AN EFFECTIVE METHOD DEVELOPMENT STRATEGY USING FUSED-CORE® COLUMNS

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ABSTRACT

The development of a stability-indicating method (related substances method) can be among the most challenging activities in support of pharmaceutical or other UHPLC method development for complex samples. The objective of such work is to develop a robust and rugged separation of all of the impurities and degradants from a drug substance or formulation, or the analytes from a multi-component mixture. To discover that your stability-indicating method does not separate all of the known and unknown impurities at a later stage can seriously affect product registration.

A sensible approach for LC separation development is to screen various selectivity parameters up front, when careful selection of the best combination of stationary phase, organic modifier, mobile phase pH, temperature and other parameters can be made.

In this presentation we will show examples of how such a method development strategy can be applied using Fused-Core stationary phase selectivities with samples such as a degraded pharmaceutical active ingredient and a double-blind-prepared mixture of acidic, basic and neutral pharmaceuticals.

OUTLINE

- **Factors That Affect Selectivity in RPLC**
  - Relative impact of various parameters
- **Review of method development strategies**
  - Expected or actual sample complexity
  - Method performance requirements
    - Assay method vs. impurity profile/related substances method
  - What should performance criteria be?
- **Screening approach example**
  - Blind sample, unknown number of components
  - Gradient screening: stationary phase, organic modifier, pH
- **Degraded pharmaceutical sample**
  - Screening results for phases and organic modifier at single pH
- **Summary**

WHICH FACTORS[1] AFFECT SELECTIVITY MOST ?

MOST Influence

Isocratic Separations

- Column Stationary Phase
- Organic modifier
- Mobile phase pH (for ionised analytes only)
- % Organic modifier
- Column temperature
- Buffer choice
- Buffer concentration
- Additive concentration

LEAST Influence

Gradient Separations

- All parameters for isocratic
- Gradient steepness
- k* (that is, t_G, F, $\Delta\Phi$, V_M, MW)

$$k^* = \frac{85 \times t_G \times F}{\Delta\Phi \times V_M \times S}$$

- Instrument delay volume

[1] Adapted from 'Introduction to Modern Liquid Chromatography", 3rd Edition, Snyder, Kirkland, and Dolan, 2010, p.29, Wiley & Sons

POWER OF CHANGING MULTIPLE PARAMETERS

Relative Impact of Different Changes in RPLC Parameters on Selectivity[1]

Selectivity Parameter	Change in Parameter	Maximum $\lvert \delta \log\alpha \rvert$
pH	5 pH units	0.70
Organic modifier choice	$CH_3CN \leftrightarrow CH_3OH$	0.20
Gradient time	10-fold	0.20
Orthogonal column	ΔF_s ~65	0.19
% Organic modifier	10% (v/v)	0.08
Column temperature	20 °C	0.07
Buffer concentration	2-fold	0.02

Change of only 0.10 needed to go from co-elution to baseline resolution

$$R_s = \left(\frac{1}{4}\right)\sqrt{N}(\alpha - 1)\left(\frac{k}{1+k}\right)$$

For $\Delta R_s = 1.5$, N = 10,000 and $k \geq 1$

$(\alpha - 1) = 0.12$ and $\alpha = 1.12$

$\log \alpha = 0.05$ and Snyder proposed $|\delta \log \alpha|_{avg} \geq 0.10$

For a change in both column phase and organic modifier, the expected change is magnified

$|\delta \log \alpha|_{avg} = [(0.20)^2 + (0.19)^2]^{0.5} = 0.28$

Use of different column phases, organic modifiers, pHs, and temperatures can be powerful in changing α and R_s

[1] Adapted from Snyder et al., "Orthogonal" separations for reversed-phase liquid chromatography, Journal of Chromatography A, 1101 (2006) 122–135

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 • Hydrogen bonding
AQ-C18	proprietary	• Hydrophobic

HALO PHASES USED IN THIS WORK

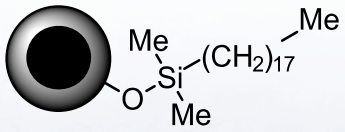
HYDROPHOBIC SUBTRACTION MODEL PARAMETERS[1]

$$\log k = \eta'\mathbf{H} - \sigma'\mathbf{S}^* + \beta'\mathbf{A} + \alpha'\mathbf{B} + \kappa'\mathbf{C} + \log k_{EB}$$

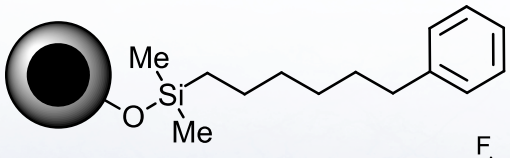
Dissimilarity vs. C18

F_s	Name	H	S*	A	B	C (pH 2.8)	C (pH 7.0)	EB retention factor	USP type	Phase type
0.00	**HALO C18**	1.10	0.04	0.00	-0.05	0.05	0.04	6.10	L1	C18
17.35	**HALO Phenyl-Hexyl**	0.78	-0.09	-0.23	0.00	0.10	0.45	3.50	L11	Phenyl
22.78	**HALO ES-CN**	0.57	-0.11	-0.34	0.02	0.13	1.15	1.88	L10	CN
52.83	**HALO RP-Amide**	0.85	0.08	-0.38	0.19	-0.41	0.31	4.60	L60	EP
94.45	**HALO PFP**	0.70	-0.12	-0.07	-0.06	1.17	0.97	2.30	L43	F

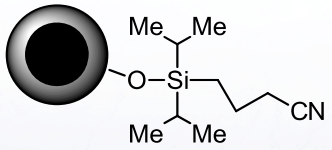
HALO C18

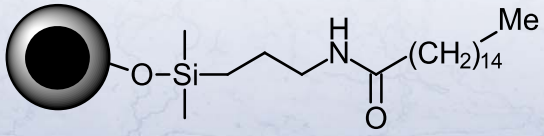


HALO Phenyl-Hexyl

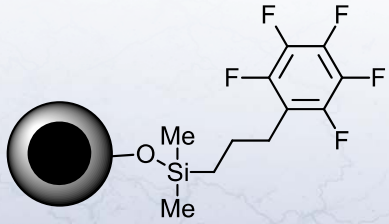


HALO ES-CN





HALO RP-Amide



HALO PFP

[1] www.hplccolumns.org

APPROACH DICTATED BY SAMPLE COMPLEXITY

Simple

- 1 stationary phase
- 1 organic modifier
- 1 pH
- Use broad gradient range
- Assess need for isocratic vs. gradient

