HALO®

advancedmaterialstechnology

macmod
SMARTER CHROMATOGRAPHY

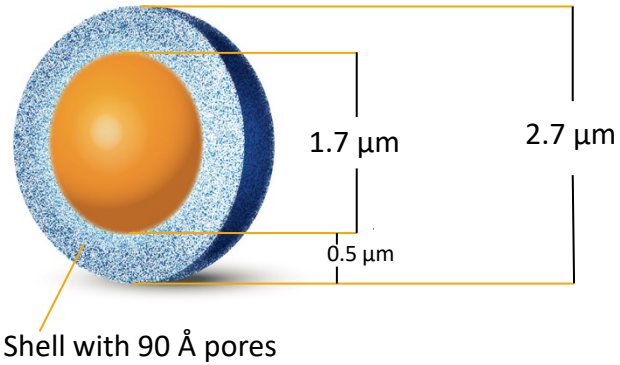
Improving Both Legacy and New HPLC Methods with Superficially Porous Particle Columns

Stephanie A. Schuster, Ph.D.
Advanced Materials Technology, Inc.
Wilmington, DE 19810
February 20, 2019

Outline

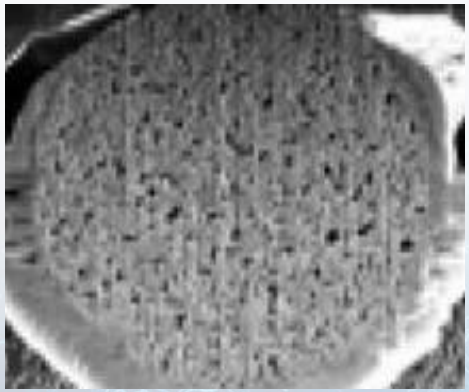
- Advantages of Superficially Porous Particle (SPP) over Fully Porous Particle (FPP) Columns
- Method Transfer/Translation Considerations
- USP Modernization Efforts
  - Allowable changes to USP Methods
  - Case Studies: FPP to SPP
- New Method Development
- Conclusion/Summary

Superficially Porous Particles

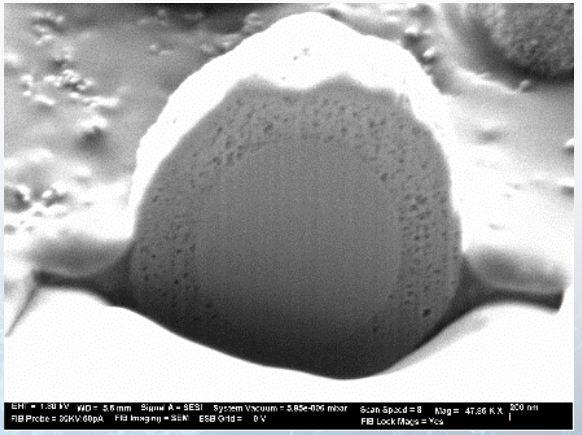


- Solid silica core
- Porous silica shell
- Shell thickness and pore size are tightly controlled

Fully Porous Particle (FPP)



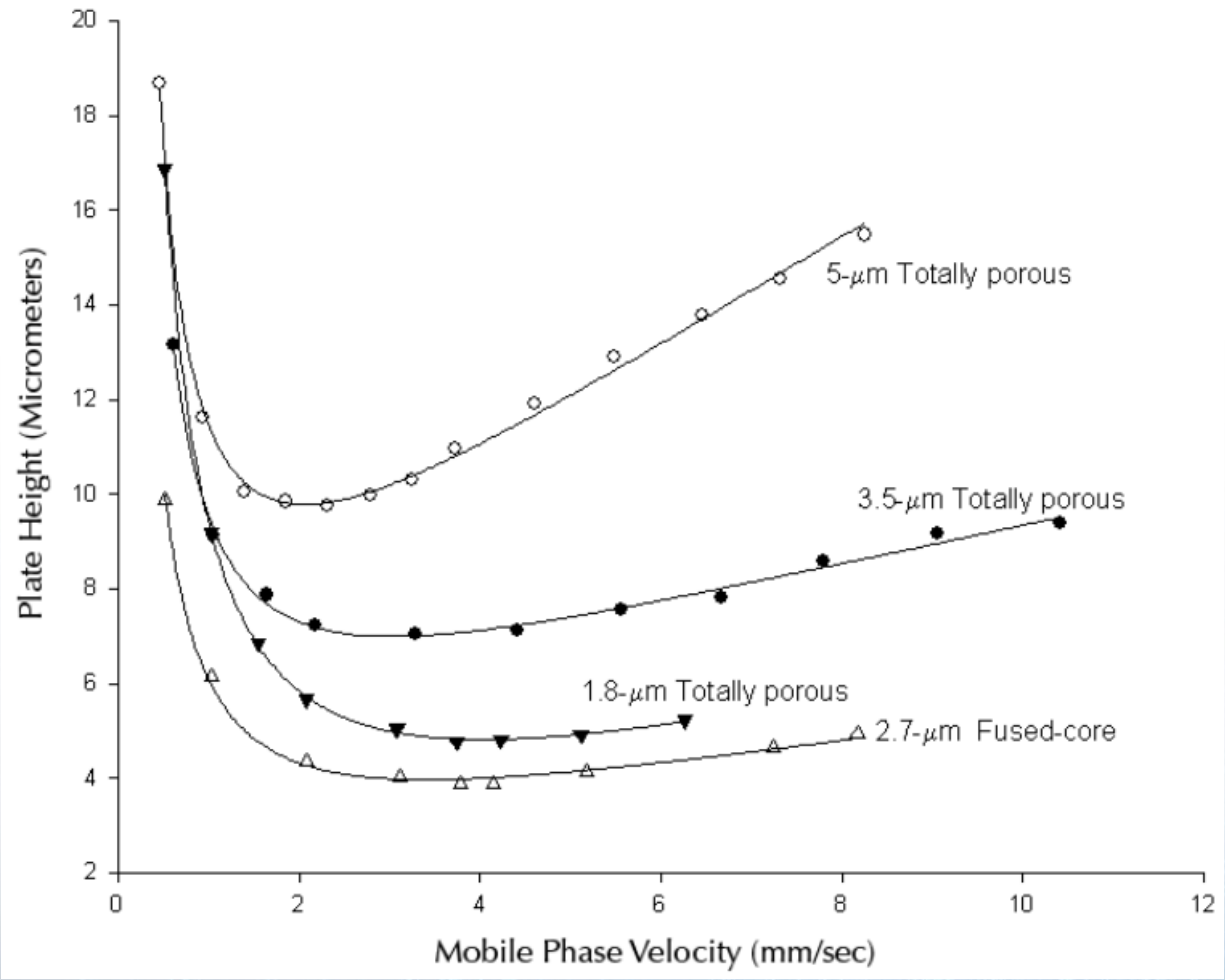
HALO 90 Å, 2.7 µm



Advantages of Superficially Porous Particle Columns vs. FPP Columns

- SPP columns provide faster separations and sharper, more efficient peaks compared to FPP columns without the need for higher pressure or extra operator training
- SPP columns have ½ to 1/3 the back pressure of sub-2-µm FPP columns
  - Enables use of legacy HPLC instruments with 400-600 bar limits
  - Permits faster flow rates/increased throughput
- SPP columns use 2-µm inlet frits that are less subject to pluggage than the 0.2 or 0.5-µm frits needed with sub-2-µm columns

van Deemter Comparisons: SPP vs. FPP



Effect of Particle Size and Type

Columns: 50 x 4.6 mm,
Non-core C18, 5 µm;
Non-core C18, 3.5 µm;
Non-core C18, 1.8 µm;
HALO C18, 2.7 µm

Solute: naphthalene
Mobile phase: 60% ACN/40% water
24 °C

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

van Deemter Equation

H = height equivalent to theoretical plate
A = eddy diffusion term **30 - 40% smaller**
B = longitudinal diffusion term **25 - 30% smaller**
C = resistance to mass transfer term
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

Method Transfer vs. Method Translation

- **Method transfer**
  - Move method from one column brand and particle size to another
  - Implement method in a different laboratory, different company or country
- **Method translation**
  - Move method from one particle size and/or column geometry to another with the same column brand
  - Move same column geometry and particle size to a different instrument brand (Δ delay volume, dispersion, etc.)
- **Typical Scenarios**
  - Transfer an HPLC method to a UHPLC column and system
    - e.g., FPP or SPP column to UHPLC SPP column
  - Translate a UHPLC method to an HPLC column and system
    - e.g., from R&D to QC
  - Direct implementation of an existing method
    - Only extracolumn volume, dispersion, delay volume and system max. pressure considerations

Questions to Ask
Method Transfer and Translation

- What is the goal of transferring or translating the method?
  - Increased speed, improved resolution, increased sensitivity
- Can the new instrument handle the pressure that the proposed new column will generate?
- Can you meet or exceed the original column's efficiency using the new instrument?
- Does the new instrument have low enough extracolumn dispersion to allow the required efficiency or can the system be optimized to minimize extracolumn dispersion?
- Can the new instrument deliver the correct column temperature to match that of the original instrument?
  - How do the setpoint temperatures compare vs. actual temperatures for the instrument(s)?

Important Method and Instrumental Parameters to Consider for Method Transfer and Translation

Isocratic Methods

- **Maximum Instrument Pressure**
  - Practical maximum operating pressure usually 75–80% of instrument maximum
- **Extracolumn volume**
  - Tubing
    - ID and Length
    - Homogeneous or heterogeneous IDs in sample flow path
  - Flow cell volume and path length
  - Injection volume
  - Injector type
    - Flow through needle vs. loop fill
- **Extracolumn dispersion**
  - Function of flow rate
  - Data Rate and Response Time
  - Instrument type
- **Column Heater Type and calibration**
  - Forced air, block/contact heater, heat tape wrap, etc.
  - Actual temperature vs. set point
- **Frictional Heating**
  - Effects on efficiency, peak width and selectivity

Gradient Methods

- **Same as for isocratic methods, except:**
  - Less impact on "efficiency" and peak capacity from precolumn tubing dispersion
- **Delay volume (aka dwell volume)**
  - High pressure mixing
    - Mixer volume
  - Low pressure mixing
  - Often a function of backpressure
    - $\propto$ column length
    - $\propto$ flow rate
    - $\propto$ 1/particle size, d_p

Instrument Optimization

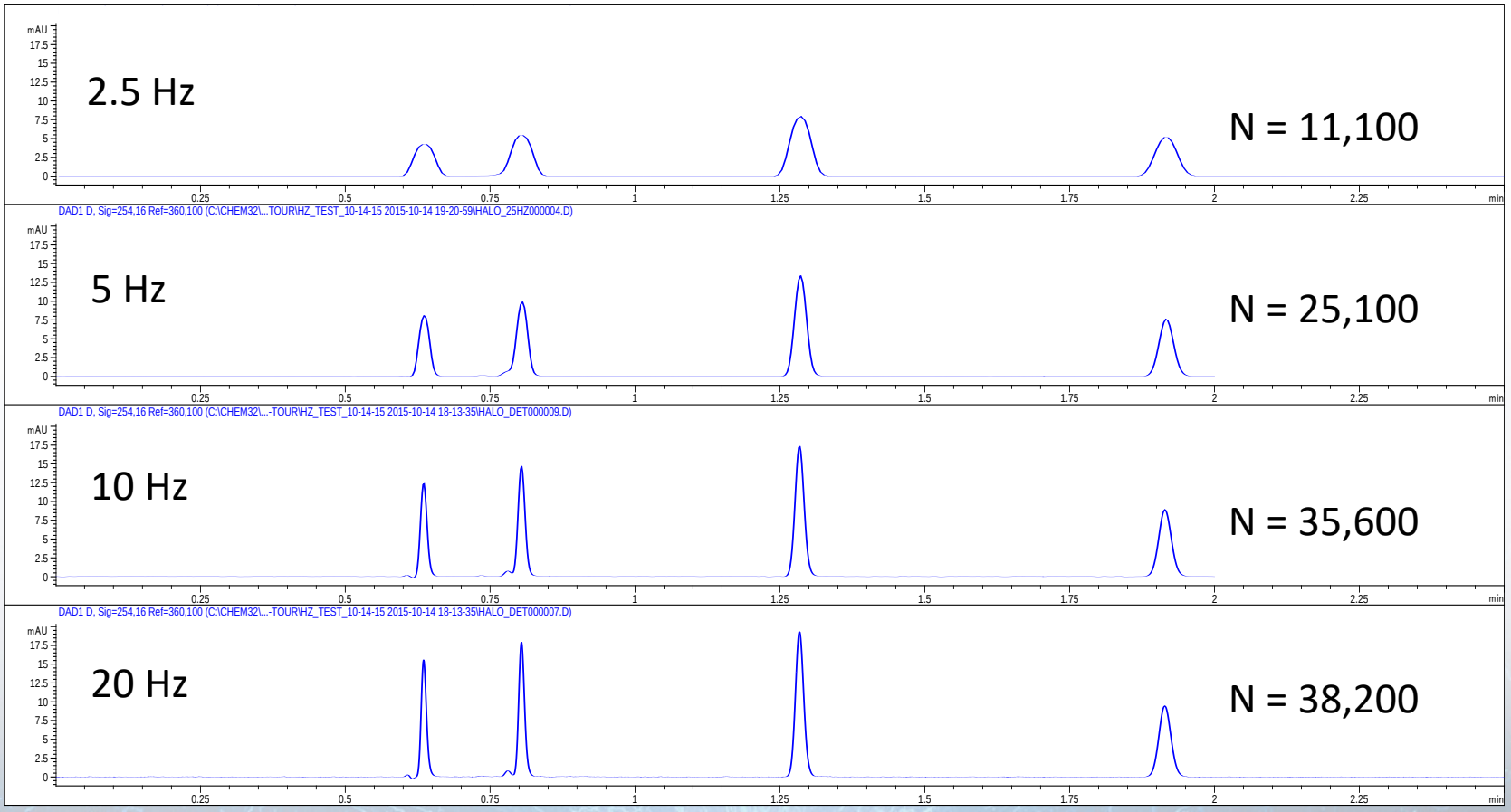
- Connections – minimize volume
  - Connection from column to flow cell is more important than from autosampler to column
  - Use a smaller volume pre-column heat exchanger (1.6 µL, if necessary)
- Data acquisition – appropriate to peak width
  - Increase the detector time constant to monitor fast-eluting peaks
  - Data rate should be at least 20 Hz for best results

