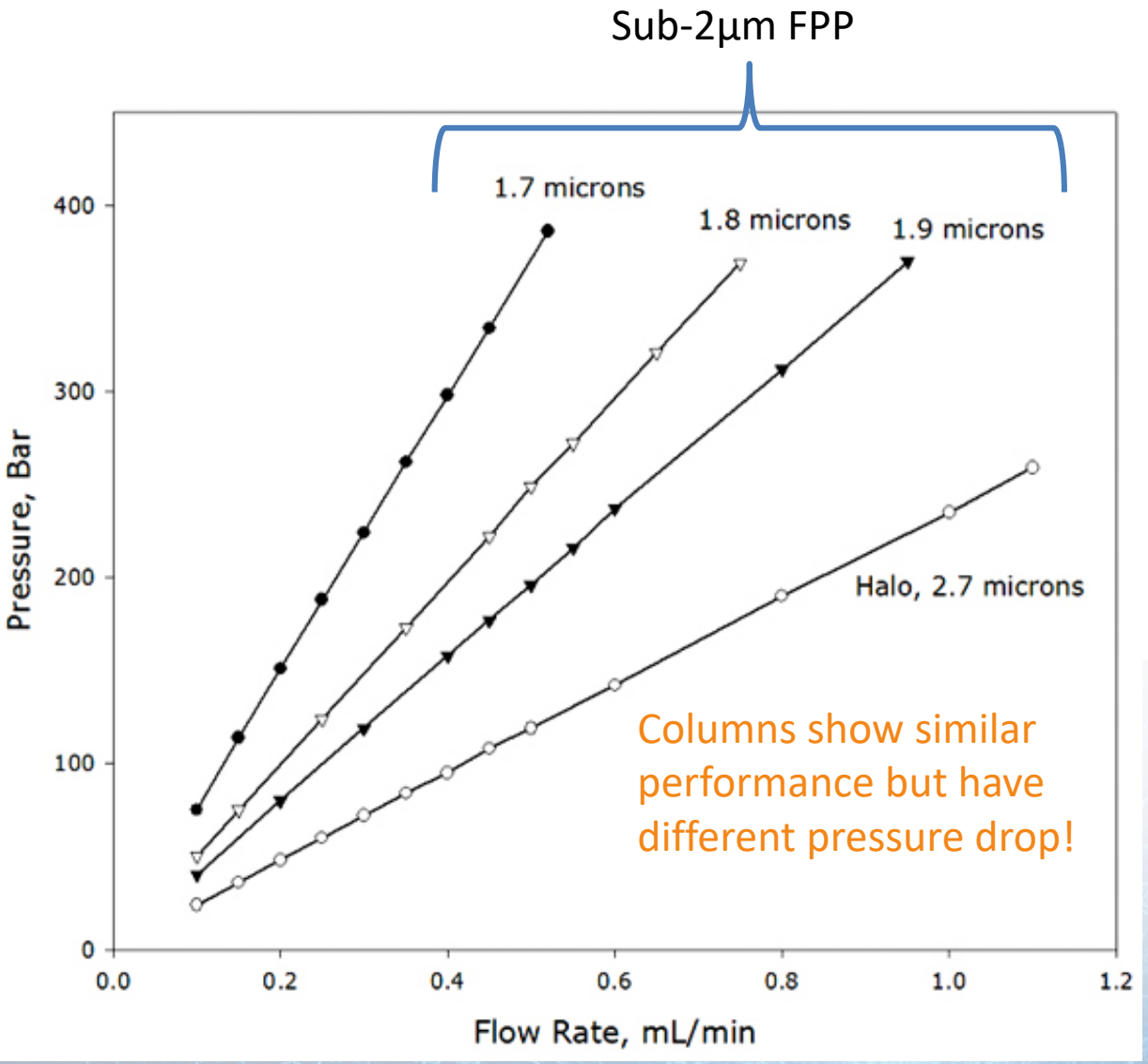
H = plate height H = L/N

- **A** = eddy diffusion term
  (30 - 40% smaller vs FPP)
- **B** = longitudinal diffusion term
  (25 - 30% smaller vs FPP)
- **C** = resistance to mass transfer
  (smaller due to shorter flow path)

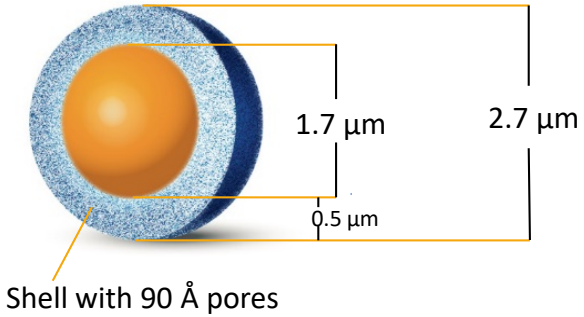
µ = mobile phase linear velocity (L/t_0)