$\frac{\Delta t_R}{t_G} \leq 0.25$, then isocratic

$\frac{\Delta t_R}{t_G} \geq 0.40$, then gradient

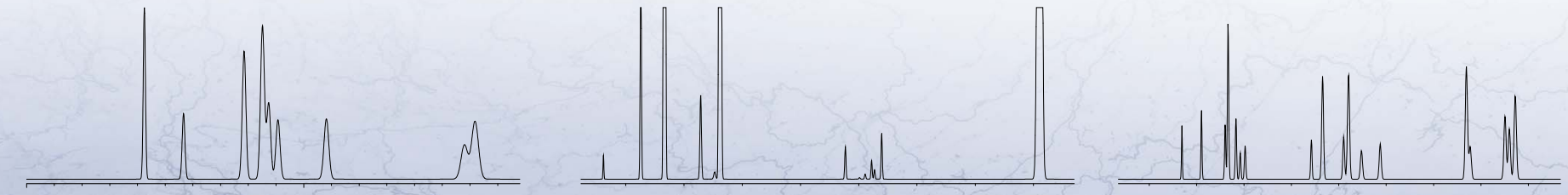
- Isocratic?
  - Identify isocratic conditions for evaluation
  - Compare %Bs at, above and below suspected or predicted conditions
- Gradient?
  - Optimize gradient slope and range
  - Assess impact of temperature/optimize

Moderate Complexity

- 2 or more stationary phases
- 2 organic modifiers and blend
- 1 or more pHs
- Use broad gradient range to screen phases, organic modifiers, pHs
- Compare results based on peak performance

Very Complex

- 2 or more stationary phases
- 2 organic modifiers and blend
- 2 or more pHs
- Use broad gradient range to screen phases, organic modifiers, pHs
- Compare results based on peak performance



HOW SHOULD SCREENING RESULTS BE EVALUATED OR GRADED?

Performance Objectives

- Best overall peak shape
- Highest # of peaks observed
- Highest limiting resolution
- Best overall average resolution
- Highest likelihood for improvement or optimization

"Must Not" Haves

- Poor peak shape
- Significant peak bunching

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 %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

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

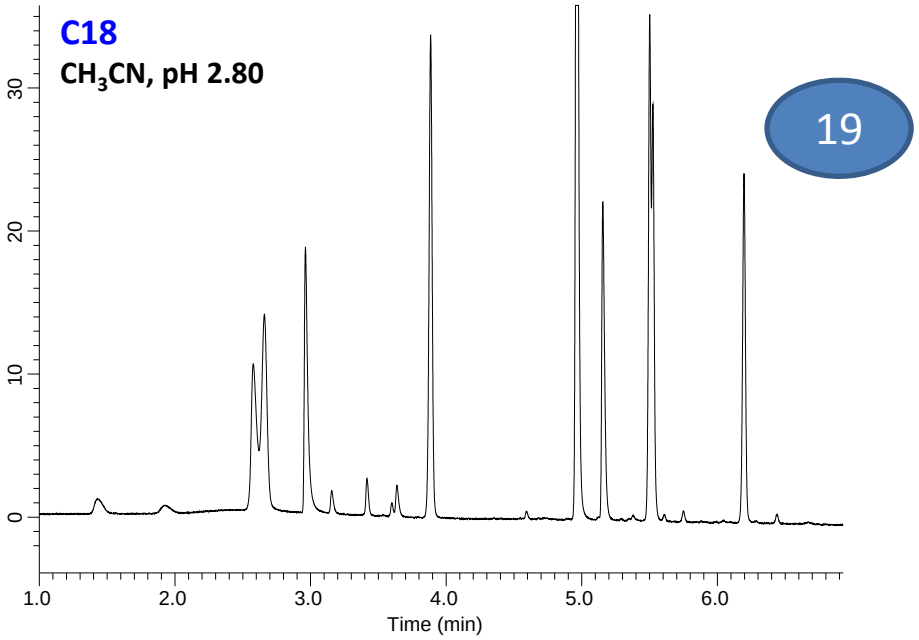
Compare different phases with each modifier at the same pH