Effect of Data Acquisition Rate on Efficiency

HALO C18
4.6 x 150 mm 2.7 µm
1.8 mL/min

70/30 ACN/water
30 °C

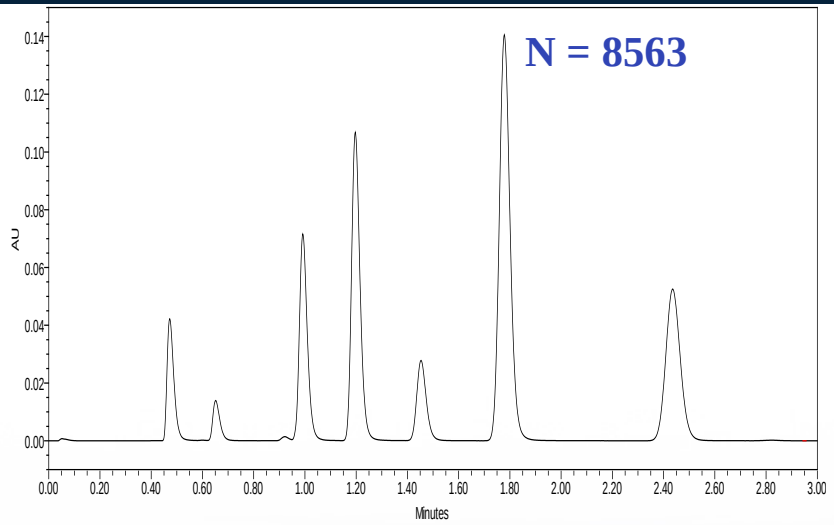
Sample contains uracil, phenol, 1-chloro-4-nitrobenzene, and naphthalene



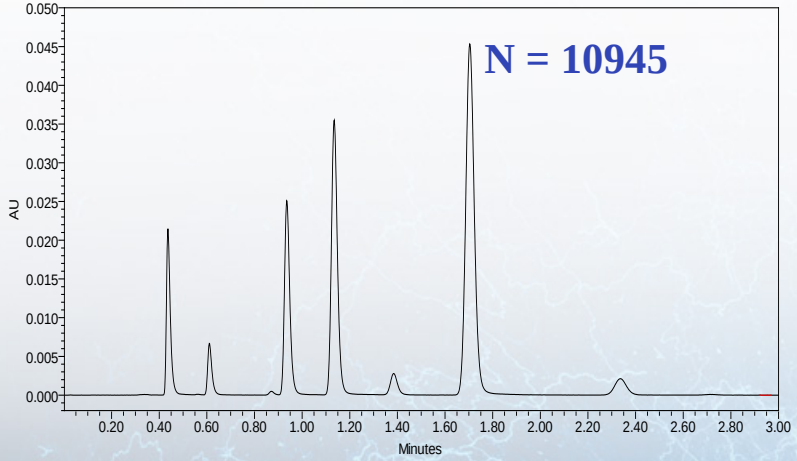
Instrument Optimization

- Type of pumps and mixing
  - Quaternary vs. Binary
  - Convergence block vs. Mixer
- Volume of flow cell
  - Use a smaller volume (250 nL-5 µL)
- Injection type
  - Needle in flow (adds volume, but improved carryover) vs. fixed loop (minimizes volume, but carryover could be problematic, depending on the compound)
- Injection volume
  - Use smallest practical injection volume keeping in mind the precision of the autosampler
- Sample Solvent
  - Keep volume small as practical if stronger than (starting) mobile phase, and matched or weaker than mobile phase for improved sensitivity/LOD/LOQ
  - Use “POISE” with aqueous or weak “chaser”

Effect of Reducing Flow Cell and Tubing Volume



Tubing: Standard 0.009” ID tubing standard
Flow Cell: 10 µL standard



Tubing pre-column 0.009” ID standard
Tubing post-column 100 µm x 300 mm
Flow Cell: 2.6 µL

HPLC (standard configuration)
Isocratic Separations with HALO C18,
4.6 x 50 mm, 2.7 µm

Mobile phase:	50:50 ACN/water
Flow rate:	1.0 mL/min.
Column temp.:	30°C
Injection vol.:	1 µL
Data rate:	10 Hz
Time Constant:	0.1 sec.
Response Time:	0.227 sec.

N = USP Plates

30% average increase in plates is observed by reducing the excess volume in the system!

Compounds in elution order: uracil, benzyl alcohol, benzonitrile, nitrobenzene, anisole, 1-chloro-4-nitrobenzene, and toluene

USP Modernization Efforts

- USP is in the process of modernizing existing monographs to use current U/HPLC columns
- Combined effort between USP and several partners in industry
- Harmonization among USP, EP, and JP

Review of Allowable Changes to Methods USP <621> Guidelines

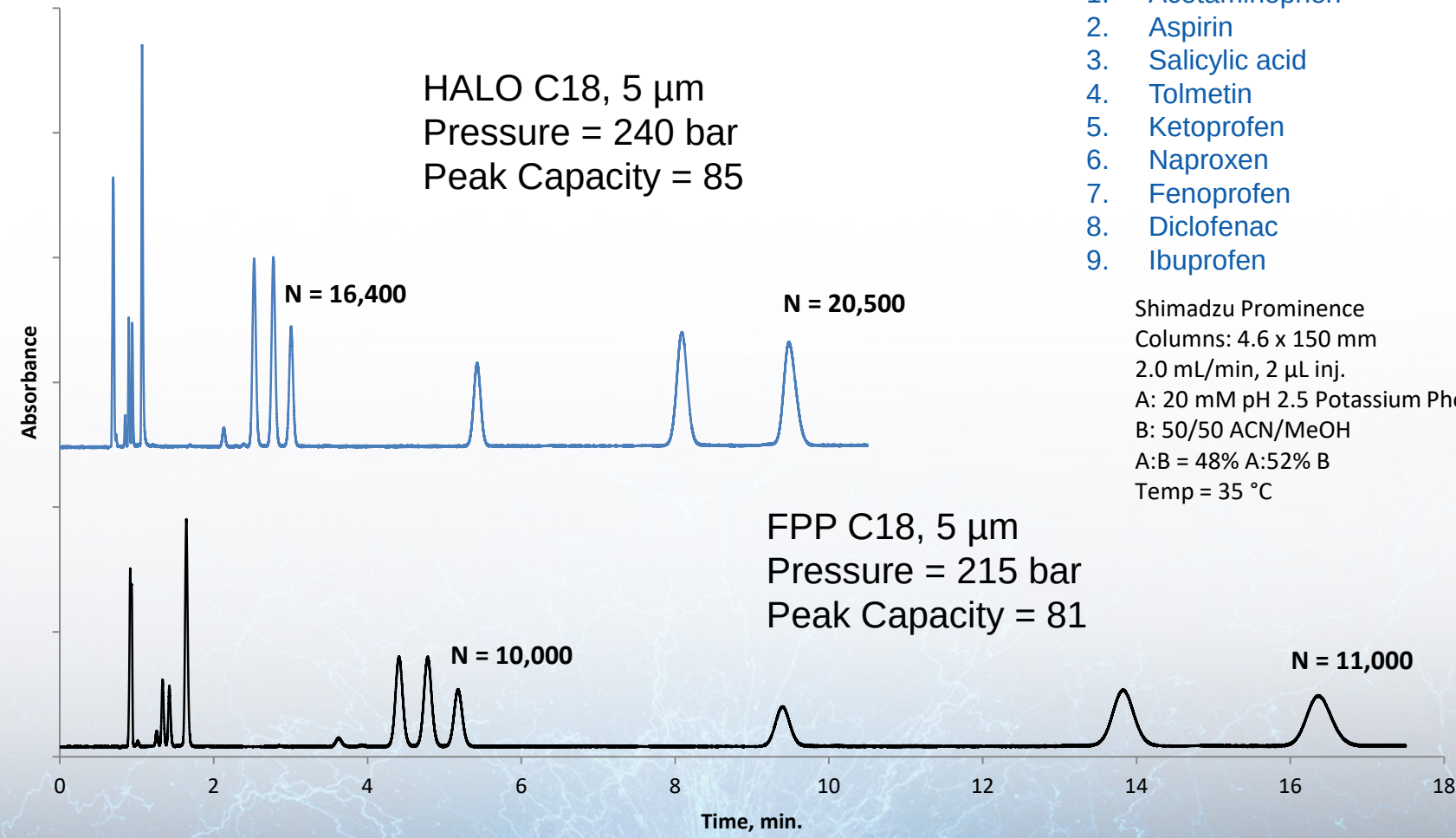
- Changes to USP Methods are only allowed for isocratic separations*
- Particle size and/or the length of the column
  - Ratio of the column length (L) to the particle size (dp) is the same or in range between -25% to +50% of the prescribed L/dp ratio.
  - Alternatively (as for the application of particle-size adjustment to superficially porous particles), other combinations of L and dp can be used provided that the number of theoretical plates (N) is within -25% to +50%, relative to the prescribed column.
- Bonded phase may not be changed
- Temperature may be adjusted ± 10 °C
- Flow rate may be adjusted ± 50%

*Harmonization efforts between USP, the European Pharmacopeia (Phr. Eur) and the Japanese Pharmacopeia (JP) are working to also allow changes to gradient methods

CASE STUDIES: EXAMPLES OF LEGACY FPP SEPARATIONS TRANSFERRED TO SPP COLUMNS

Case Study: Isocratic NSAIDs Separation from 5 µm FPP to 5 µm SPP

> 60% improved efficiency and about one-half separation time using Fused-Core particles.



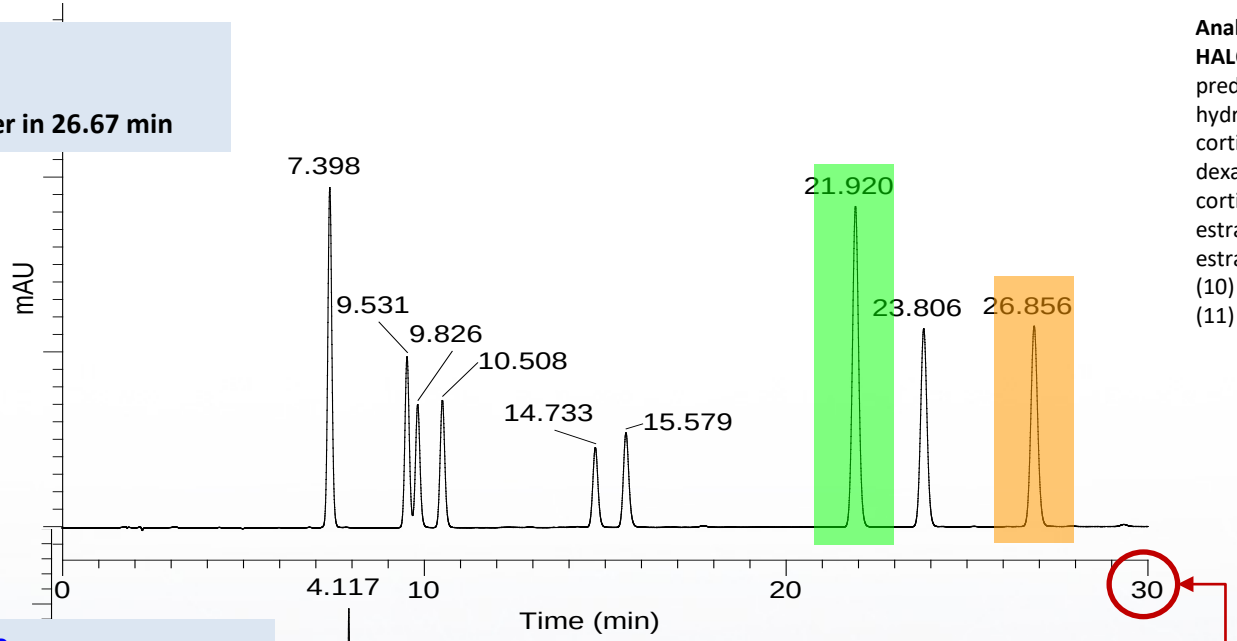
Peak Identities

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

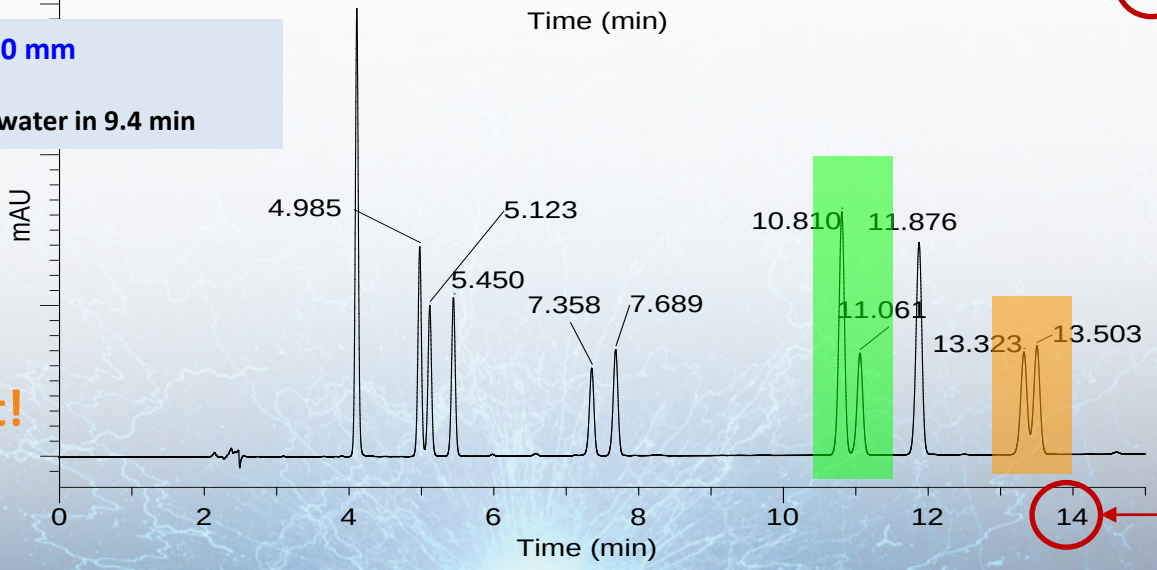
Case Study: Gradient Steroids Separation from 5 µm FPP to 5 µm SPP