J.J. DeStefano, T.J. Langlois, & J.J. Kirkland, ***J. Chromatogr. Sci.***, 2008, 46(3), 254-260

Low Backpressure of SPP vs FPP



HALO® Fused-Core® Particle



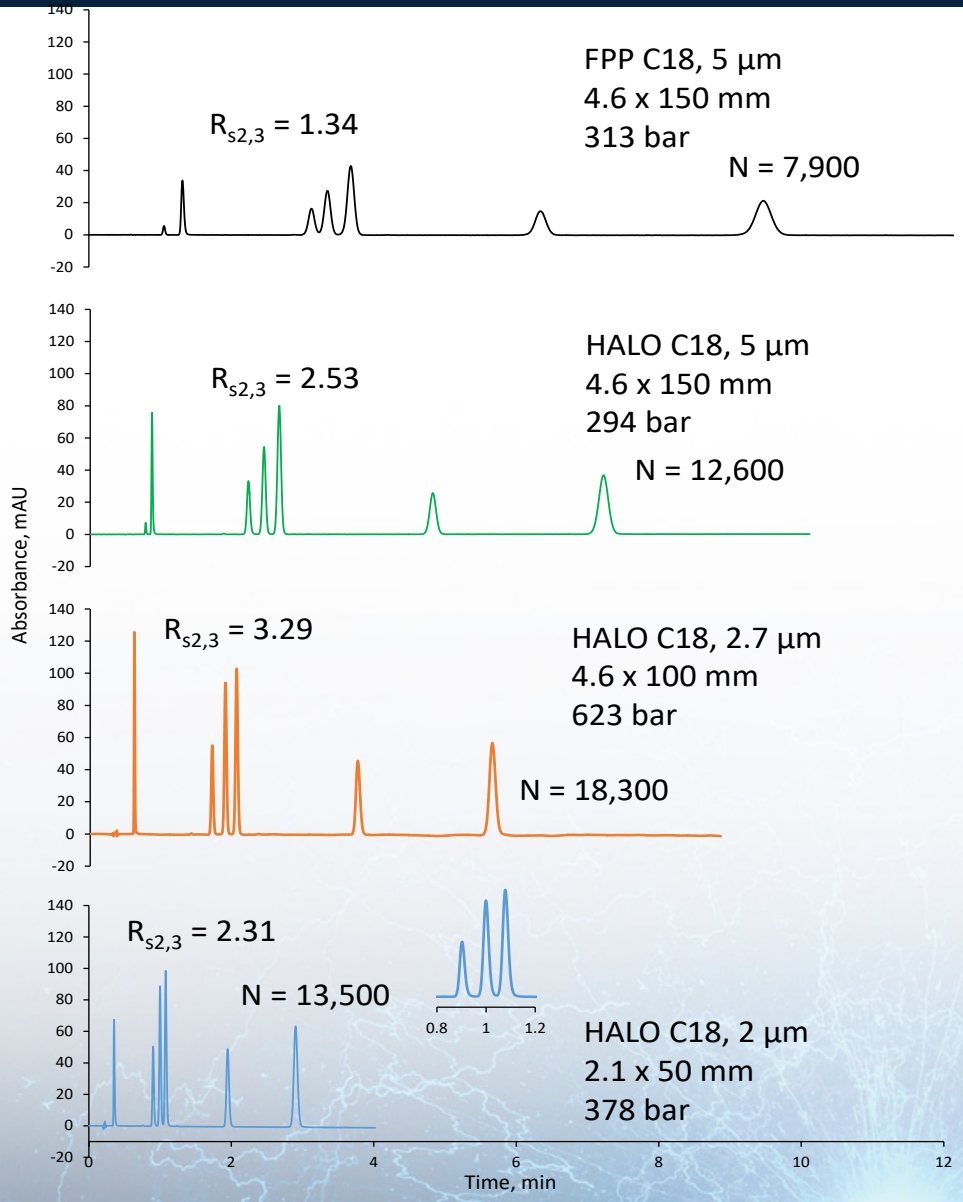
Columns: 50 x 2.1 mm, C18
Mobile Phase:
70% ACN, 30% Water
Temperature: 24° C

Summary of USP Modernization Efforts* (Following USP-NF Chapter 621 Guidelines)

- Particle size and/or column length may be changed if ratio of column length (L) to particle diameter (d_p) is the same or in range between -25% to +50% of the prescribed L/d_p ratio. L/d_p is proportional to column resolving power.
- Bonded phase may not be changed to another L Code.
- Temperature may be adjusted ± 10 °C.
- Flow rate may be adjusted ± 50%.
- Mobile phase may be adjusted but cannot exceed ±10% (or introduce new chemical modifiers).

* USP moves to encourage adoption of modern HPLC columns and particles in USP Monograph Methods. Changes currently allowed only for isocratic methods; efforts are underway by USP to establish guidelines for changing gradient methods.