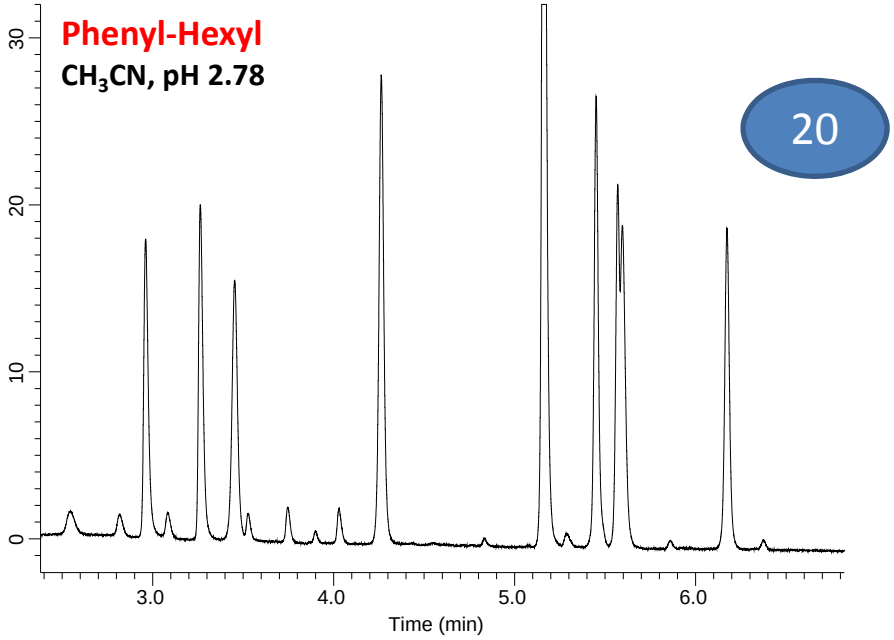
2nd Approach

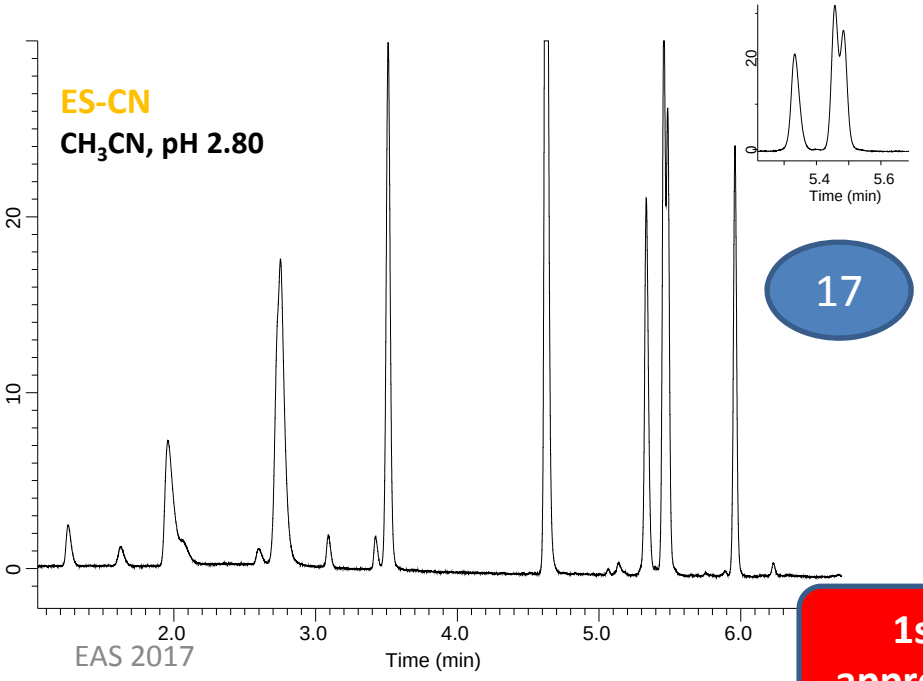
C18 CH_3CN pH 2.8	**C18** CH_3CN pH 3.8
C18 CH_3CN pH 4.8	**C18** CH_3CN pH 5.8