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

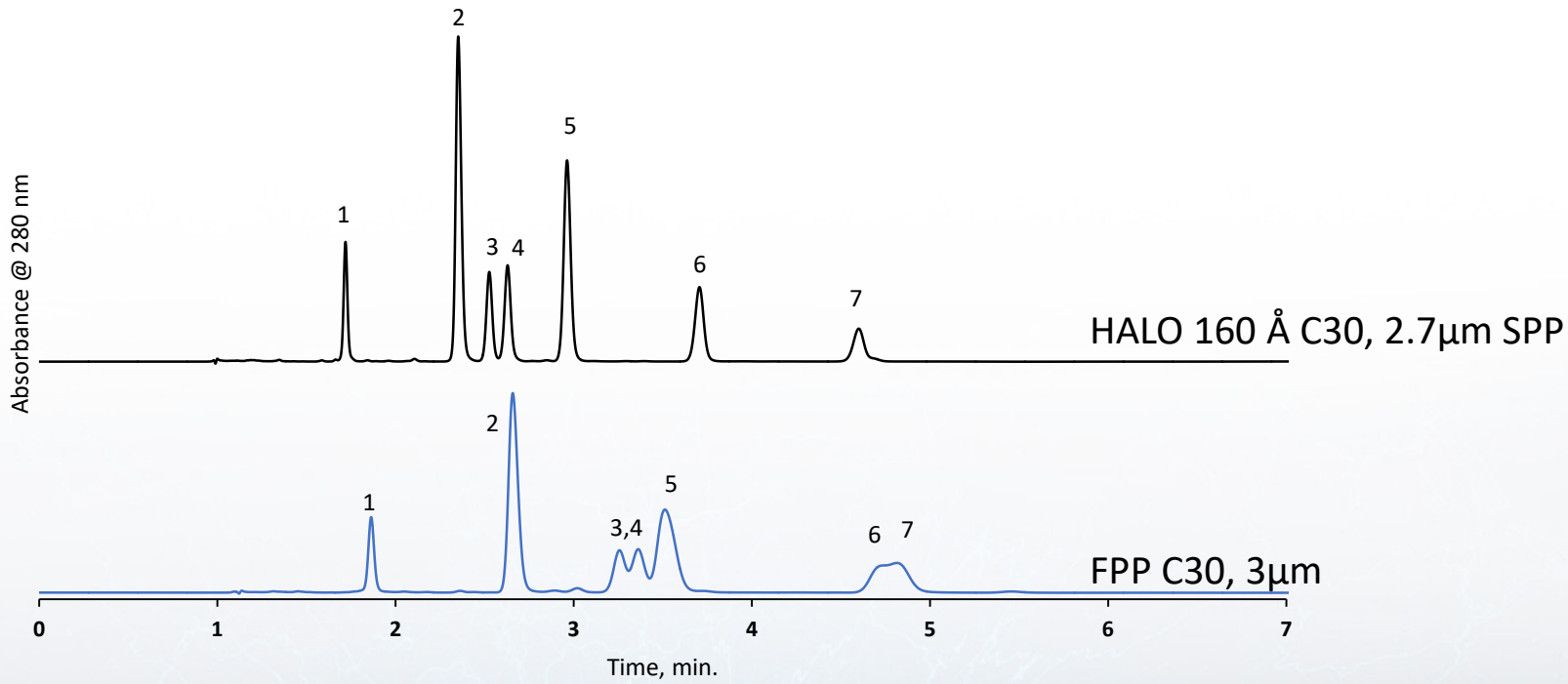


SPP method

- 2x faster!
- 3x less solvent!

Case Study: Isocratic Fat Soluble Vitamins Separation from 3 µm FPP to 2.7 µm SPP

Sharper peaks and increased resolution with the C30 Fused-Core column!

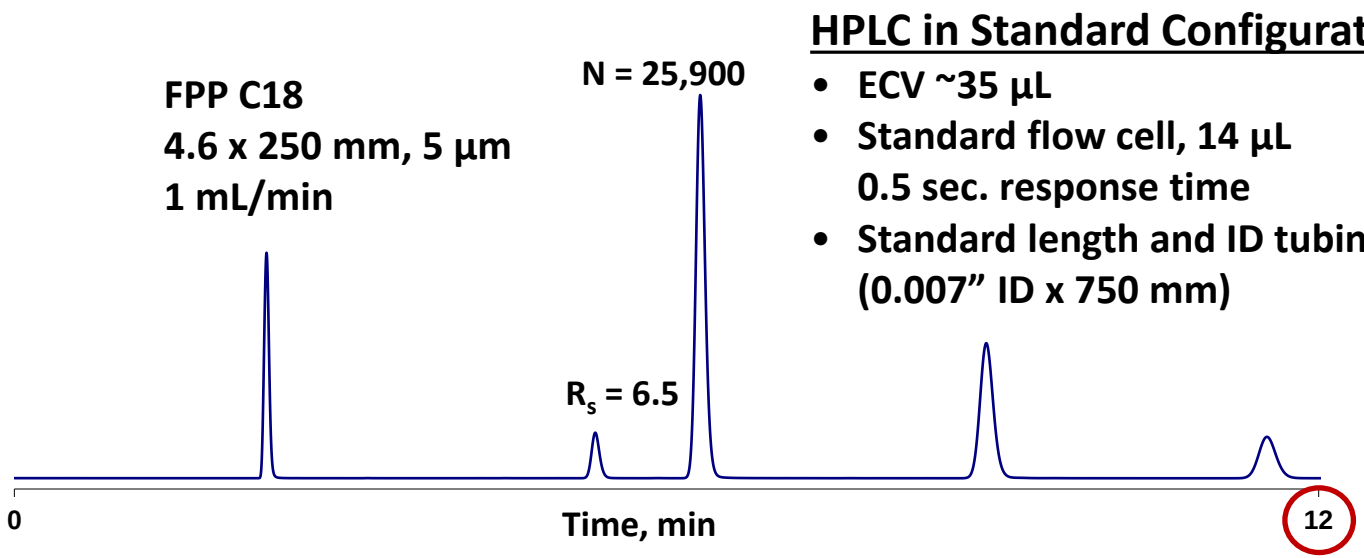


Isocratic: 100% Methanol
Wavelength: 280nm
Injection: 2 µl
Temperature: 30°C
Flow Rate: 1.5 mL/min.
Columns: 4.6 x 150 mm

PEAK IDENTITIES:

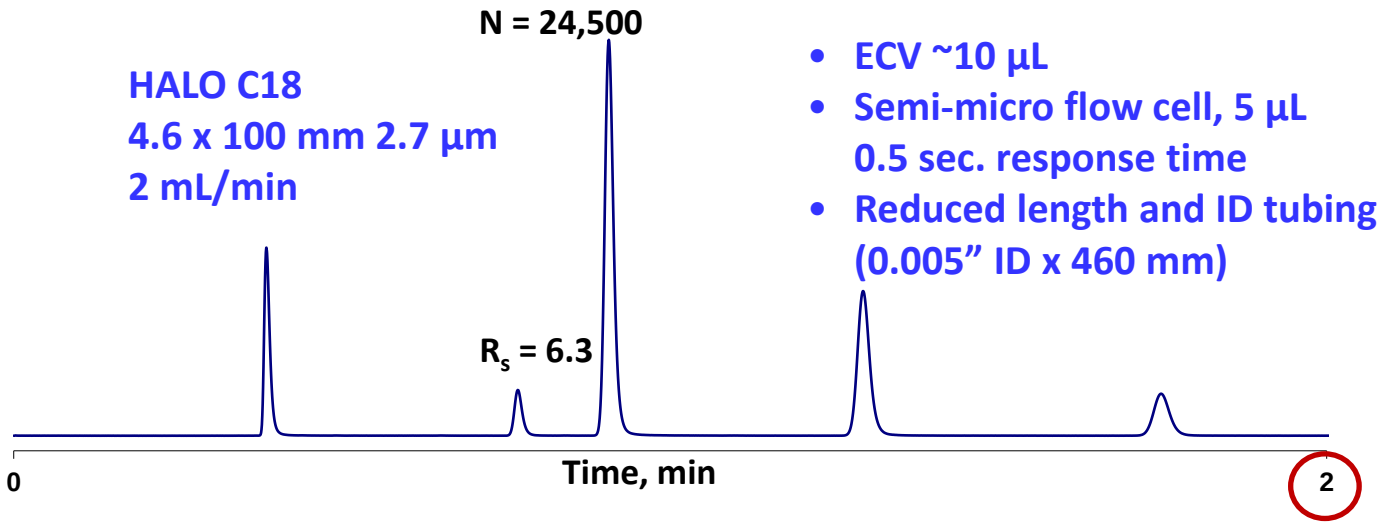
1. Retinyl acetate (A)
2. Delta tocopherol (E)
3. Ergocalciferol (D2)
4. Cholecalciferol (D3)
5. Alpha tocopherol (E)
6. DL-alpha-tocopherol acetate (E)
7. 2,3-*trans*-phylloquinone (K)

Optimized Fused-Core Separation Yields up to 6-fold Increase in Throughput



HPLC in Standard Configuration

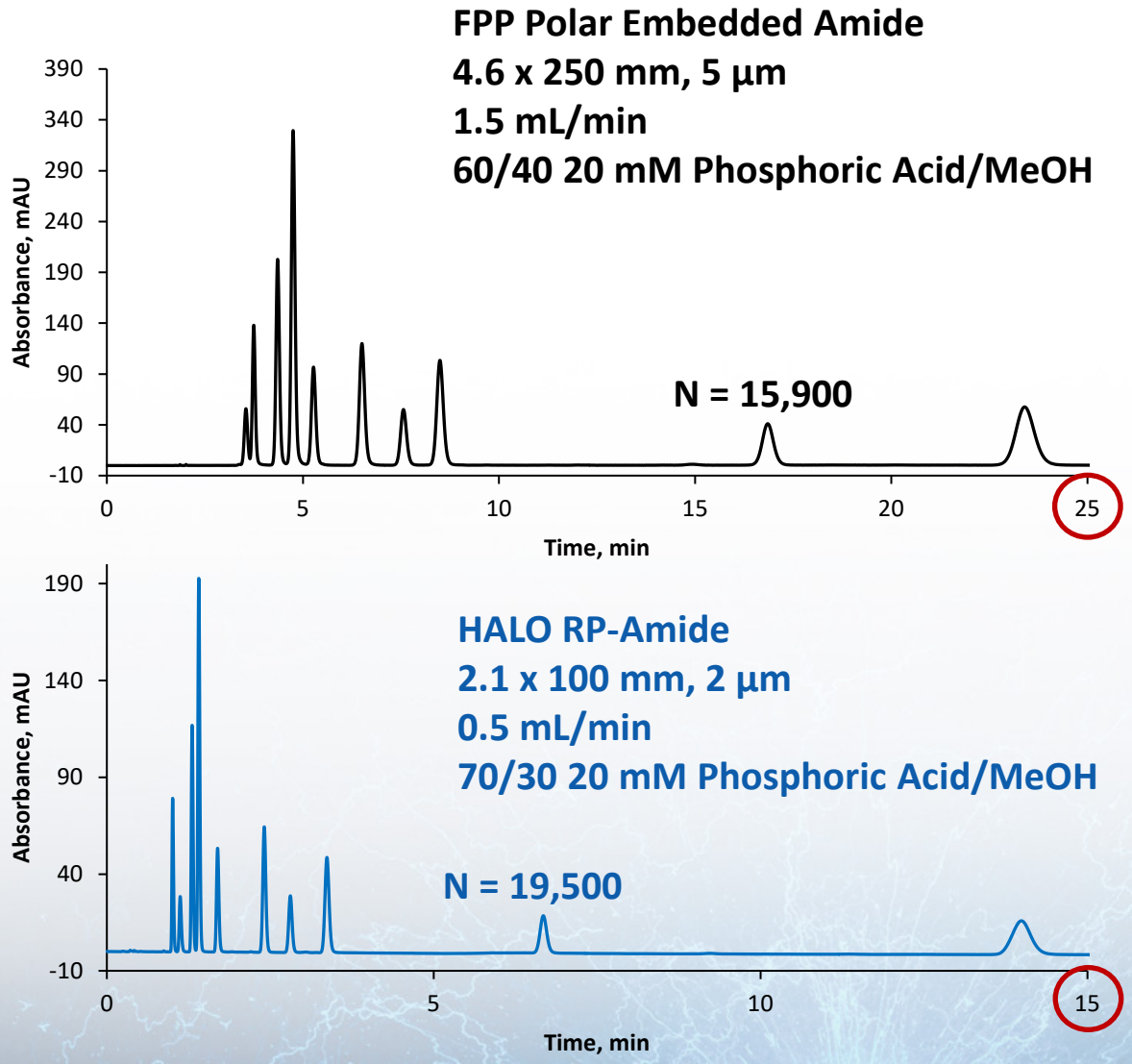
- ECV ~35 µL
- Standard flow cell, 14 µL
  0.5 sec. response time
- Standard length and ID tubing
  (0.007" ID x 750 mm)



HPLC in Ultra-Low ECV Configuration

- ECV ~10 µL
- Semi-micro flow cell, 5 µL
  0.5 sec. response time
- Reduced length and ID tubing
  (0.005" ID x 460 mm)