L/d_p Ratios When SPP is Same or Smaller



L/d_p = 150/.005 = 30,000

For -25 to +50%, L/dp can be 22,500-45,000

L/d_p = 150/0.005 = 30,000

L/d_p criteria met

37% higher plates

L/d_p = 100/.0027 = 37,037

L/d_p criteria met

57% higher plates

L/d_p = 50/0.002 = 25,000

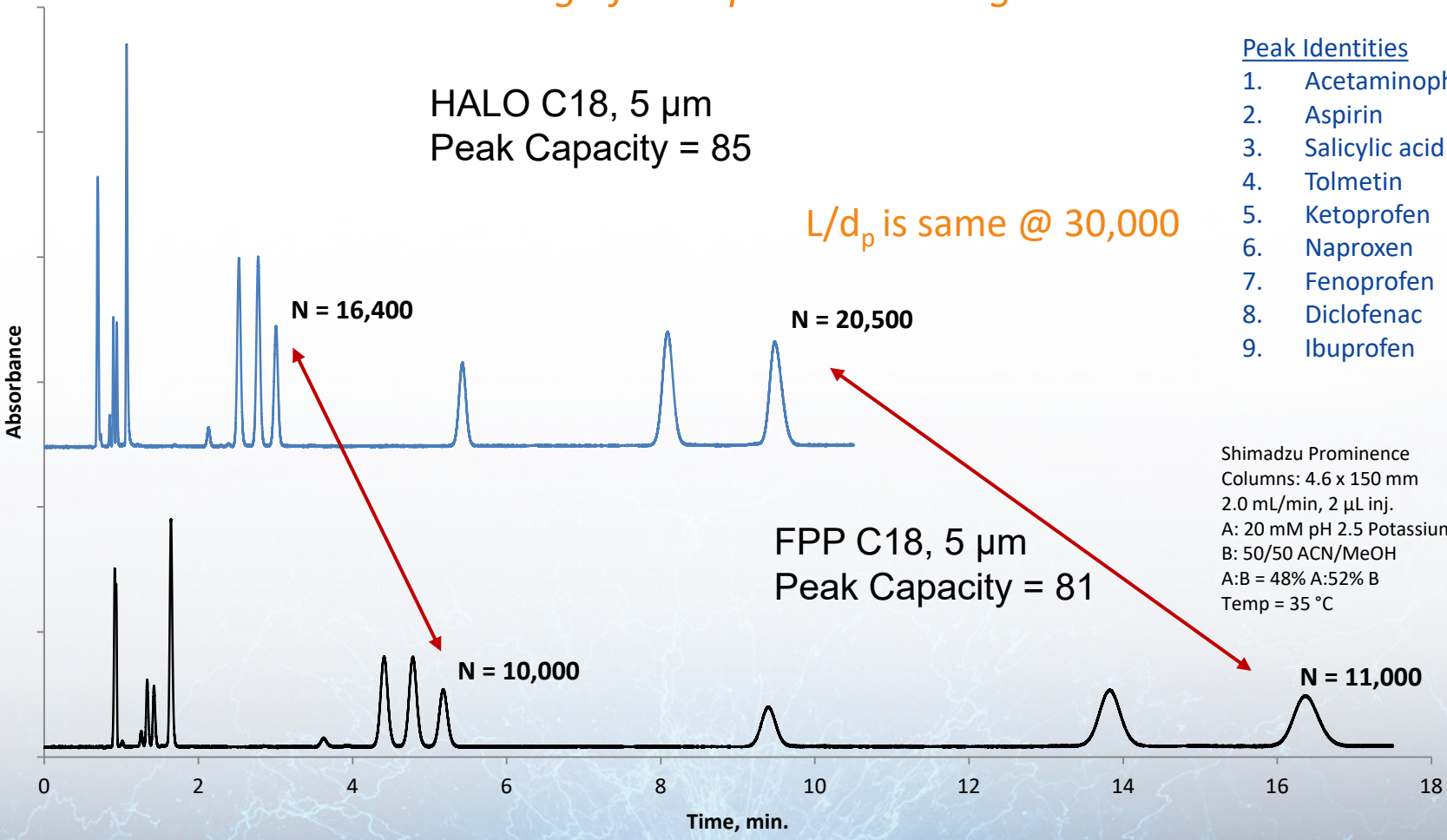
L/d_p criteria met

3x times faster

41% higher plates

When Should 5 µm SPP Columns Be Used?

To <u>increase</u> efficiency, speed and response of 5µm FPP methods without significant pressure change.