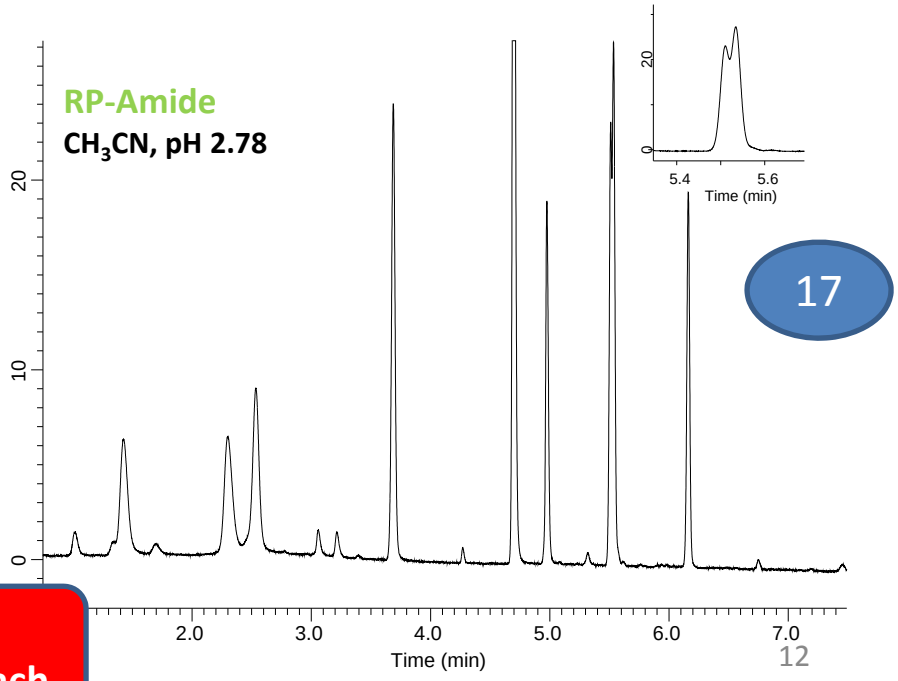
And so on for CH_3OH and other phases

Compare different pHs for same phase with each modifier separately

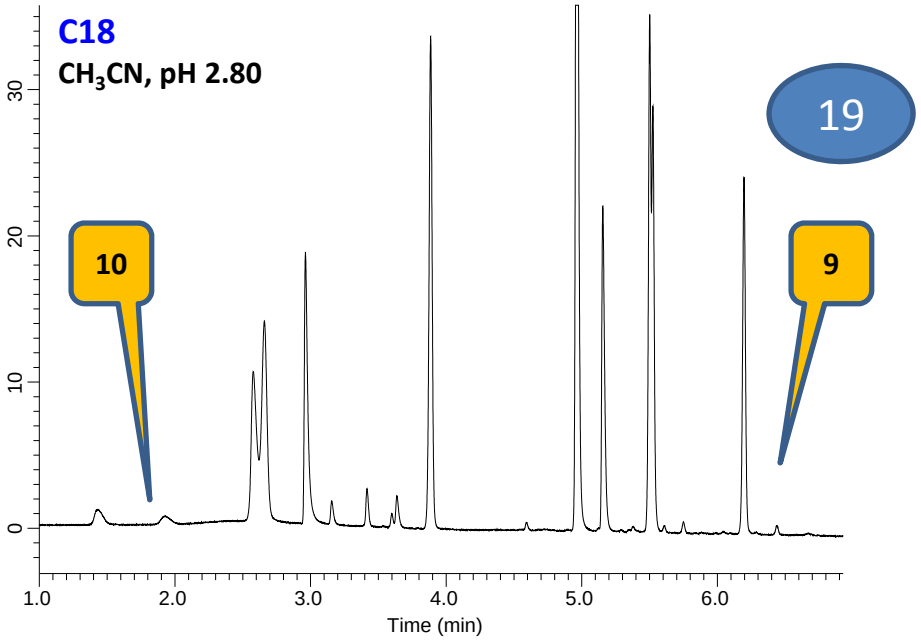


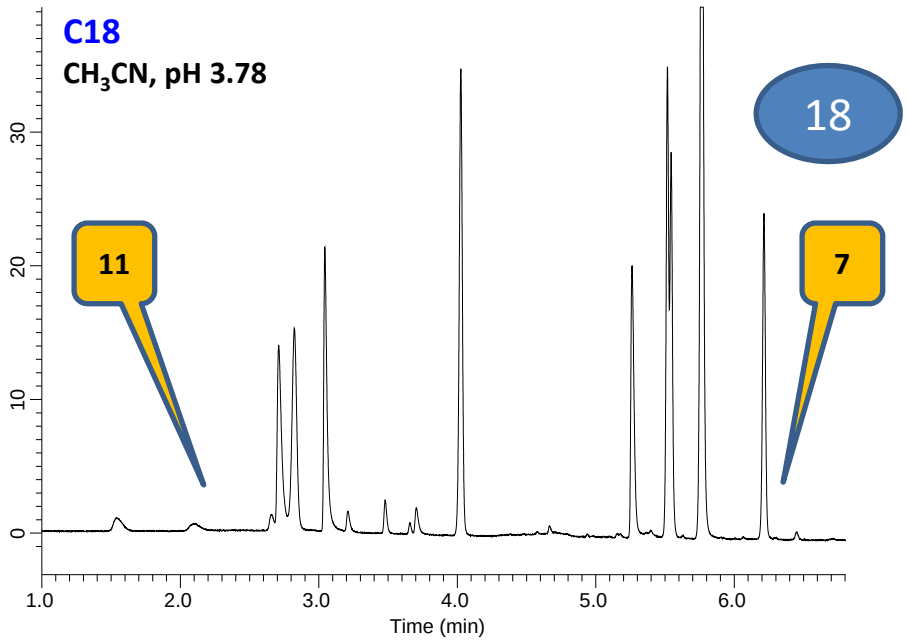