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

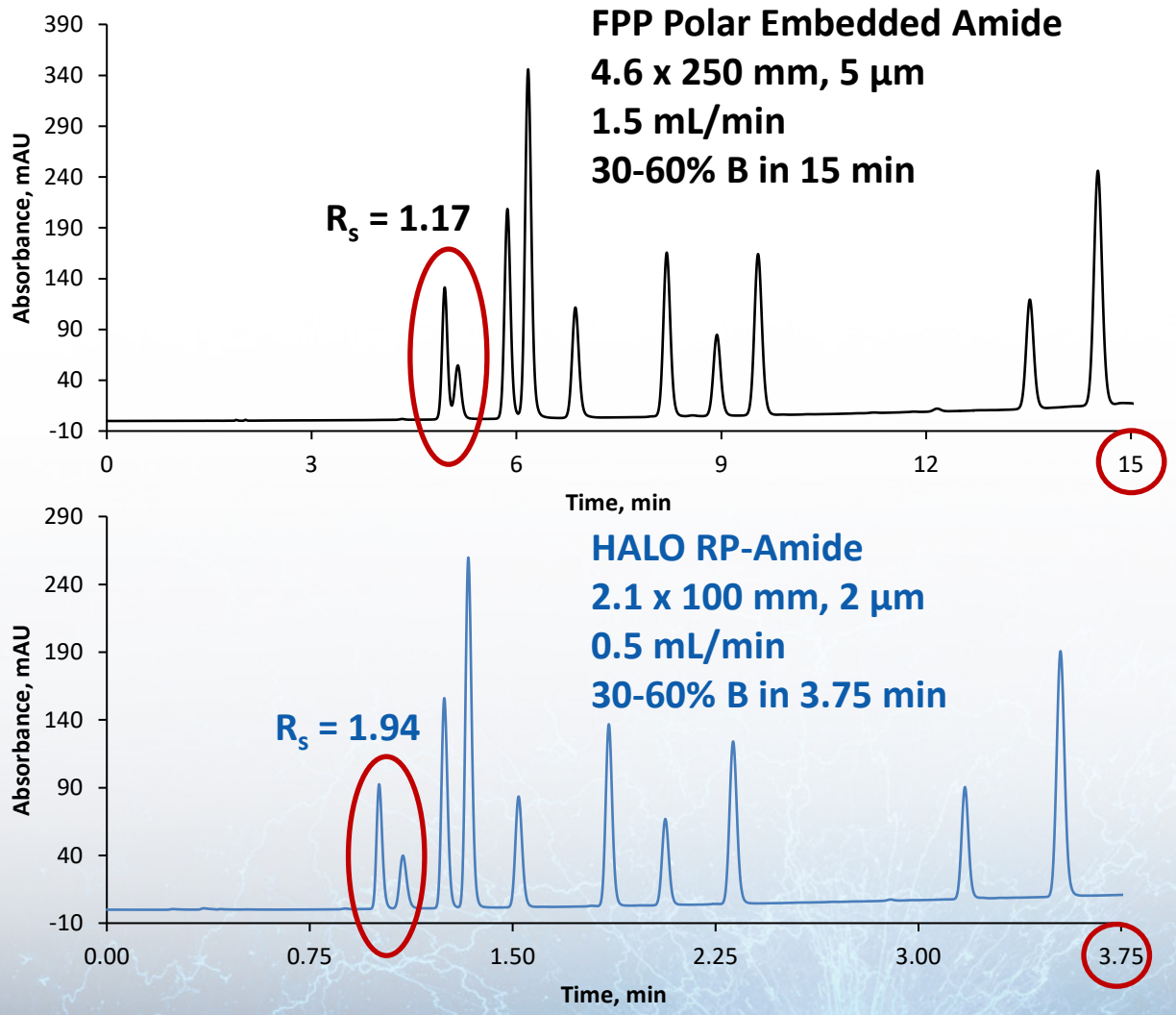


SPP method

- 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



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

NEW HPLC METHOD DEVELOPMENT WITH SPP COLUMNS

New Method Development

- Depends on complexity of sample
- Gradient approach is best when you don't know how many components etc.
- Single screening gradient on one phase can give you a quick look at sample
- Use of multiple phases and conditions can help you select best combo(s) to move forward

HALO Phases for Reversed-Phase HPLC and UHPLC

Packing Description	Bonded Phase	Types of Interactions
C18	C18 (dimethyloctadecylsilane)	• Hydrophobic
C8	C8 (dimethyloctylsilane)	• Hydrophobic
Phenyl-Hexyl	Phenyl-Hexyl (dimethylphenylhexylsilane)	• Hydrophobic • π - π
ES-CN	ES-CN (di-isopropylcyanopropylsilane)	• Hydrophobic • Dipole-dipole
PFP	PFP (pentafluorophenylpropylsilane)	• Hydrophobic • π - π • Dipole-dipole • Hydrogen bonding
RP-Amide	C16 Amide	• Hydrophobic
AQ-C18	proprietary	• Hydrophobic
Biphenyl	Biphenyl (dimethylbiphenylsilane)	• Hydrophobic • π – π
C30	C30 (Triacontyldimethylsilane)	• Hydrophobic

Contrived, Complex, Blindly-prepared Mixture 13-20 compounds: Acids, Bases and Neutrals

Strategy

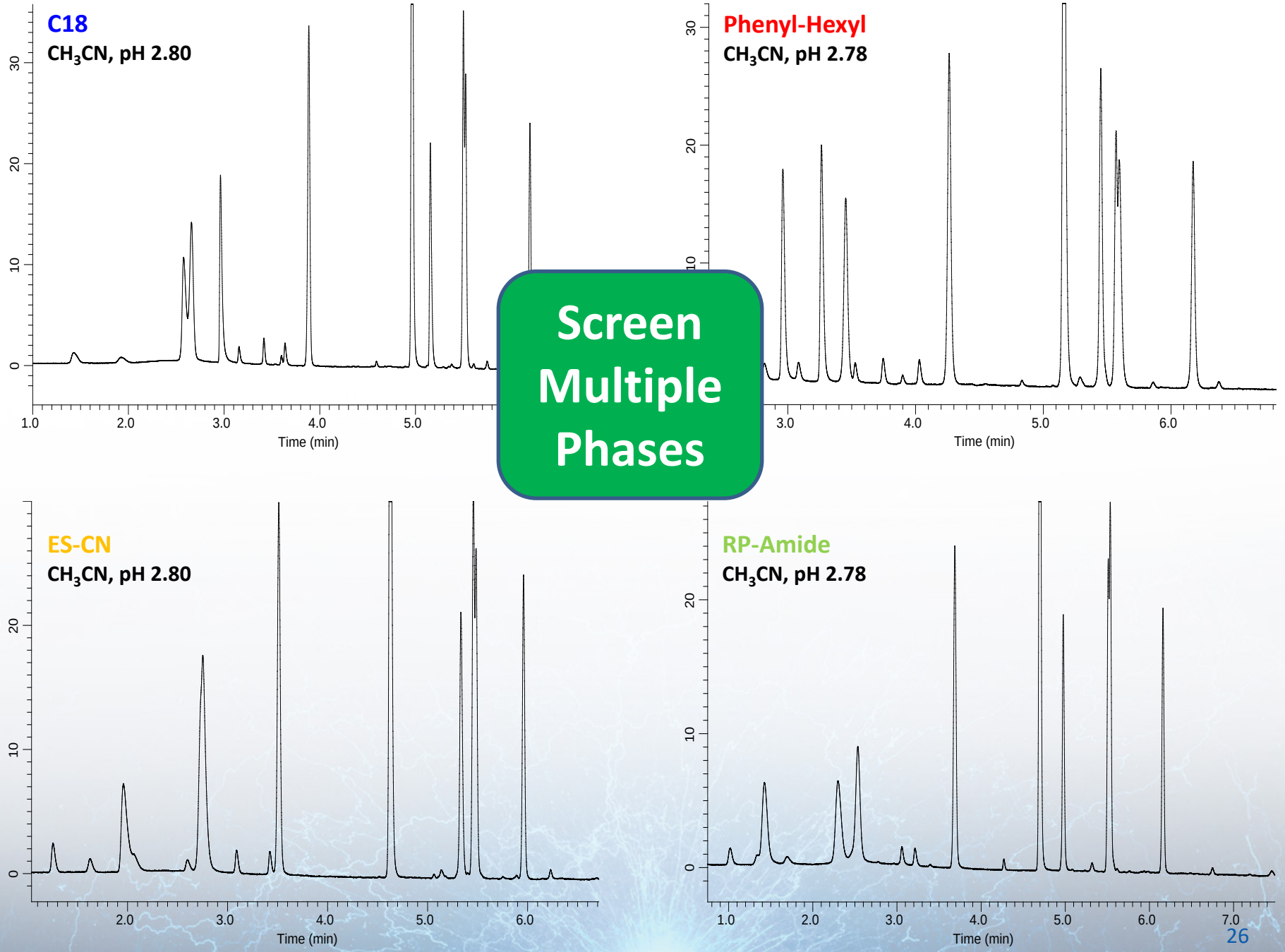
- Screened four HALO phases
  - C18
  - Phenyl-Hexyl
  - ES-CN
  - RP-Amide
- Different organic modifiers
  - CH_3CN, CH_3OH
- Different pHs with LC-MS compatible buffers
  - pH 2.8, 3.8 (NH_4COOH)
  - 4.8 and 5.8 (NH_4OAc)
- Identify one or more possible combinations for further improvement/optimization

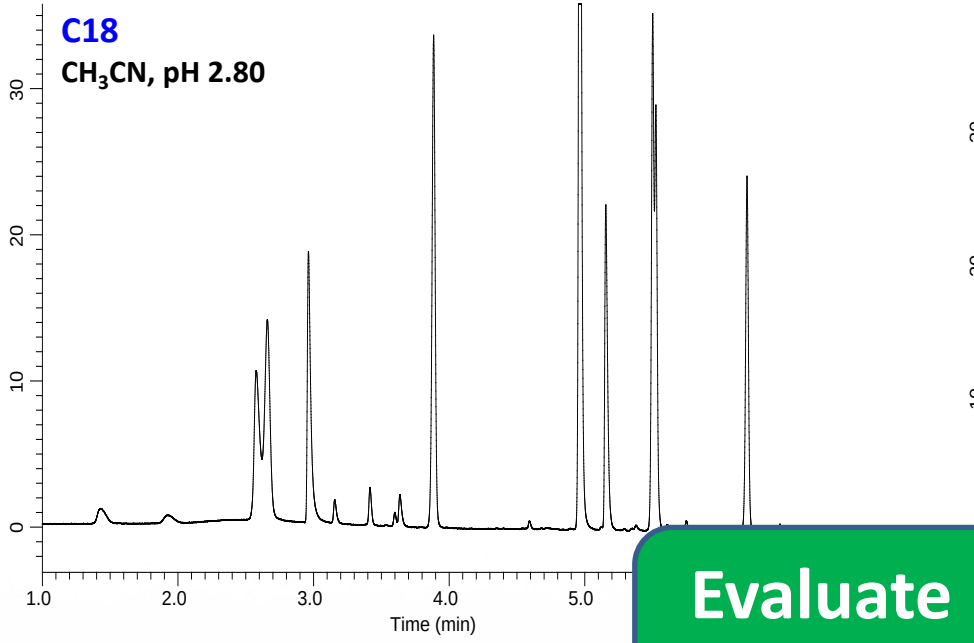
Columns: 3 x 50 mm, 2.7 µm
Flow Rate: 0.6 mL/min
Temperature: 30 °C
Gradient: 2–90% organic/buffer
Gradient Time: 10 min
Initial Hold: 1 min

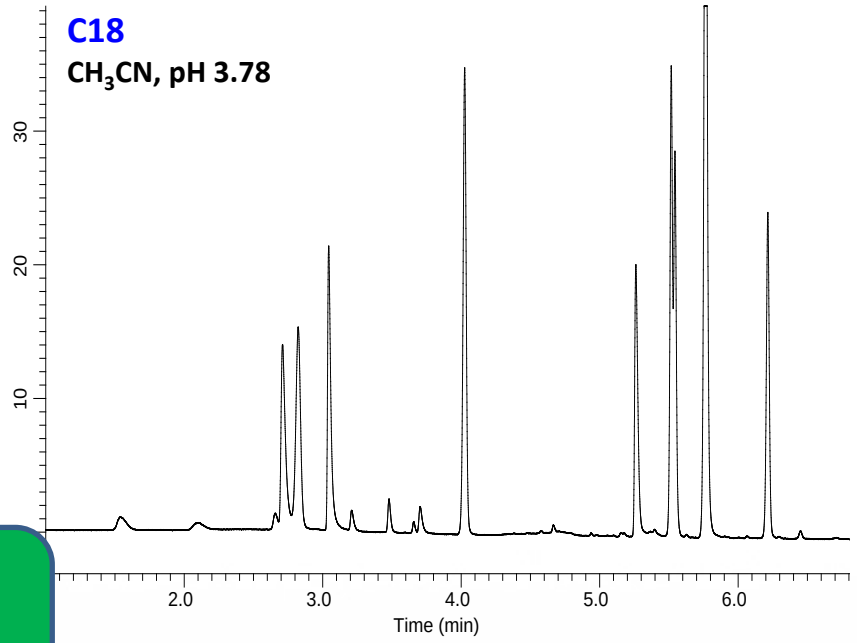
Agilent 1200 binary 600 bar system

- Delay volume: 0.74 mL (from DryLab runs)
- Hold 1 min at 2% B initial x 0.6 mL/min = 0.6 mL
- Effective delay volume: 1.34 mL

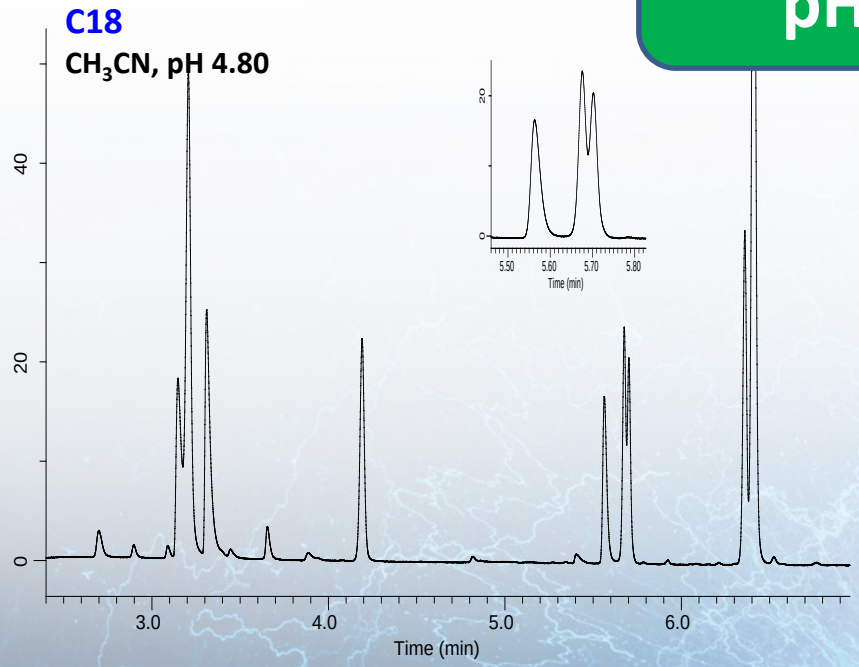
Time	%B				
0	2		Phases	4	
1	2		Modifiers	2	
11	90		pHs	4	
12	90		# injections	2	
12.5	2		Total Runs	64	
5	Post Time				
17.5	min		Total Time	1120	min
			Total hrs	18.7	hr