Peak Identities

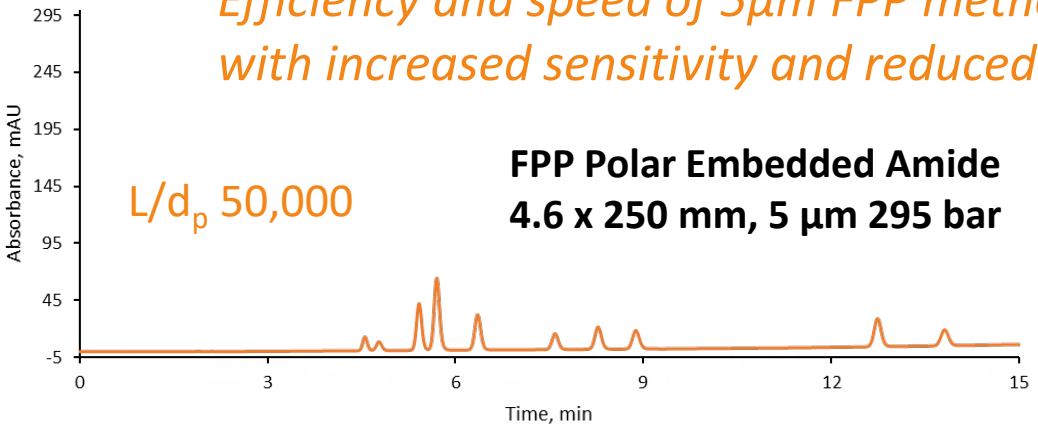
1. Acetaminophen
2. Aspirin
3. Salicylic acid
4. Tolmetin
5. Ketoprofen
6. Naproxen
7. Fenoprofen
8. Diclofenac
9. Ibuprofen

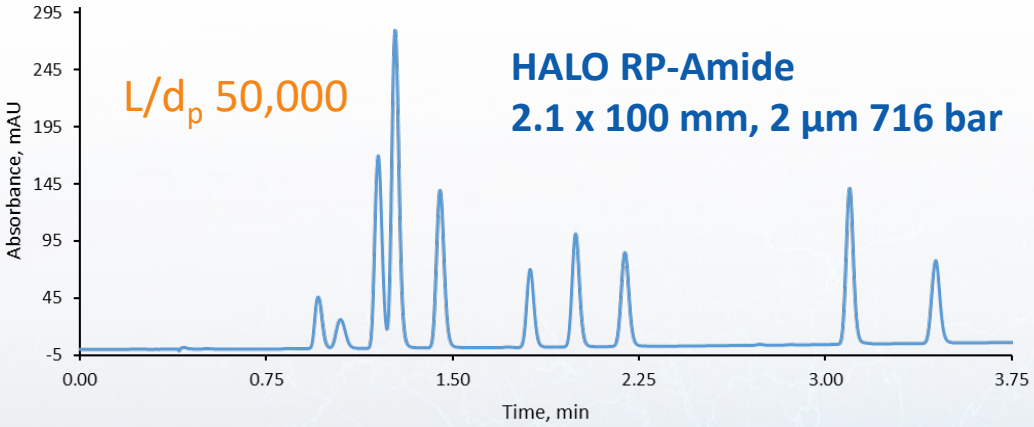
Shimadzu Prominence
Columns: 4.6 x 150 mm
2.0 mL/min, 2 µL inj.
A: 20 mM pH 2.5 Potassium Phosphate
B: 50/50 ACN/MeOH
A:B = 48% A:52% B
Temp = 35 °C

60% more efficiency with shorter separation time

What If UHPLC is Available?

Efficiency and speed of 5µm FPP methods can be increased further with increased sensitivity and reduced solvent consumption.