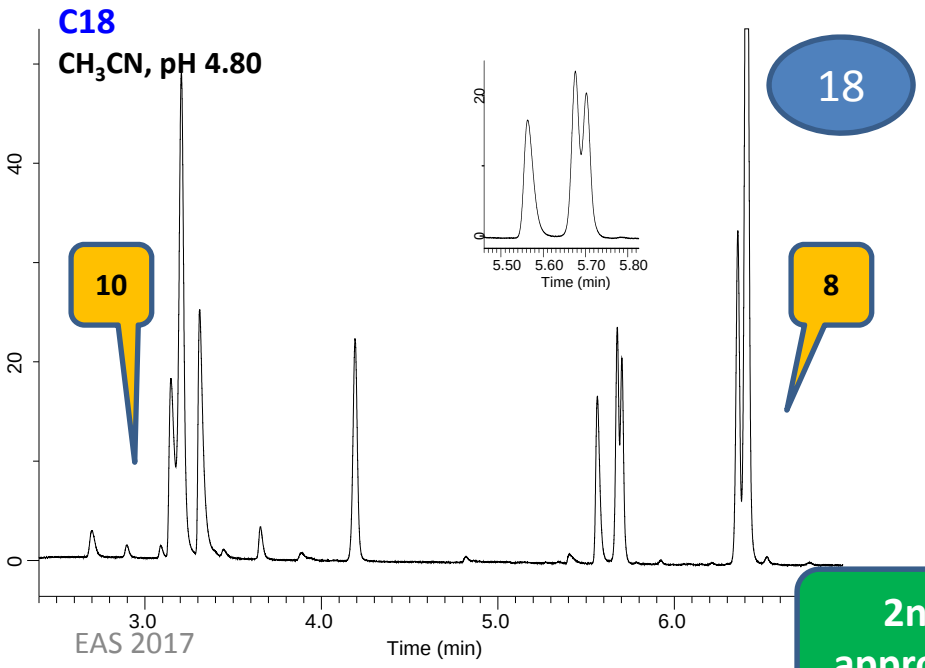


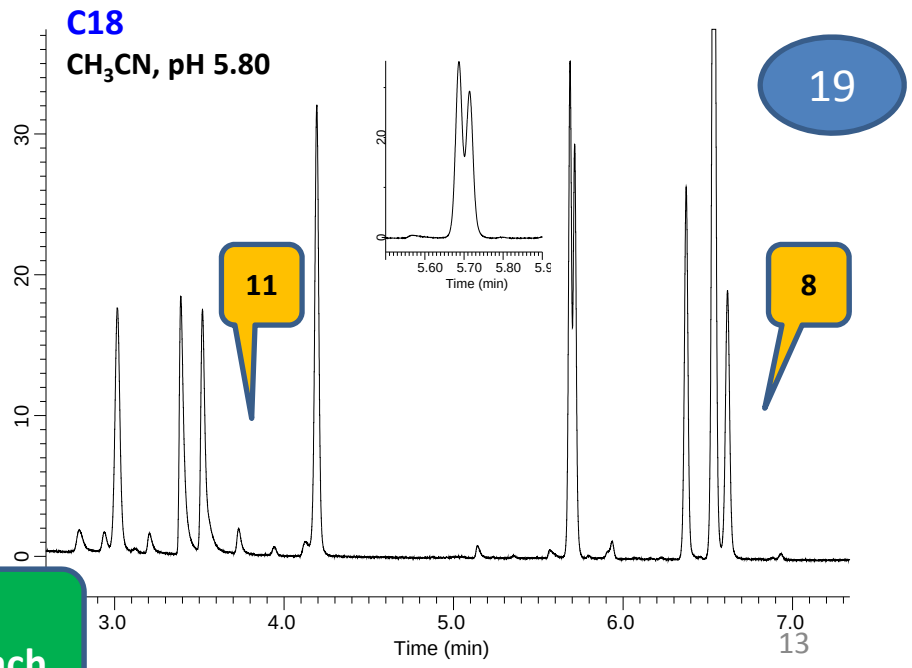


1st approach

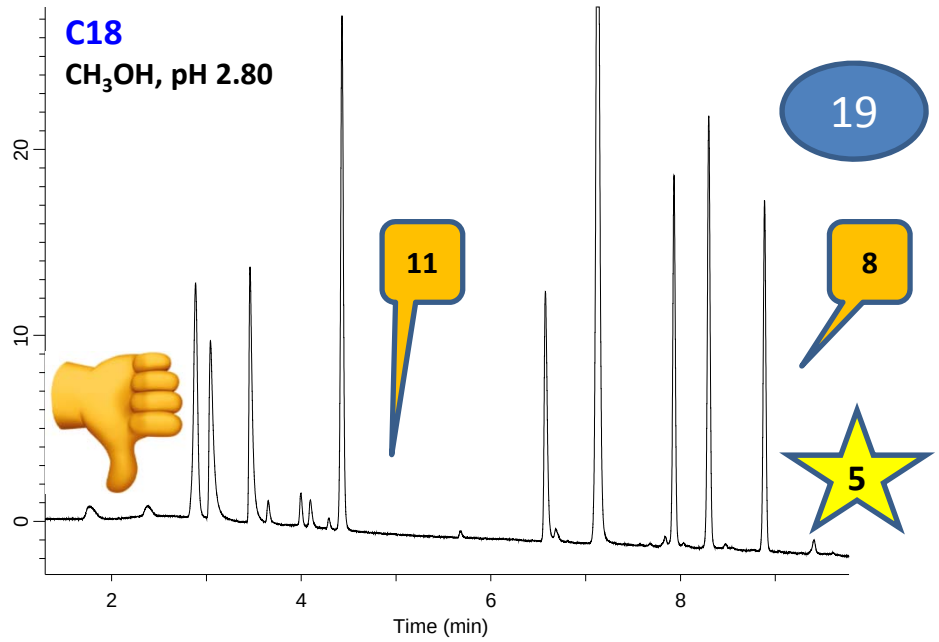


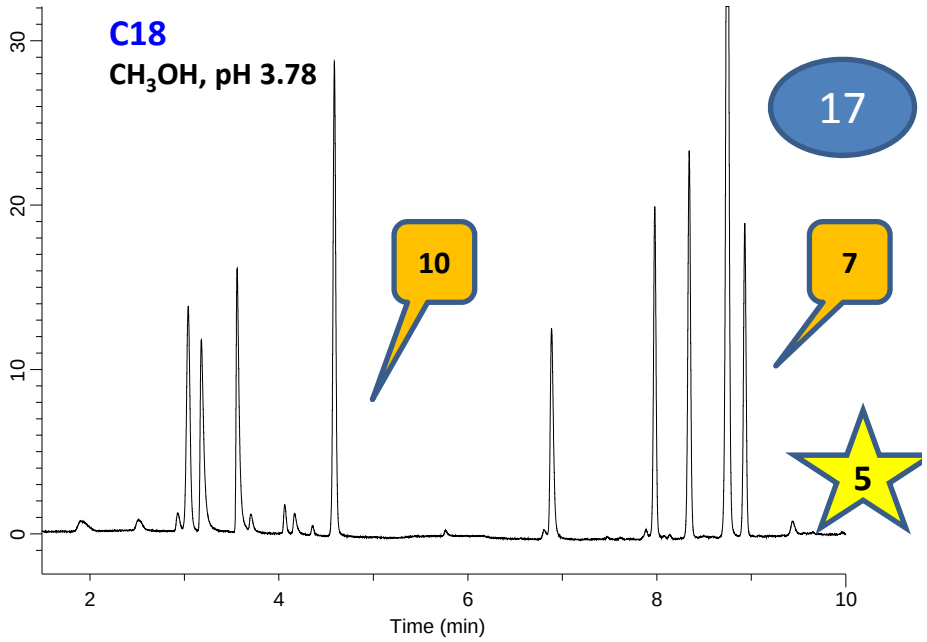


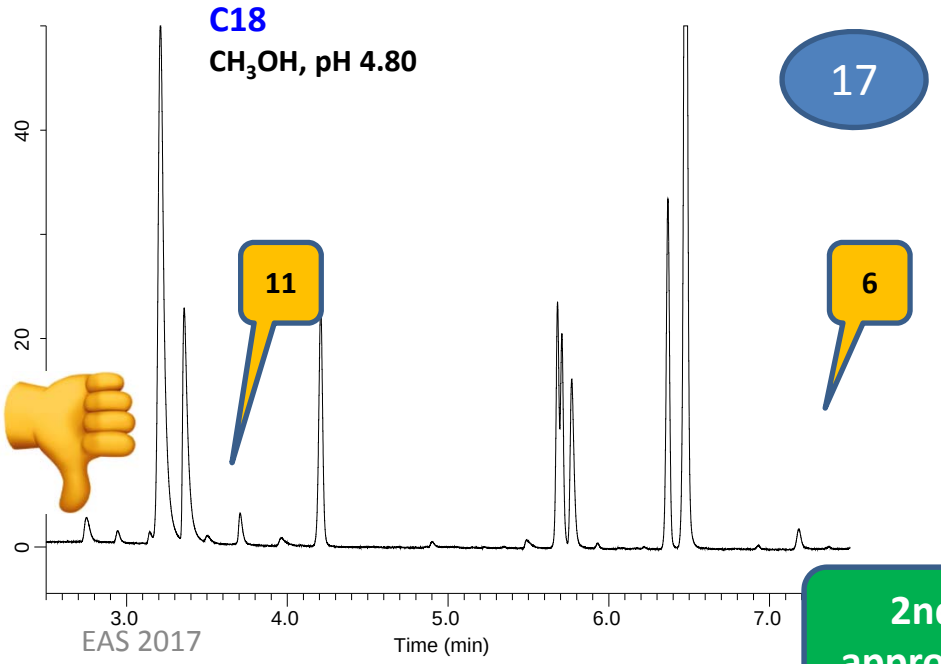