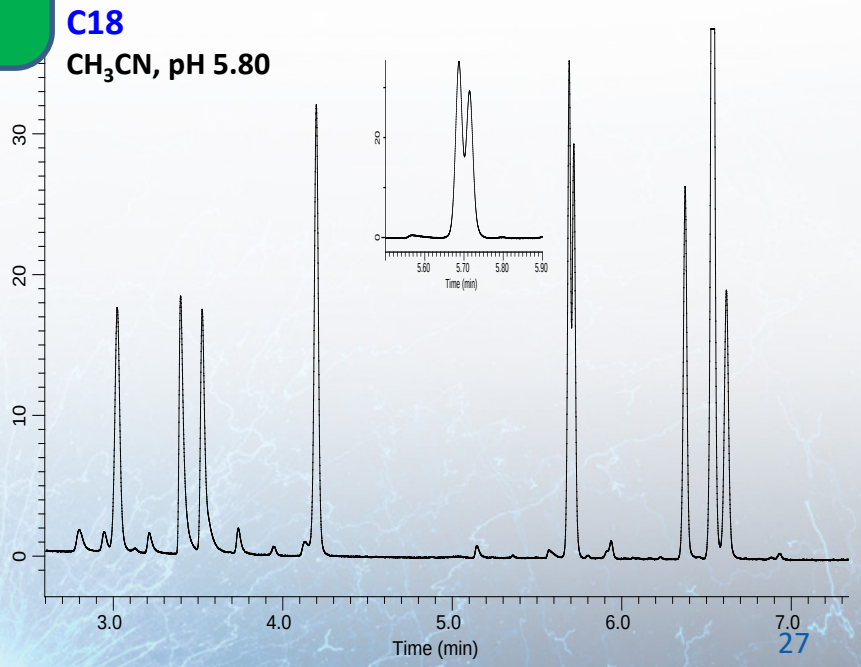

C18
CH3CN, pH 2.80
Phenyl-Hexyl
CH3CN, pH 2.78
Screen Multiple Phases
ES-CN
CH3CN, pH 2.80
RP-Amide
CH3CN, pH 2.78
Time (min)

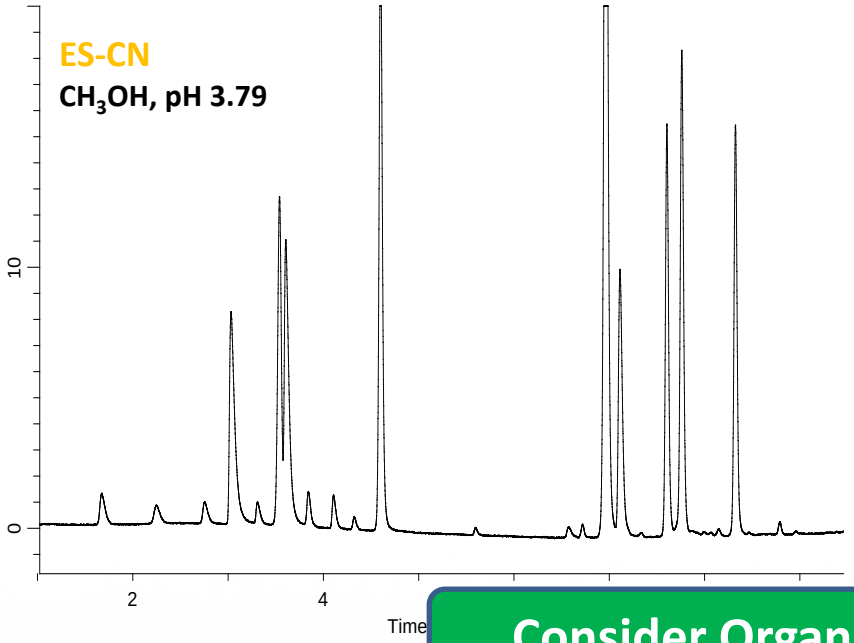

C18
CH3CN, pH 2.80
Time (min)

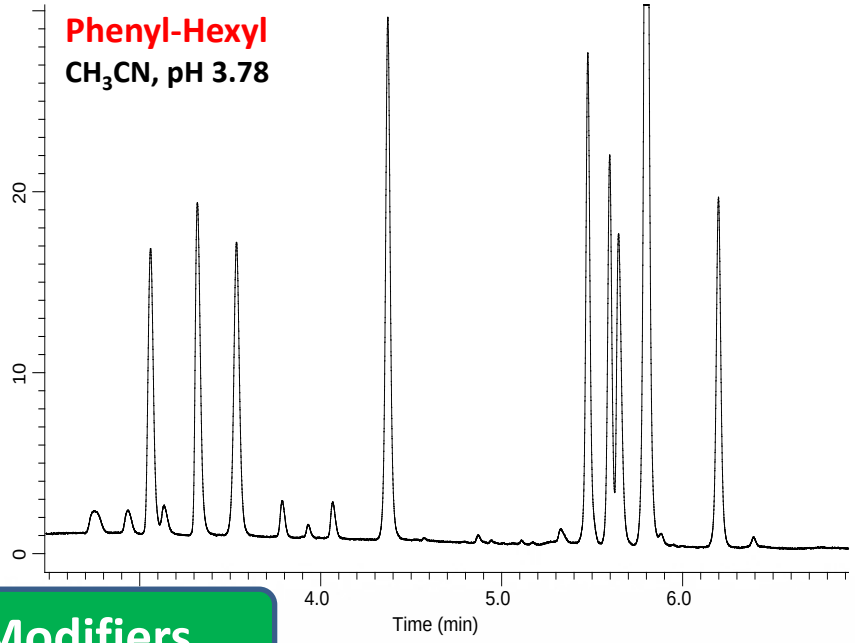

C18
CH3CN, pH 3.78
Time (min)

Evaluate pH

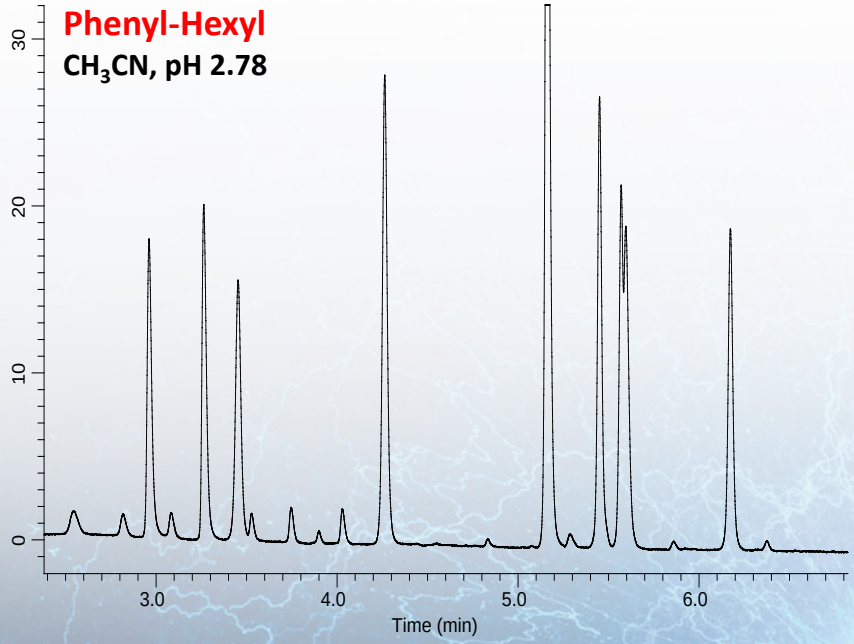

C18
CH3CN, pH 4.80
Time (min)

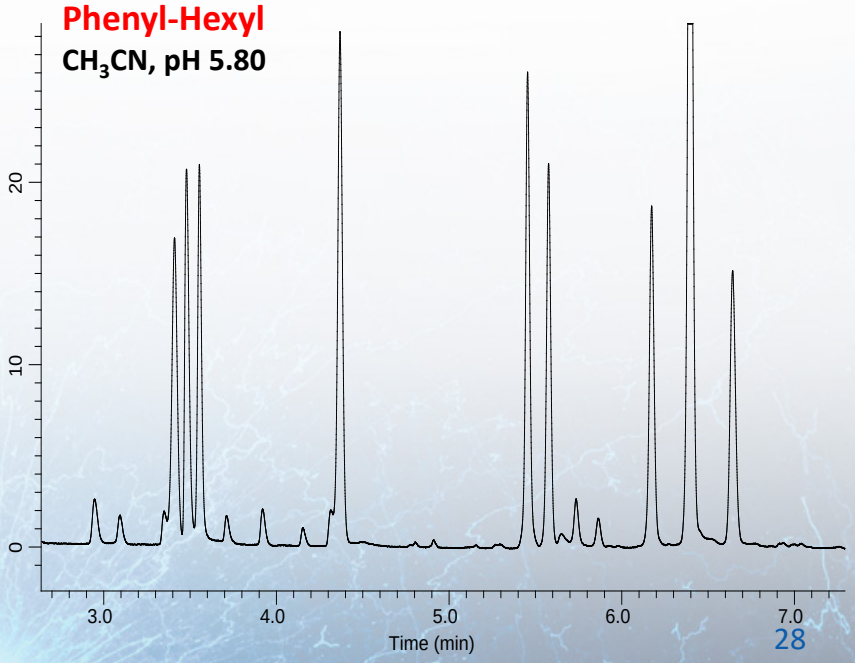

C18
CH3CN, pH 5.80
Time (min)





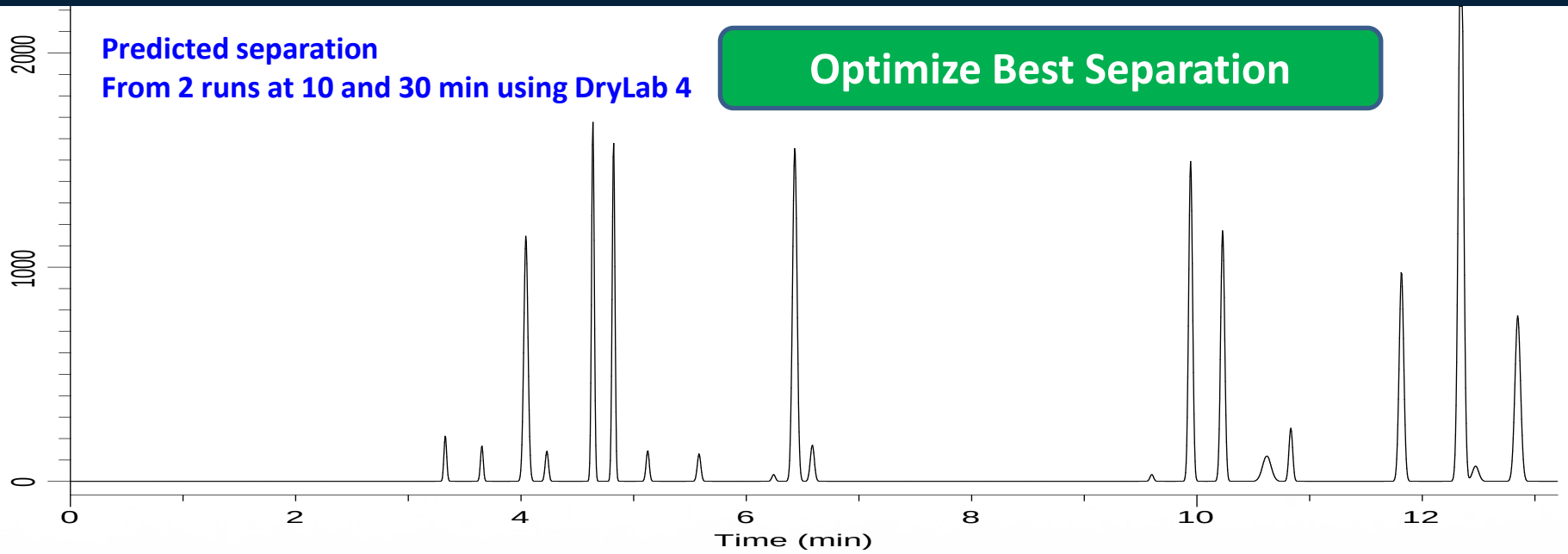
Consider Organic Modifiers



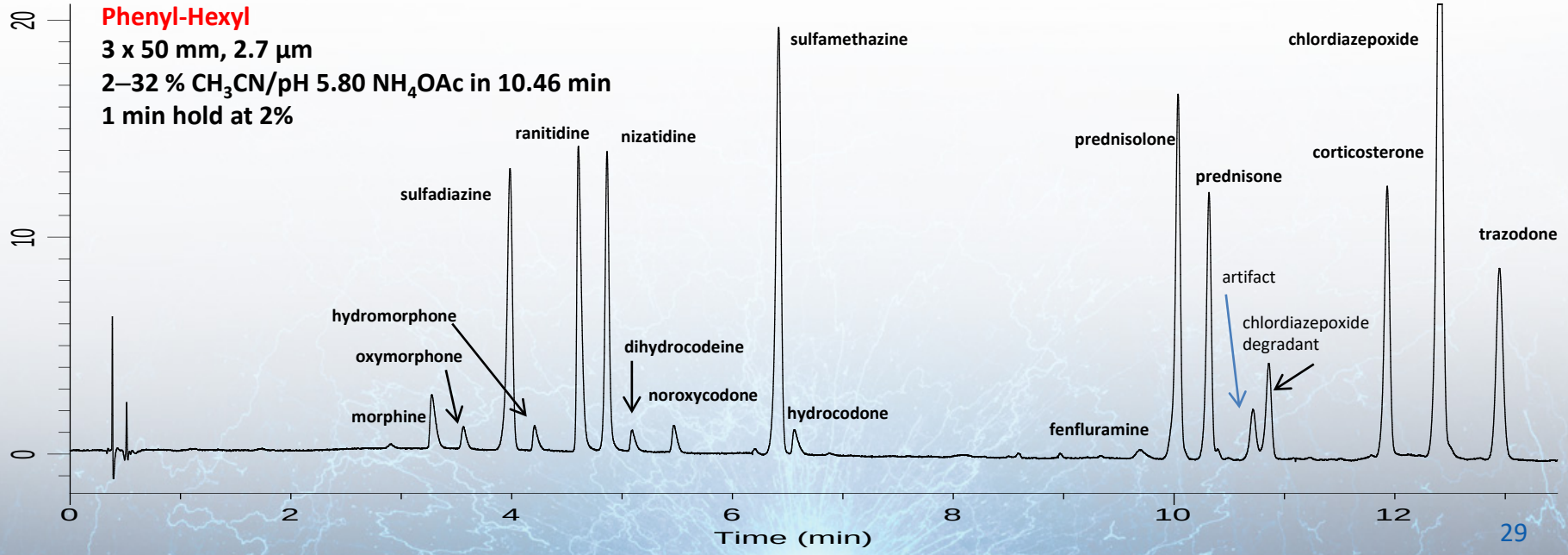


Predicted separation
From 2 runs at 10 and 30 min using DryLab 4

Optimize Best Separation



Phenyl-Hexyl
3 x 50 mm, 2.7 µm
2–32 % CH_3CN/pH 5.80 NH_4OAc in 10.46 min
1 min hold at 2%