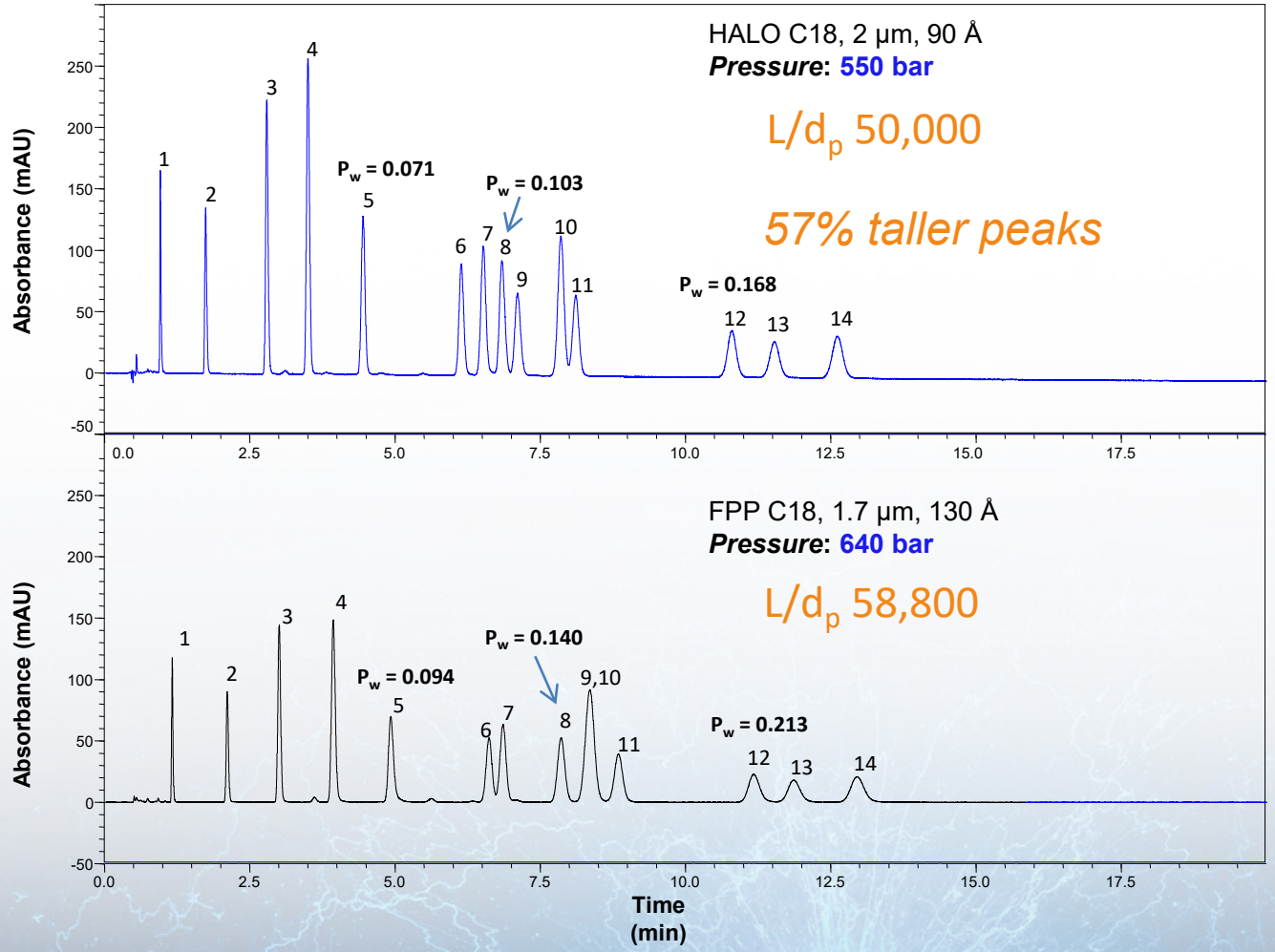
- Significant improvements in speed by moving from 5µm FPP to 2.7µm or even 2µm SPP.
- Note equivalent selectivity for polar embedded phase on SPP.

- **4x faster**
- **12x less solvent**
- **14.5x gain in sensitivity**

"This particle lets you do "UHPLC-like" separations on a standard system or do ultrafast HPLC on a UHPLC system" -Customer Comment

When Should 2 µm SPP Columns Be Used?

To <u>decrease</u> pressure and peak width of 1.7 µm FPP methods



Instrument: Shimadzu Nexera
Column: 2.1 x 100 mm
Mobile Phase: Water/MeOH (72/28)
Flow rate: 0.4 mL/min
Temperature: 42 °C
Detection: 254 nm
Injection volume: 1.0 µL