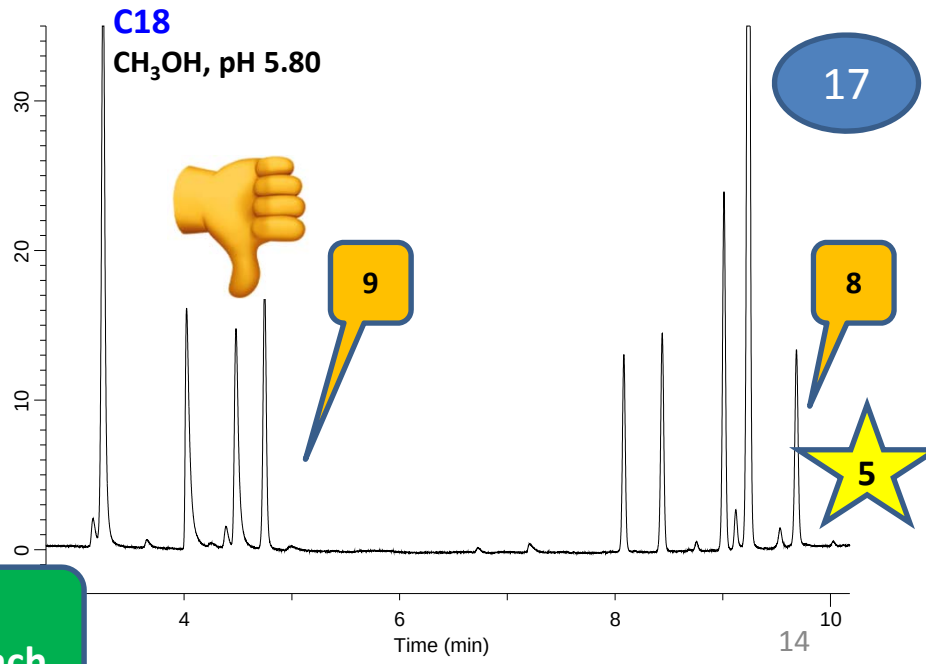


2nd approach

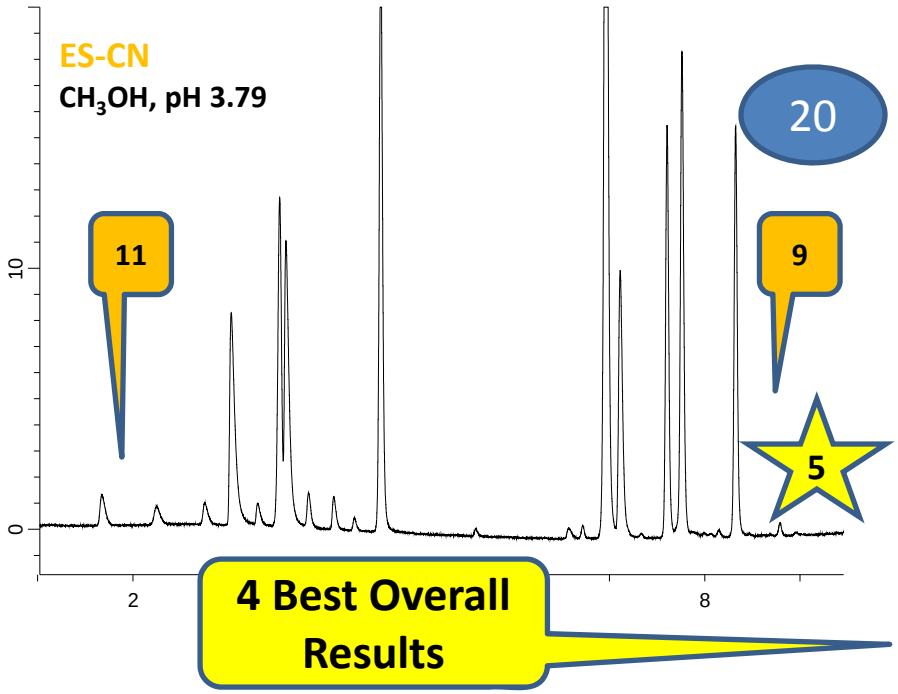


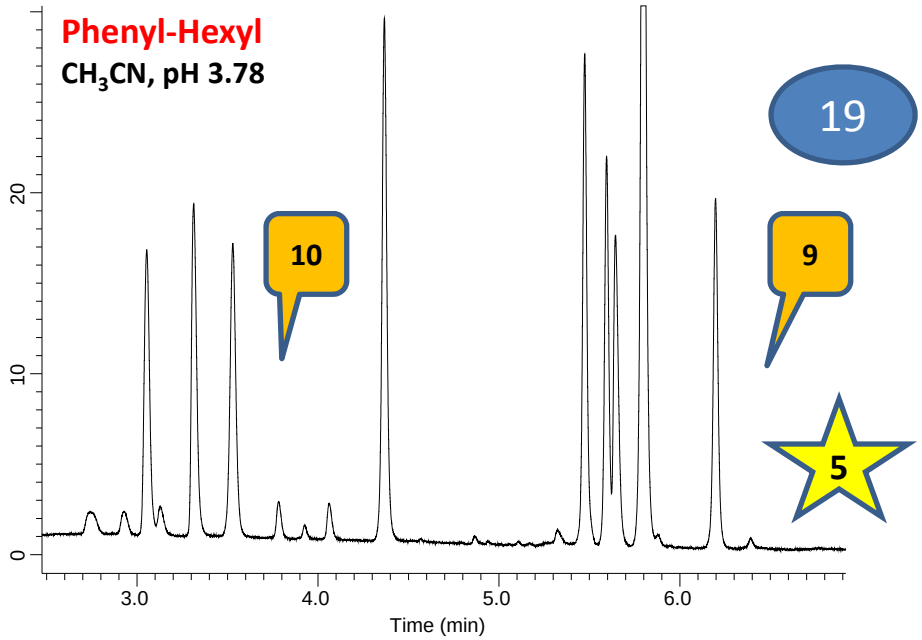




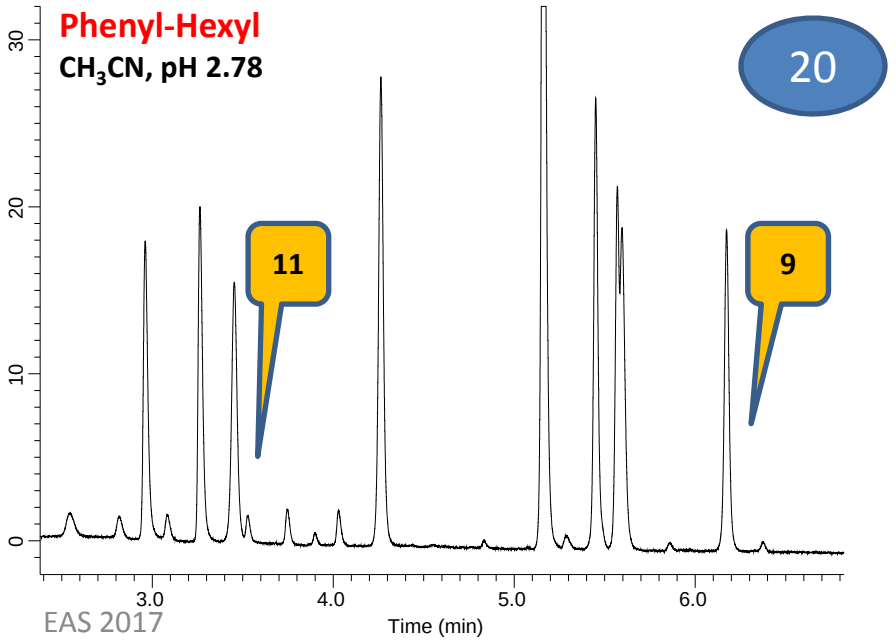


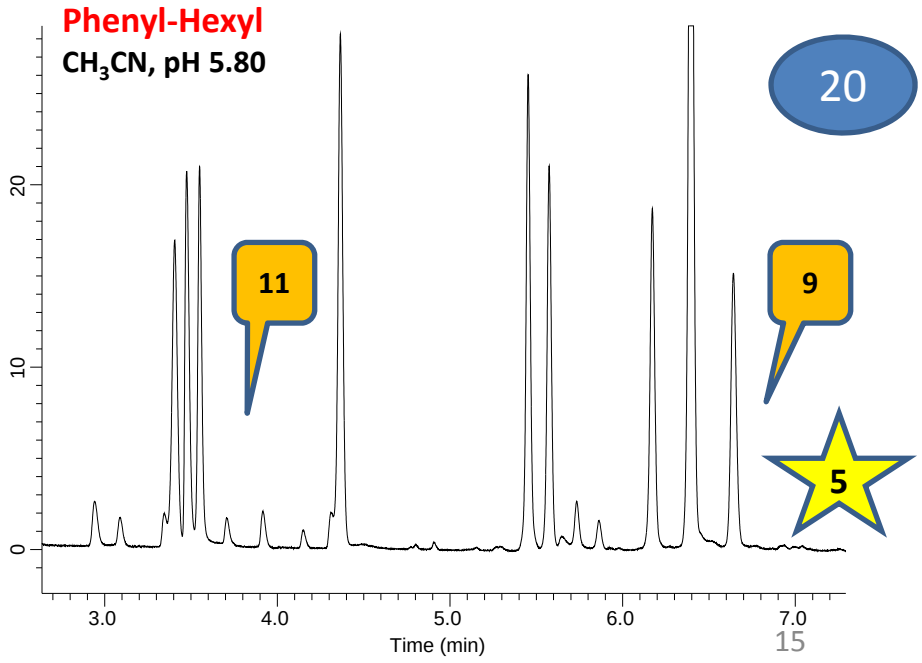