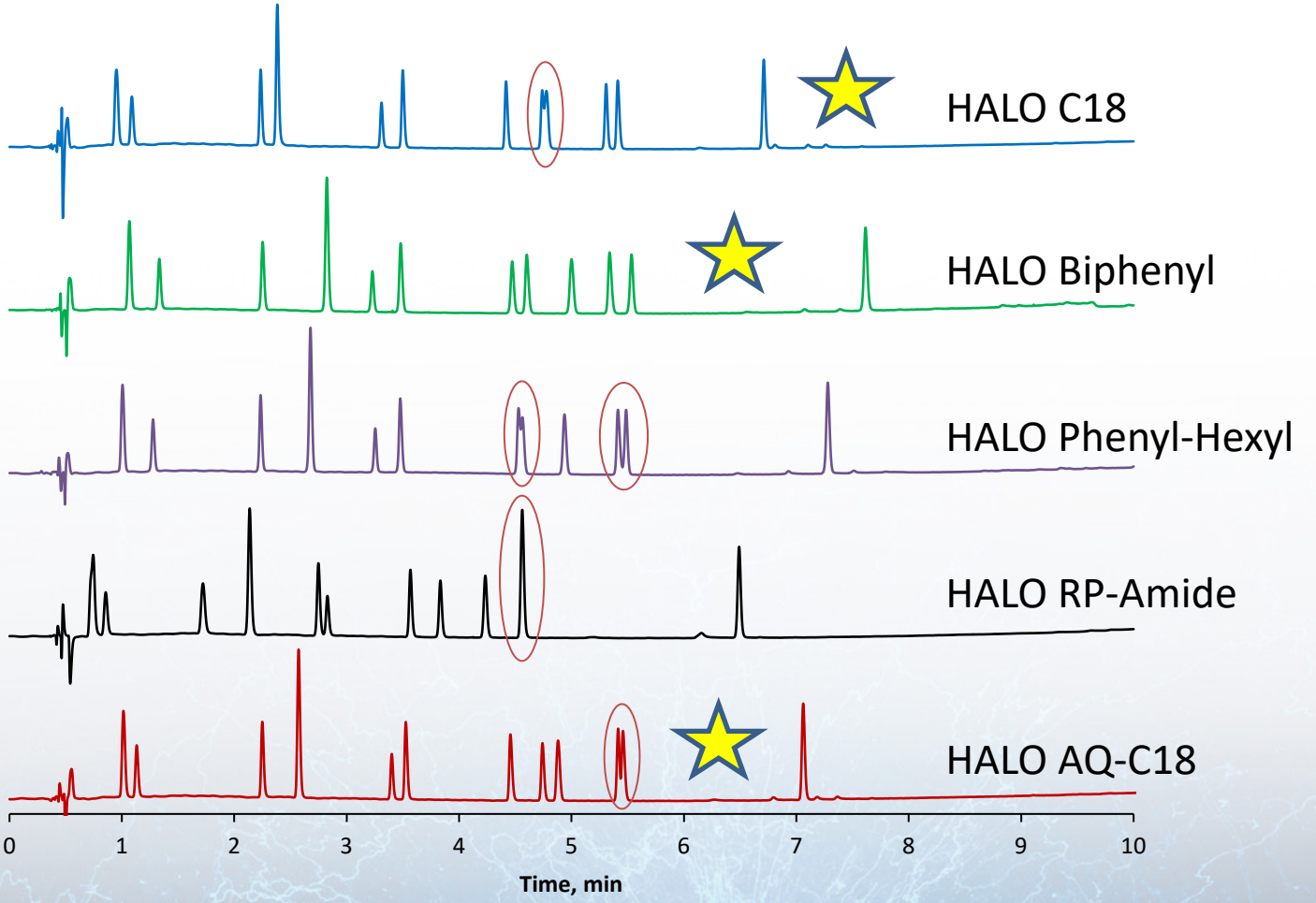


Application Example

- Evaluation of 5 HALO phases with 12 β-blockers
- 2.1 x 100 mm columns
- Gradient with ACN/0.1% TFA

Screening β-blockers with 5 different stationary phases

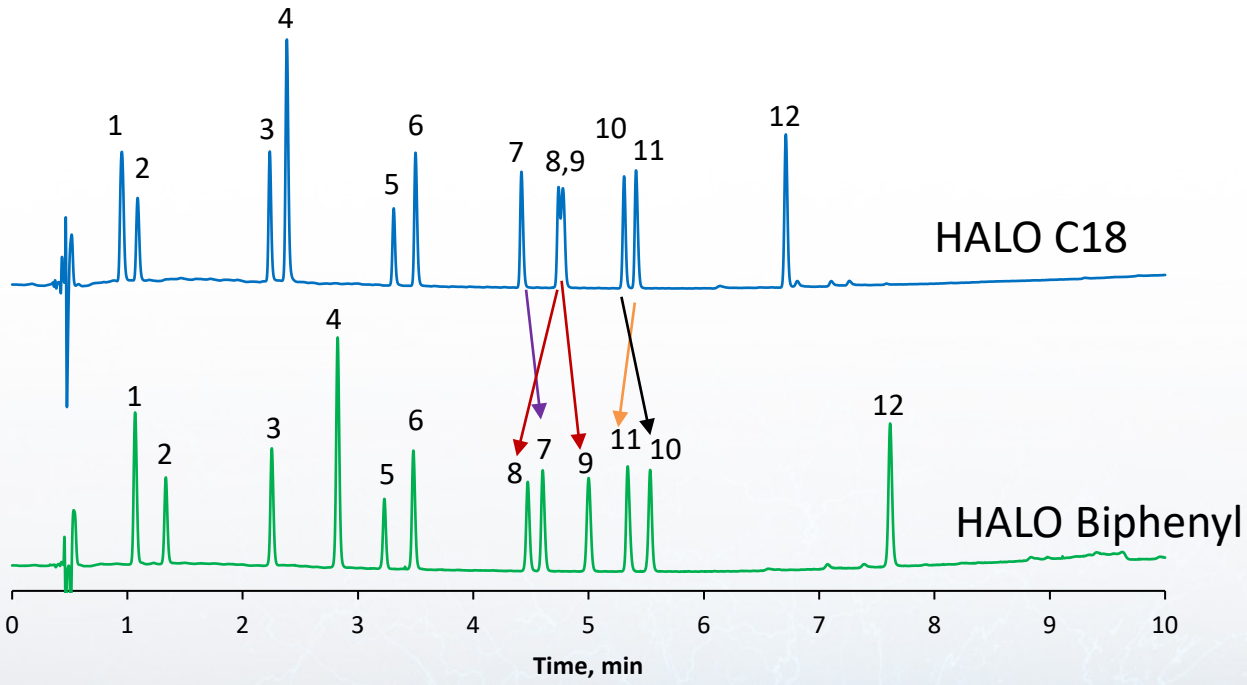
2.7 µm HALO, phases as indicated, 2.1 x 100 mm SPP columns
1 µL , 0.50 mL/min, 35 °C, 220 nm
Gradient from 10-50% CH_3CN/water/0.1% TFA in 10 min



Sample contains atenolol, sotalol, nadolol, pindolol, acebutolol, metoprolol, oxprenolol, labetalol, bisoprolol, propranolol, alprenolol, carvedilol

Screening β-blockers: C18 compared to Biphenyl

In addition to particle technology, the available phase chemistries enable faster and more comprehensive method development



Peak Identities:

1. Atenolol
2. Sotalol
3. Nadolol
4. Pindolol
5. Acebutolol
6. Metoprolol
7. Oxprenolol
8. Bisoprolol
9. Labetalol
10. Propranolol
11. Alprenolol
12. Carvedilol

2.7 µm HALO, phases as indicated, 2.1 x 100 mm SPP columns
1 µL , 0.50 mL/min, 35 °C, 220 nm
Gradient from 10-50% CH_3CN/water/0.1% TFA in 10 min

Summary and Conclusions

- Use of Fused-Core columns to modernize legacy methods enables faster separations, equivalent or better efficiencies, and increased resolution
- Use of different column selectivities, with different organic modifiers and pHs, can be an effective approach for ensuring:
  - **all sample components can be "seen" and,**
  - **acceptable combination(s) of column/modifier/pH can be found**
- For moderately complex and very complex samples, it can be effective to screen different stationary phase types, organic modifiers and pHs to identify a promising combination for further refinement or optimization
  - **Related substance methods**
  - **Multiple active ingredient drug products (OTCs)**
  - **Impurity profiles**
  - **Forensic analyses**
  - **Environmental samples**
- Short, efficient, narrow-ID Fused-Core columns allow faster screening of various combinations of conditions and faster answers to (U)HPLC challenges

Acknowledgements

- Advanced Materials Technology, Inc.
  - Joe DeStefano
  - Tim Langlois
  - Barry Boyes
  - Stephanie Rosenberg
  - Conner McHale
  - Robert Moran
  - Will Miles
- Mac-Mod Analytical, Inc.
  - Alex Nasseh
  - Tom Waeghe
  - Geoff Faden

Questions?

Pressure Estimation

To estimate pressure for a given column length and particle size, you need to know the following:

- **Flow rate (linear velocity)**
- **Column porosity (to calculate linear velocity)**
- **Column temperature**
- **Mobile phase viscosity as f(T)**
  - There are tables available for binary mixtures of ACN and MeOH with water
  - Tables for ternary mixtures (ACN, MeOH, water) or for binary mixtures of other solvents such as IPA, ethanol or THF with water are much harder to find.
- **Column Permeability (flow resistance parameter) is the most difficult to estimate**
- **If you have a column for a given product, you can estimate the permeability (flow resistance parameter) from the QC test conditions and reported pressure.**

Example

HALO 2 µm, 2.1 x 150 mm

- Mobile Phase A: ammonium formate, 10 mM, pH 3.7
- Mobile Phase B: CH_3CN
- Mobile phase composition: 50% B
- Flow Rate: 0.5 mL/min
- Temperature: 50 °C
- Viscosity, η: 0.51 cP
- Porosity: 0.506
- $V_M = \pi \times ID^2 \times L/(4 \times 1000) = 0.263$ mL
- $t_0 = 0.263/0.5 = 0.526$ min
- μ (mm/sec) = 150 mm/(0.526 x 60 sec/min) = 4.75 mm/sec

Φ Flow resistance parameter estimated at 600

$$\Delta P = \frac{\Phi \times \eta \times \mu \times L}{100 \times (d_p)^2}$$

$$\Delta P = \frac{600 \times 0.51 \times 4.75 \times 150}{100 \times 2.0^2} = 545 \text{ bar}$$

Efficiency Measurement or Theoretical Efficiency Estimation

- Theoretical plates, $N = L/(d_p \times h)$
- Column QC test report provides N and flow rate, but not dispersion of instrument used
- Conservative estimates of h for SPP particles
  - **2 µm**
    - 2.1 mm, 1.7
    - 3.0 mm, 1.6
  - **2.7 µm**
    - 2.1 mm, 1.7
    - 3.0 mm, 1.6
    - 4.6 mm, 1.4
  - **5 µm**
    - 2.1 mm, 1.7
    - 3.0 mm, 1.3
    - 4.6 mm, 1.3
- TPP Particles
  - **1.7 and 1.8 µm:** h ≈ 1.8–2.8
  - **3 µm:** h ≈ 2.2–2.3
  - **5 µm:** h ≈ 2.3–2.5
- Reduced plate height (*h*) varies with column diameter (4.6 < 3.0 < 2.1 mm ID)
- Easier to pack larger particles and larger ID columns to give higher N and lower *h* values

Some Examples

HALO 5 µm, 3 x 150 mm

- N ≈ 150 mm x 1000*/(1.3 x 4.6) ≈ 25,080

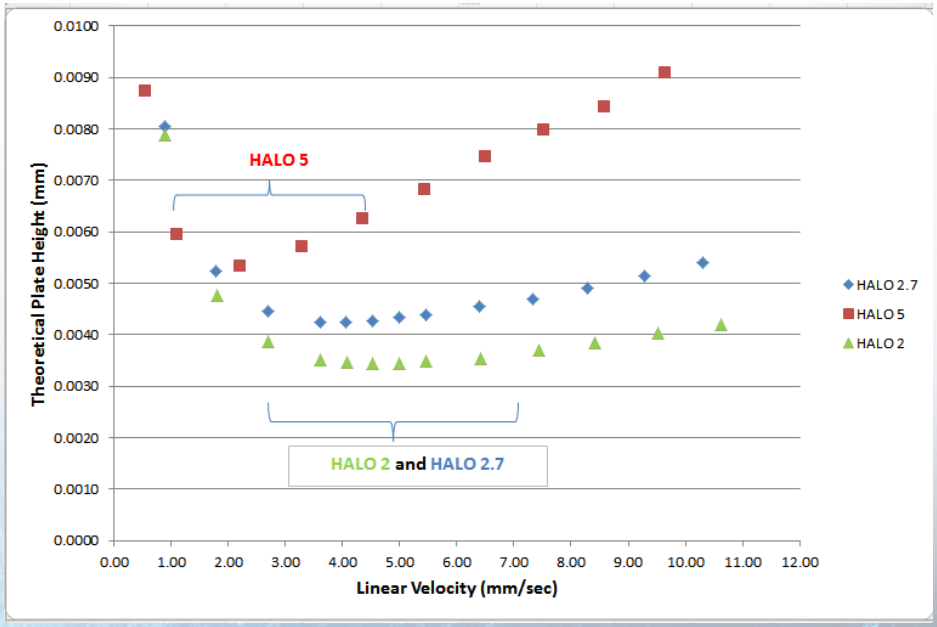
HALO 2 µm, 3 x 150 mm

N ≈ 150 mm x 1000*/(1.7 x 2) ≈ 44,120

HALO 2.7 µm, 4.6 x 250 mm

N ≈ 250 mm x 1000*/(1.4 x 2.7) ≈ 66,140!