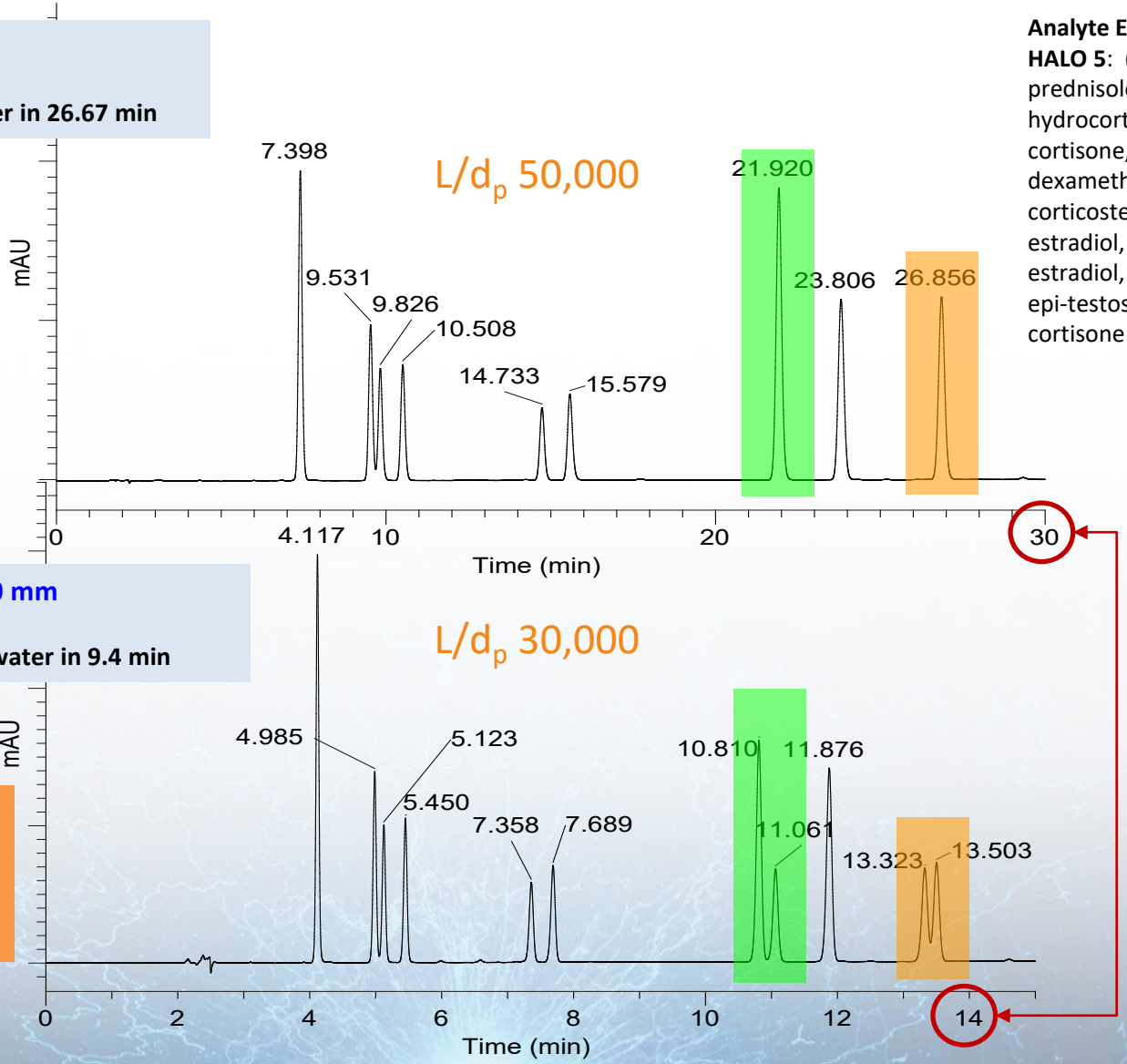
Peak Identities

1. *HMX*
2. *RDX*
3. *1,3,5-Trinitrobenzene*
4. *1,3-Dinitrobenzene*
5. *Nitrobenzene*
6. *Tetryl*
7. *2,4,6-Trinitrotoluene*
8. *2-Amino-4,6-Dinitrotoluene*
9. *4-Amino-2,6-dinitrotoluene*
10. *2,4-Dinitrotoluene*
11. *2,6-Dinitrotoluene*
12. *2-Nitrotoluene*
13. *4-Nitrotoluene*
14. *3-Nitrotoluene*

Higher efficiency and sensitivity at lower pressure

Case Study: Gradient Steroids Separation from 5 µm FPP to 5 µm SPP

FPP C18, 5 µm, 4.6 x 250 mm
10 µL, 1.5 mL/min, 20 °C
Gradient : 25–46% CH_3CN/water in 26.67 min