2nd approach

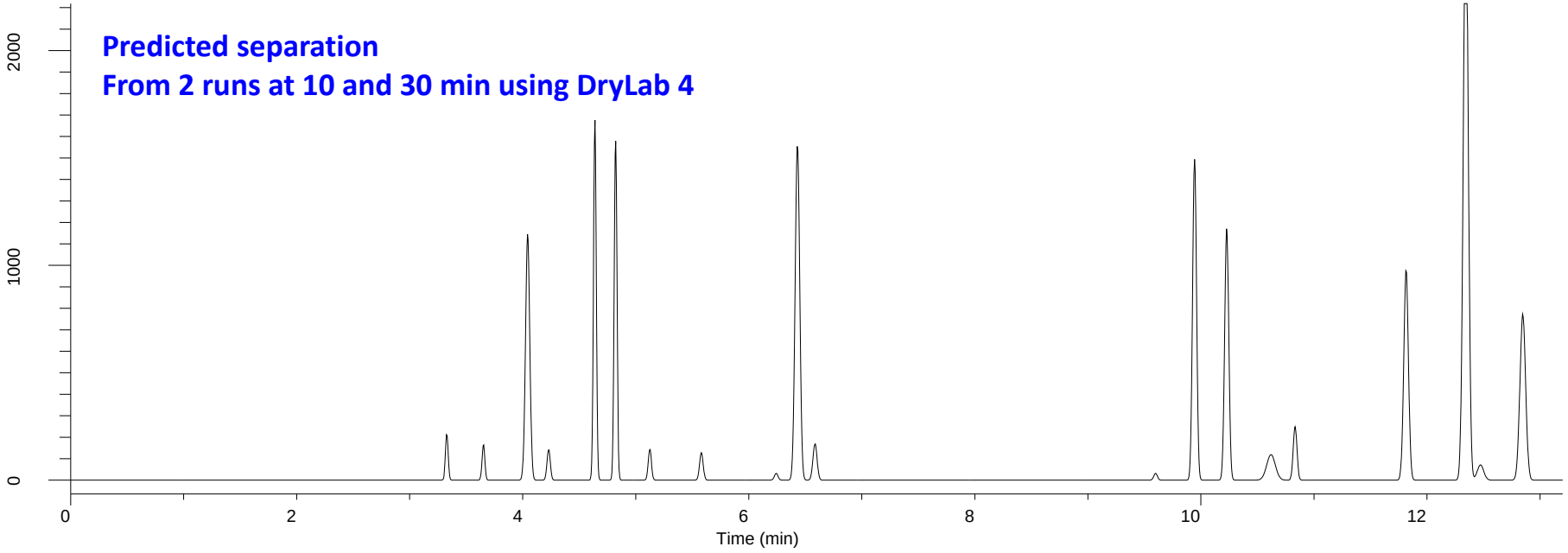


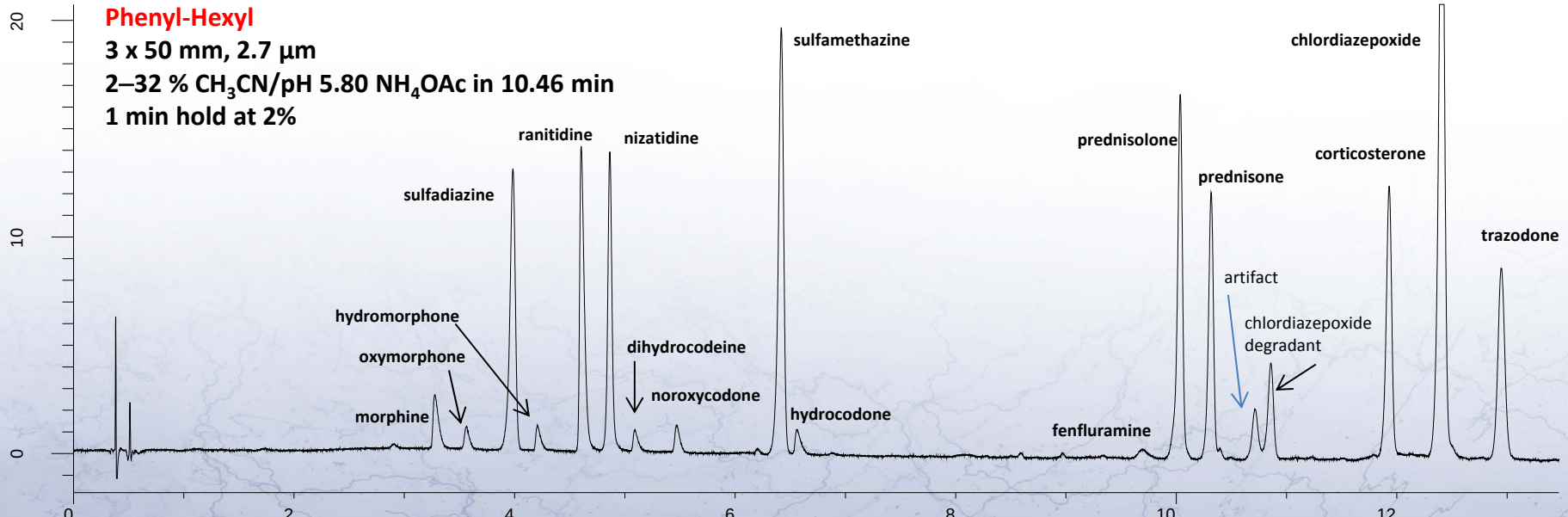


4 Best Overall Results






Predicted separation
From 2 runs at 10 and 30 min using DryLab 4
2000
1000
0
0
2
4
6
8
10
12
Time (min)