*1000 µm/mm



Guiochon-Gritti Approach for Estimating Extracolumn Dispersion

$$\sigma^2_{obs} = \sigma^2_{ec} + \sigma^2_{col} = \sigma^2_{ec} + \left(\frac{V_0^2}{N_{theoretical}}\right)(1+k)^2$$

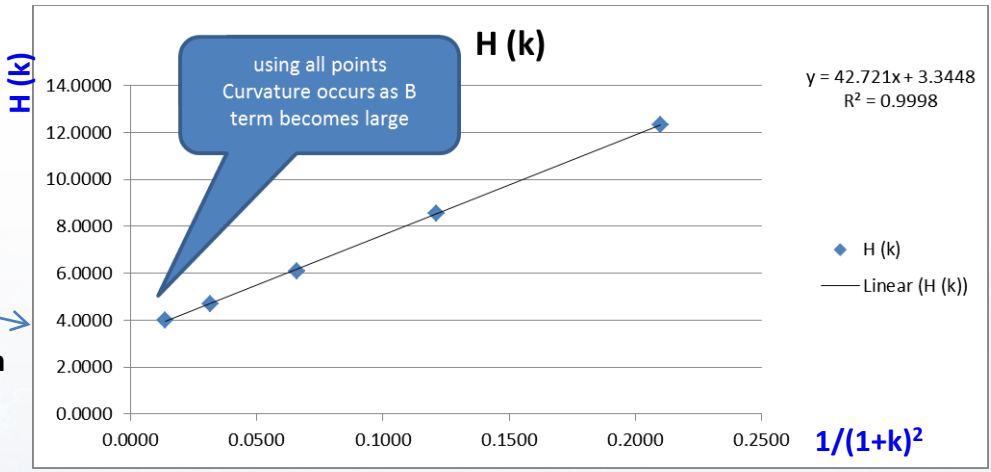
$$H_{obs}(k) = H_{theoretical} + L\left(\frac{\sigma^2_{ec}}{V_0^2}\right)\left(\frac{1}{(1+k)^2}\right)$$

$$Slope = L\left(\frac{\sigma^2_{ec}}{V_0^2}\right), \sigma^2_{ec} = \frac{V_0^2(mm^3)\times slope}{L\,(mm)}$$

1. Chromatograph the mixture of homologs (plus uracil as t_0 marker) at the desired flow rate and linear velocity.
2. Obtain a performance report that shows plate count for each peak at half height
3. Plot the observed plate height in microns for each peak vs. $1/(1+k)^2$.
4. Note where the plot curves and include only those points from the first analyte forward.
5. Usually curvature occurs at or just before point for maximum plates vs. k is reached.

Example for 2.1 x 100 mm, 2 µm SPP column
(0.5 µL injection, 0.4 mL/min with 50:50 CH3CN/water, 30 °C)

Analyte	Plates	RT	k	1/(1 + k)^2	H (k)	h	% Max Plates
acetophenone	8118	1.024	1.18	0.2101	12.3183	6.1592	32%
propiophenone	11693	1.349	1.87	0.1210	8.5521	4.2761	45%
butyrophenone	16398	1.828	2.90	0.0659	6.0983	3.0492	64%
valerophenone	21408	2.632	4.61	0.0318	4.6712	2.3356	83%
hexanophenone	25054	4.000	7.52	0.0138	3.9914	1.9957	97%
heptanophenone	25738	6.295	12.41	0.0056	3.8853	1.9427	100%
octanophenone	24346	10.132	20.59	0.0021	4.1075	2.0537	95%



	L	100	mm
	V_0	187.7	µL
	V_0^2	35241.59	µL^2
	slope	42.7213	
	σ_{ec}^2	15.1	µL^2
$H_{intrinsic}$	intercept	3.34	µm
IBW	4 σ	15.5	µL
h		1.67	

H(k) = L x 1000/N(k)
$h = H(k)/d_p$

Accurate measurements of the true column efficiency and of the instrument band broadening contributions in the presence of a chromatographic column

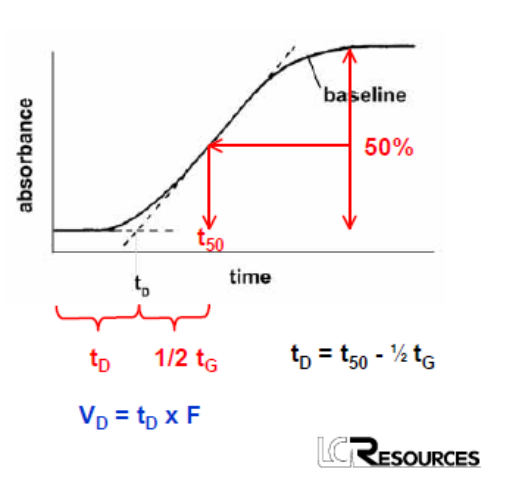
Journal of Chromatography A, 1327 (2014) 49– 56
Fabrice Gritti, Georges Guiochon

Excel calculator available on request from authors

Estimating Gradient Delay Volume (aka Dwell Volume)

Acetone Tracer Approach

- Install ZDV union in place of column
- A solvent: water
- B solvent: 0.1% (v/v) acetone in water
- Set a 0.5 or 1.0 min hold at start (0% B) to provide a flat portion initially
- Use a 10 min gradient time with hold for 5 min at %B final
- Flow Rates
  - 1 mL/min flow rate for 4.6 mm ID columns
  - 0.4 mL/min for 3 mm ID column
  - 0.2 or 0.25 mL/min for 2 mm ID columns



Note: If you use a 0.5 or 1.0 minute hold, remember to "back out" that portion of the calculated t_D and thus V_D

DryLab Software Approach

1. Sample: mixture of alkylphenones
2. Column: desired column
3. Flow rate: typical flow rate for column ID
4. Carry out 3 gradients (e.g., 5, 10 and 15 min) from 5 to 100% organic/water at the desired flow rate with column of interest.
5. Input 5 min and 10 min gradient data (RTs and PWs) into DryLab and vary dwell volume setting to obtain predicted RTs for 15 min run using those dwell volumes.
6. Find the delay volume setting that minimizes the error in RT for all peaks for predicted vs. actual 15 min run.
7. Estimate the dwell volume that minimizes the sum of the RT error differences by interpolation.
8. Input chromatograms into DryLab as CDF files or put retention times and peak widths into Excel table and paste into DryLab.
9. Note: a Microsoft Excel spreadsheet for carrying out the calculations is available from the authors based on the Reference 1 below.

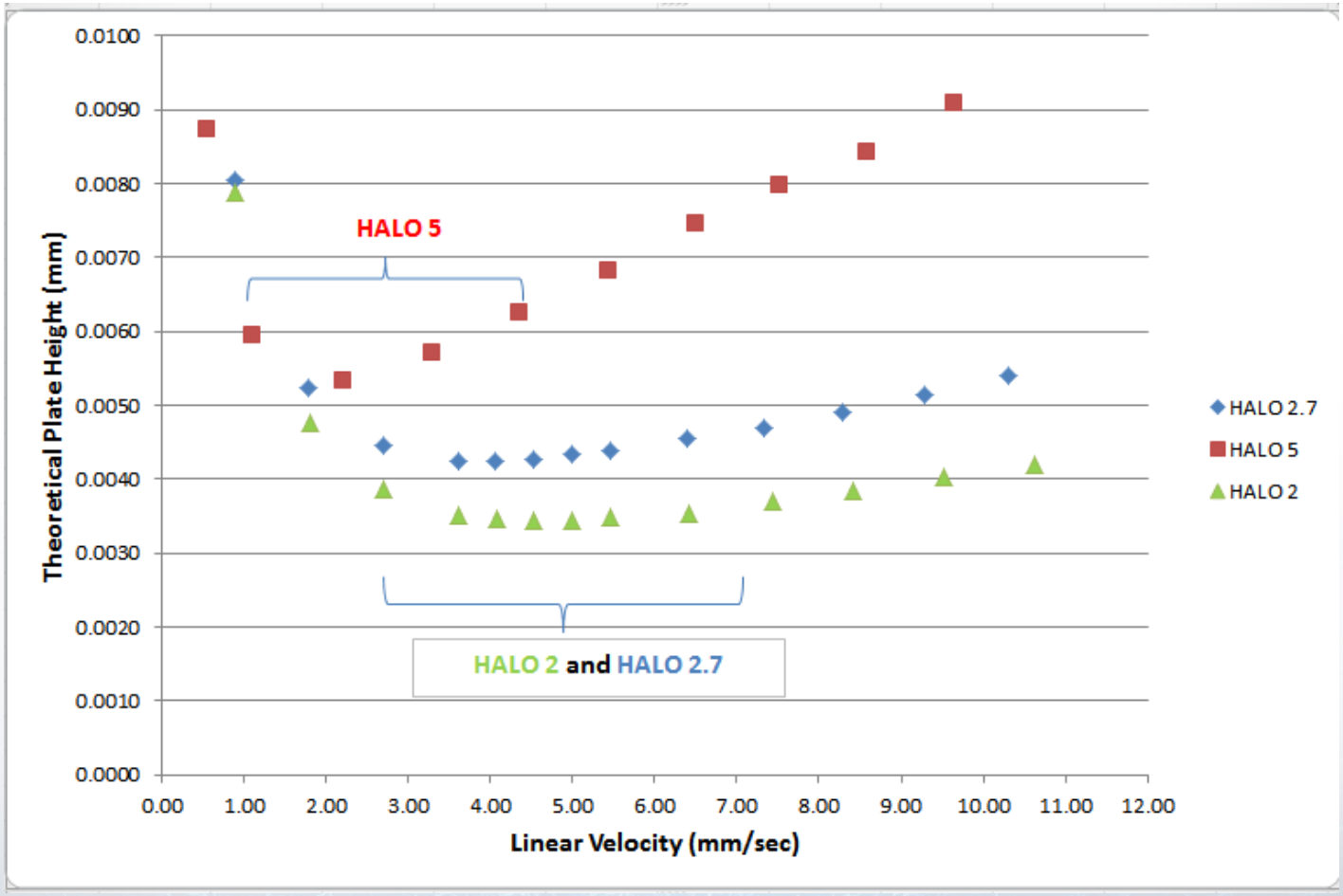
Excel calculator available on request from authors

1. **LC-GC Magazine, 1990, Vol. 8, Number 7, 524-537**
   "Reproducibility Problems in Gradient Elution Caused by Differing Equipment.
2. **J Chromatogr A. 2014 Nov 21; 1369: 73–82.**
   "Measure Your Gradient": A New Way to Measure Gradients in High Performance Liquid Chromatography by Mass Spectrometric or Absorbance Detection

Lamotrigine Case Study

- Lamotrigine – used to treat seizures and control mood swings
- USP method for extended release tablets
  - 4.6 x 150 mm, 3 µm FPP C18 column
  - L/dp = 150 mm / 3 µm = 50000
  - -25% to +50% L/dp = 37,500 to 75,000
  - For a 2.7 µm HALO column, L/dp = 150 mm / 2.7 µm = 55,556

van Deemter Comparisons: SPP of various sizes



How Should Experimental Results be Evaluated or Graded?

1st Approach

C18 CH_3CN pH 2.8	**Phenyl-Hexyl** CH_3CN pH 2.8
ES-CN CH_3CN pH 2.8	**RP-Amide** CH_3CN pH 2.8

And so on for CH_3OH and other pHs

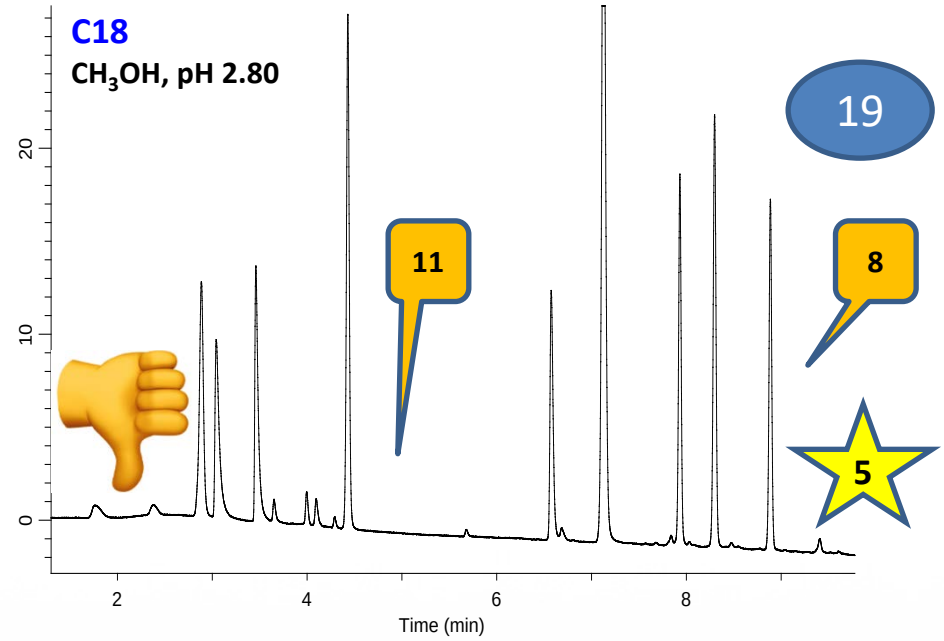
Compare different phases with each modifier at the same pH

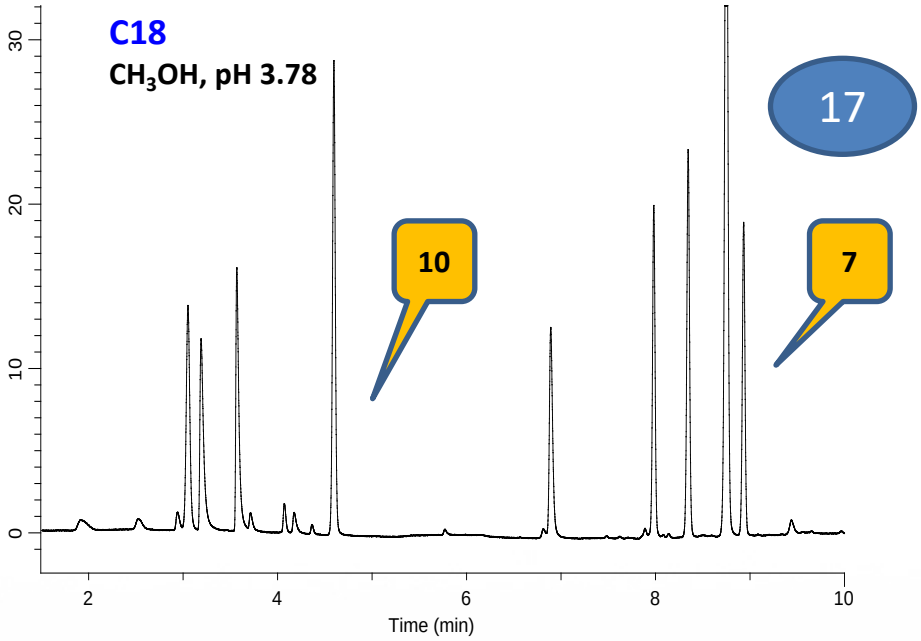
2nd Approach

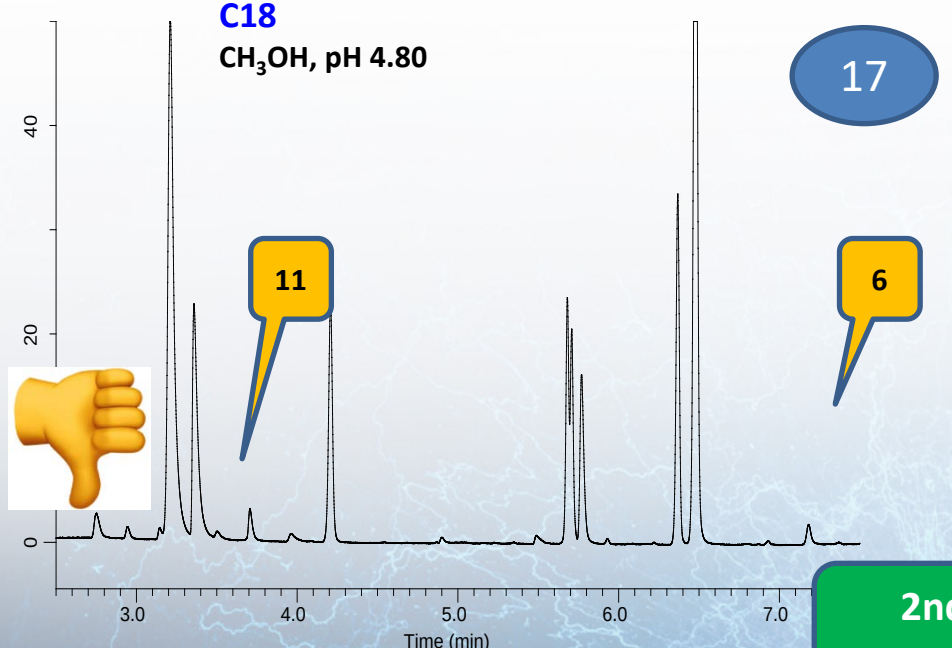
C18 CH_3CN pH 2.8	**C18** CH_3CN pH 3.8
C18 CH_3CN pH 4.8	**C18** CH_3CN pH 5.8