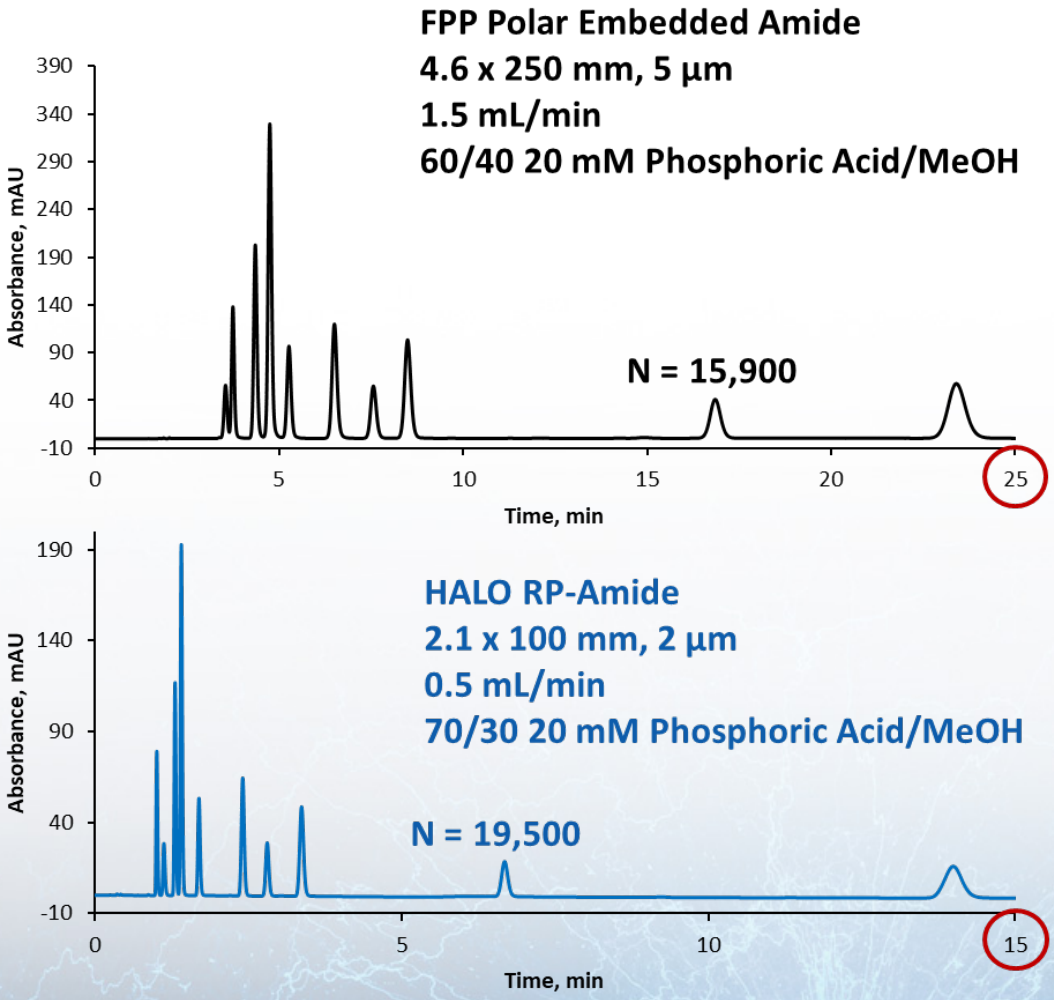


Analyte Elution order on HALO 5: (1) estriol, (2) prednisolone , (3) hydrocortisone, (4) cortisone, (5) dexamethasone, (6) corticosterone, (7) 17-β-estradiol, (8) 17-α-estradiol, (9) estrone, (10) epi-testosterone, (11) cortisone acetate

HALO 90 Å C18, 5 µm, 3 x 150 mm
2.3 µL, 1.0 mL/min, 20 °C
Gradient from 25–46% CH_3CN/water in 9.4 min