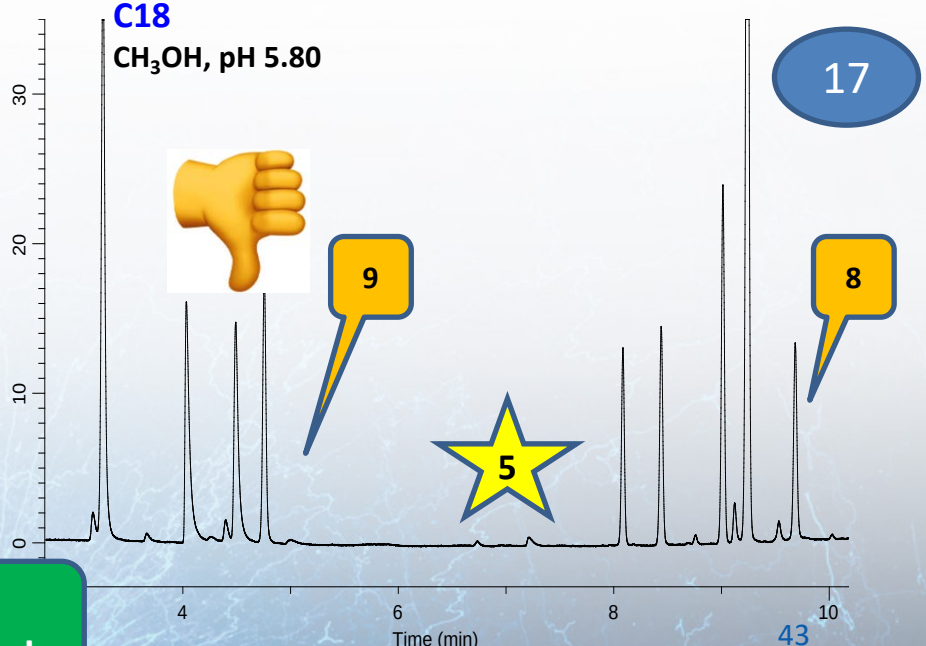
And so on for CH_3OH and other phases

Compare different pHs for same phase with each modifier separately









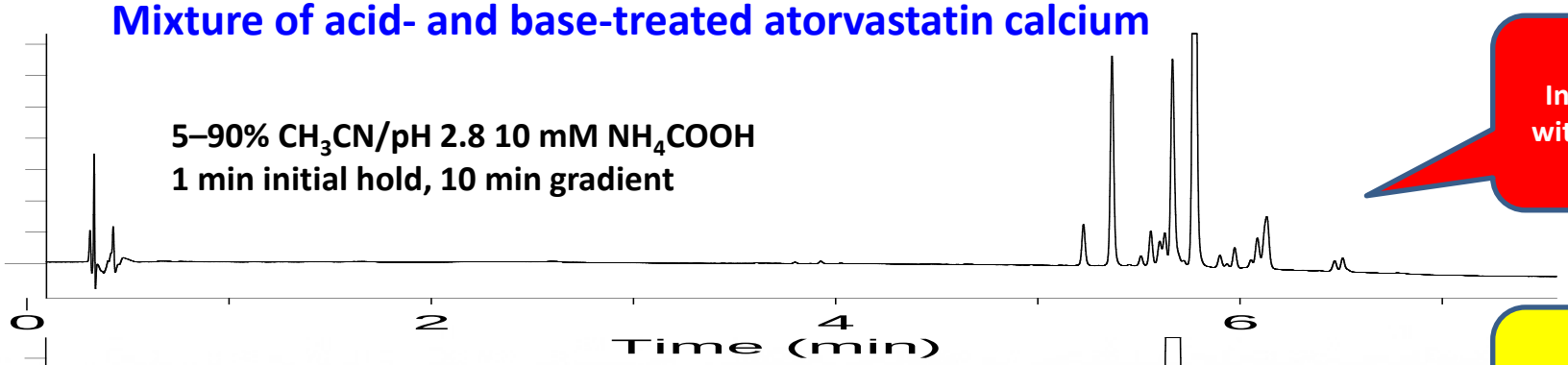
2nd approach

Application of Multiple Phases for Stability Indicating Method Development

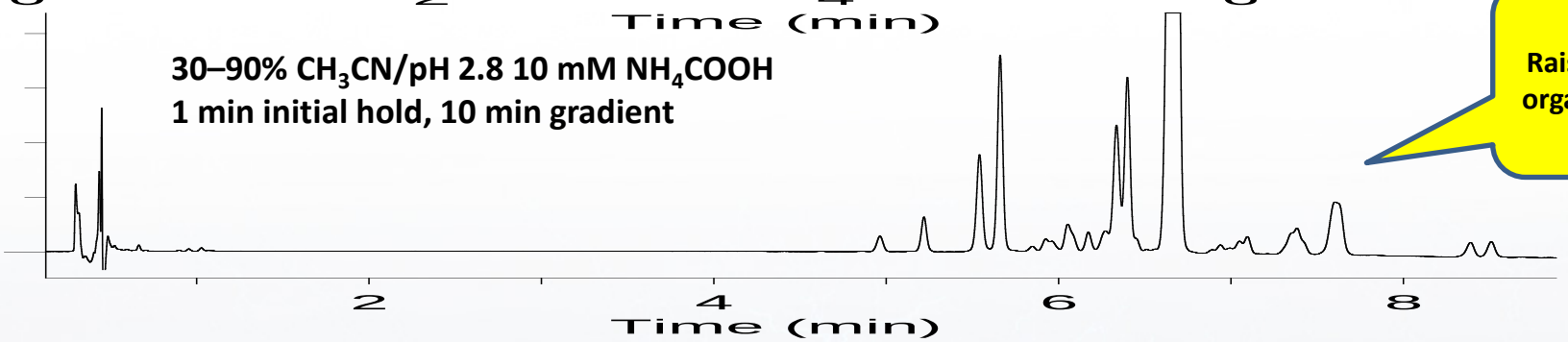
- Atorvastatin Calcium
  10 mg active/310 mg tablet
- Generate HCl-degraded and NaOH-degraded samples
- Pool acid- and base-treated samples together
- Compared five different HALO phases using both CH_3CN and CH_3OH at one pH (2.8, ammonium formate)
- Compared results and identified best option(s) for further development and optimization
- Again, used 3 x 50 mm, 2.7 µm HALO column geometry
- Initially screened C18 column using broad gradient with CH_3CN
- Fine tuned to narrower ranges
- Compared all phases using narrower range using both CH_3CN and CH_3OH

A Broad Range Gradient May Not Be as Useful When Screening More Complex Samples

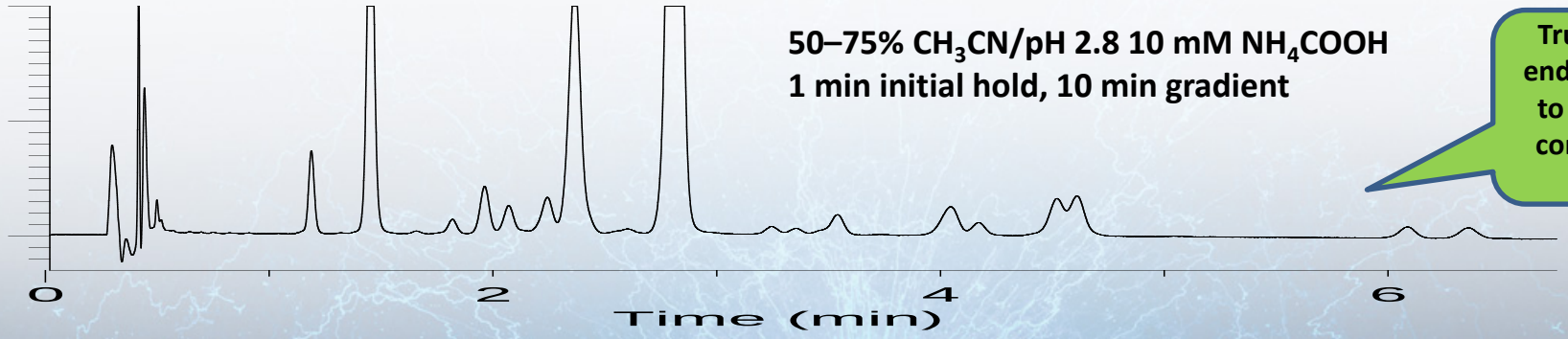
Mixture of acid- and base-treated atorvastatin calcium



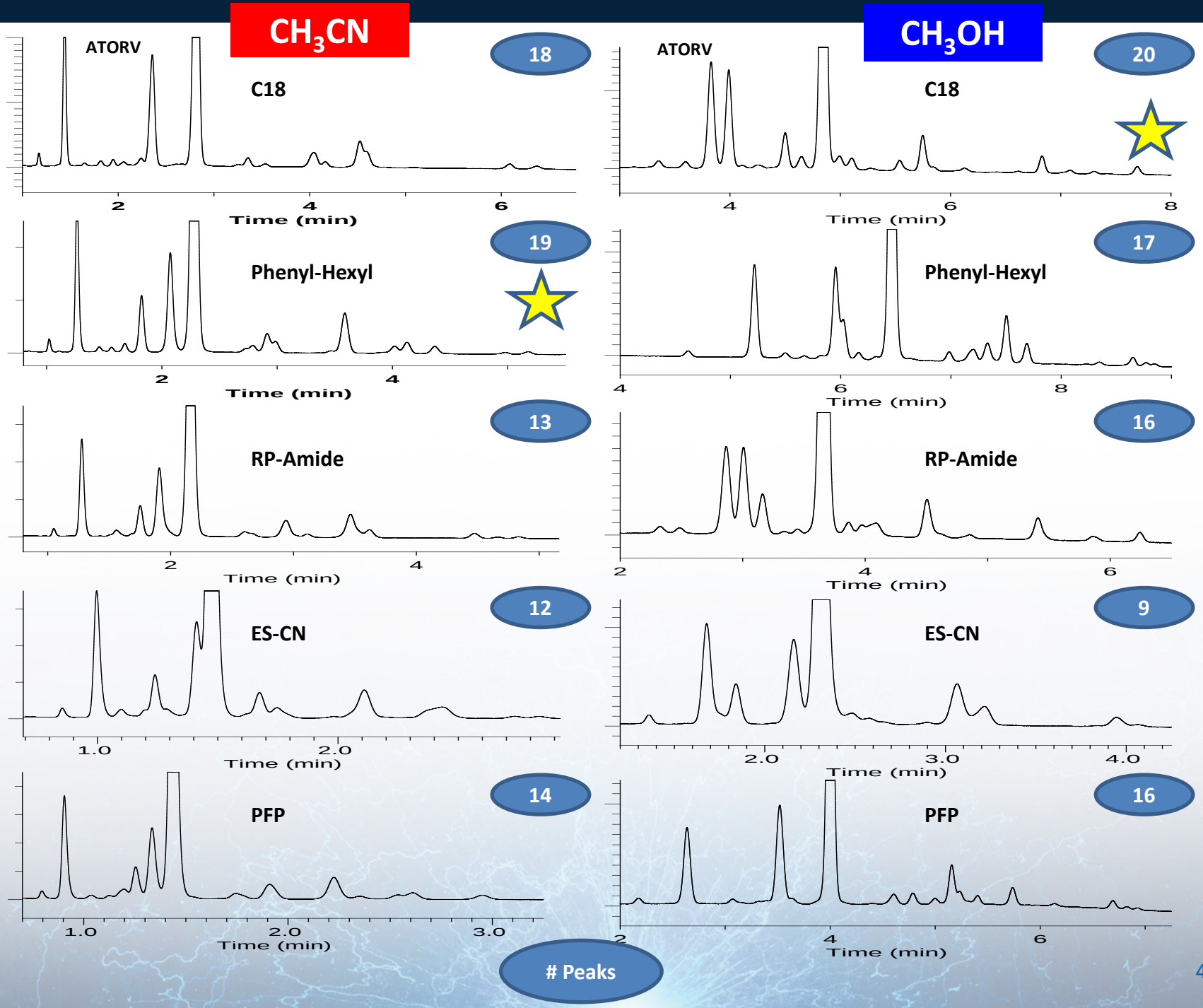
Initial screen with HALO C18



Raise starting % organic modifier



Truncate both ends of gradient to allow fairer comparison of phases


CH3CN
CH3OH
ATORV
C18
18
20
Time (min)
Phenyl-Hexyl
19
17
RP-Amide
13
16
ES-CN
12
9
PFP
14
16
Peaks

How Do You Choose Which Combination to Develop and Optimize Further?

- Compare chromatogram for number of peaks observed
- Compare shapes for all detected peaks
- Select phase/modifier combination(s)
  - # peaks separated
  - minimum critical R_s for peak pair
  - shortest analysis time
  - most peaks with acceptable USP T_f
- If no clear winning combination, carry out several gradients having differing slopes
  - For example, 50–75% in 10 minutes and 25 minutes for C18 and Phenyl-Hexyl
  - Assess whether either combination stands out vs. criteria
- Compare separation on longer column with higher efficiency