SPP method
- **2x faster!**
- **3x less solvent!**

Case Study: Isocratic Phenolic Acids Separation from 5 µm FPP to 2 µm SPP



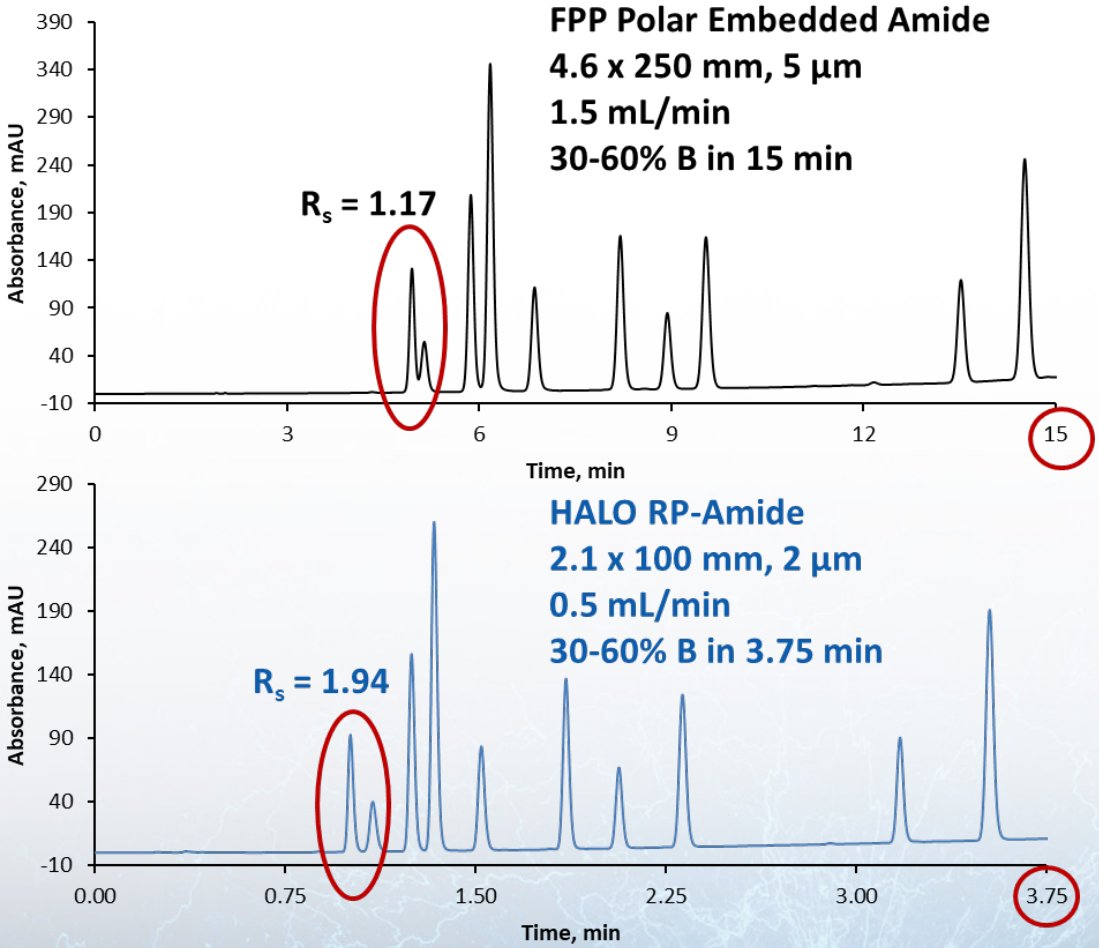
L/d_P = 50,000

L/d_P = 50,000

SPP method
- 1.7x faster
- 5x less solvent

Sample components: homovanillic acid, caffeic acid, syringic acid, vanillic acid, chlorogenic acid, sinapic acid, ferulic acid, *p*-coumaric acid, *trans*-cinnamic acid, resveratrol

Case Study: Gradient Phenolic Acids Separation from 5 µm FPP to 2 µm SPP



L/d_p = 50,000 for both columns

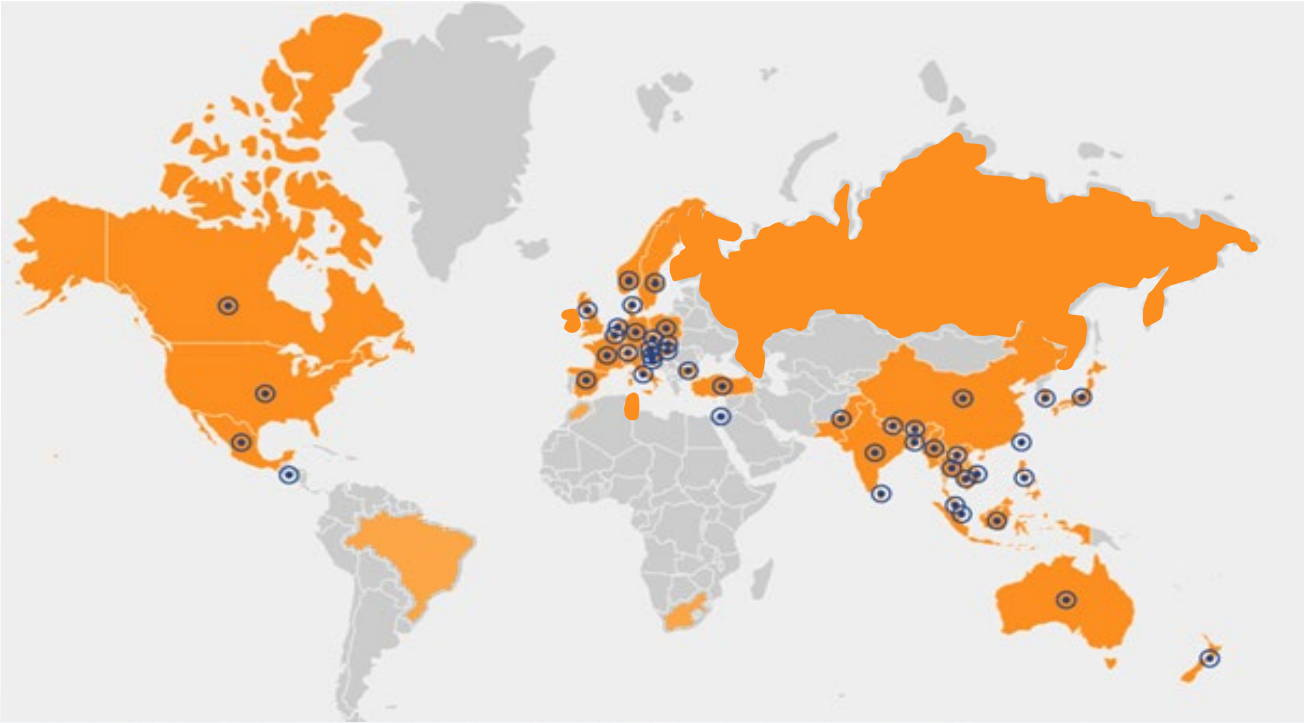
SPP method
- 4x faster
- 12x less solvent

Sample components (in order): homovanillic acid, caffeic acid, syringic acid, vanillic acid, chlorogenic acid, sinapic acid, ferulic acid, *p*-coumaric acid, *trans*-cinnamic acid, resveratrol

Summary

- Fused-Core® columns are designed for rugged, high-efficiency and high-speed separations.
  - UHPLC instruments that have been optimized for low dispersion are required to take full advantage of Fused-Core® for fast separations on short, small ID columns.
- Following new USP <621> guidelines for method modernization, many existing FPP methods can be quickly improved for speed and sensitivity using HALO® Fused-Core® column technology. Guidelines apply only to updating USP monographs.
- Examples and case studies were shown for FPP to SPP method transfer.

Global Access



HALO® is supplied through distributors in most major countries around the world and proudly holds a 99% on time (within 24 hours) shipping record!