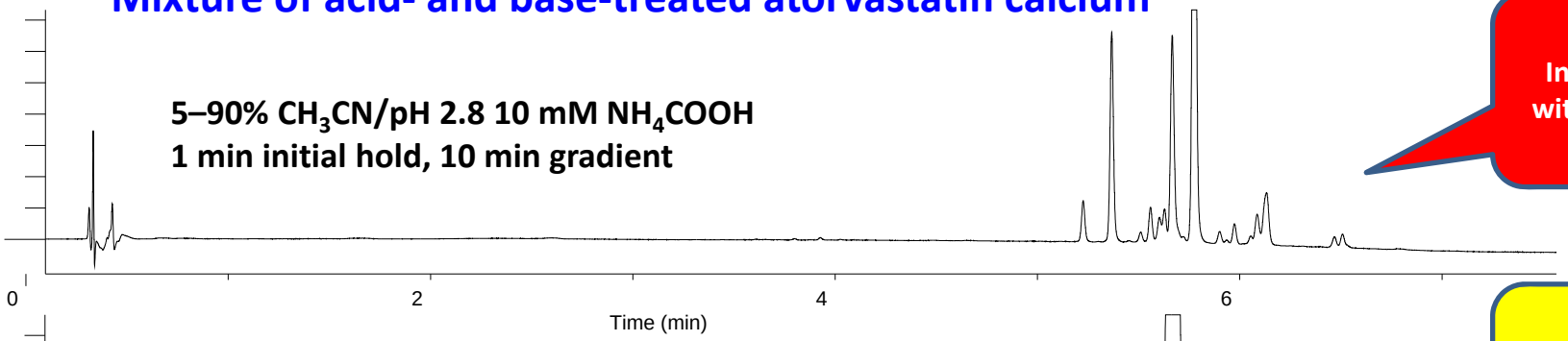

Phenyl-Hexyl
3 x 50 mm, 2.7 µm
2–32 % CH3CN/pH 5.80 NH4OAc in 10.46 min
1 min hold at 2%
sulfamethazine
chlordiazepoxide
ranitidine
nizatidine
prednisolone
sulfadiazine
prednisone
corticosterone
trazodone
artifact
hydromorphone
chlordiazepoxide
degradant
oxymorphone
dihydrocodeine
noroxycodone
morphine
hydrocodone
fenfluramine
20
10
0
0
2
4
6
8
10
12
Time (min)

APPLICATION OF MULTIPLE PHASES FOR STABILITY INDICATING METHOD DEVELOPMENT

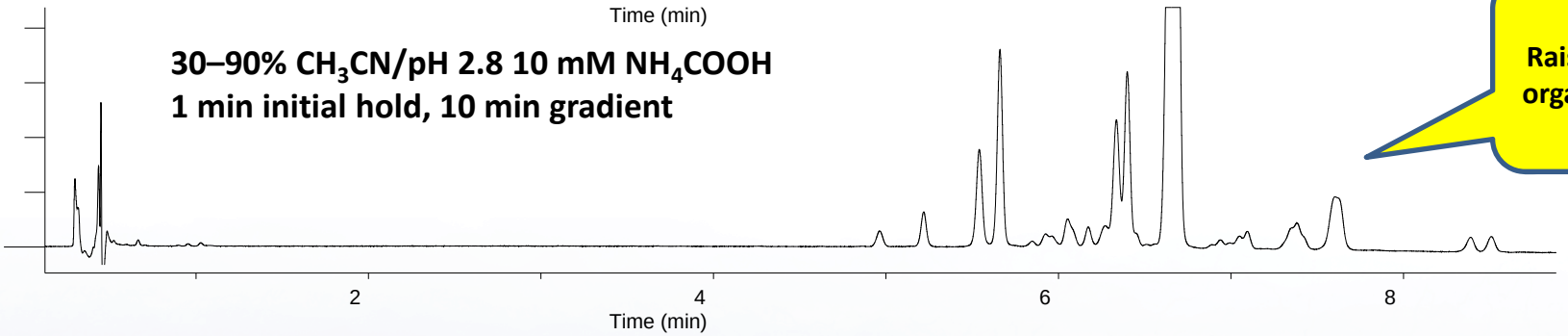
- Atorvastatin Calcium
  10 mg active/310 mg tablet
- Generate HCl-degraded and NaOH-degraded samples
- Pool acid- and base-treated samples together
- Compared five different HALO phases using both CH_3CN and CH_3OH at one pH (2.8, ammonium formate)
- Compared results and identified best option(s) for 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

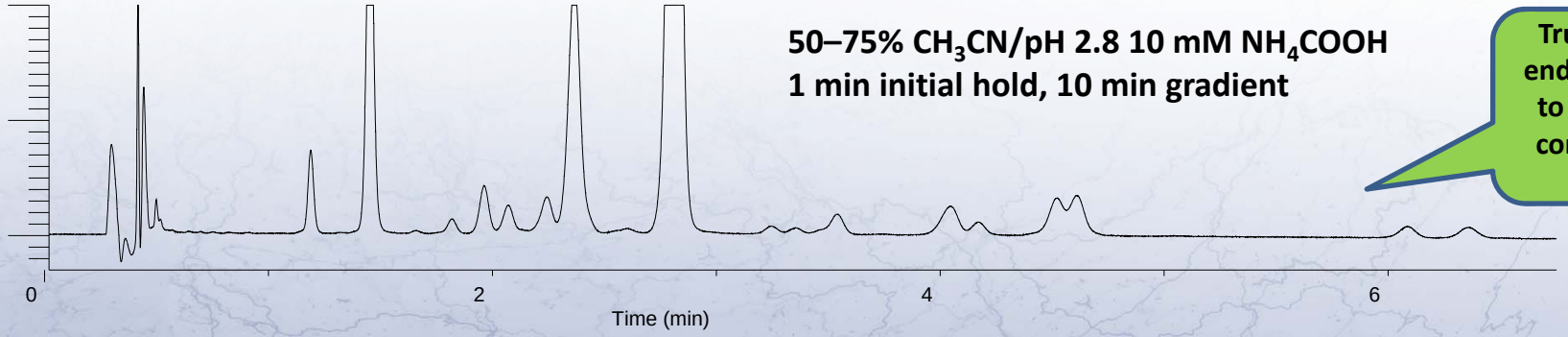
Mixture of acid- and base-treated atorvastatin calcium



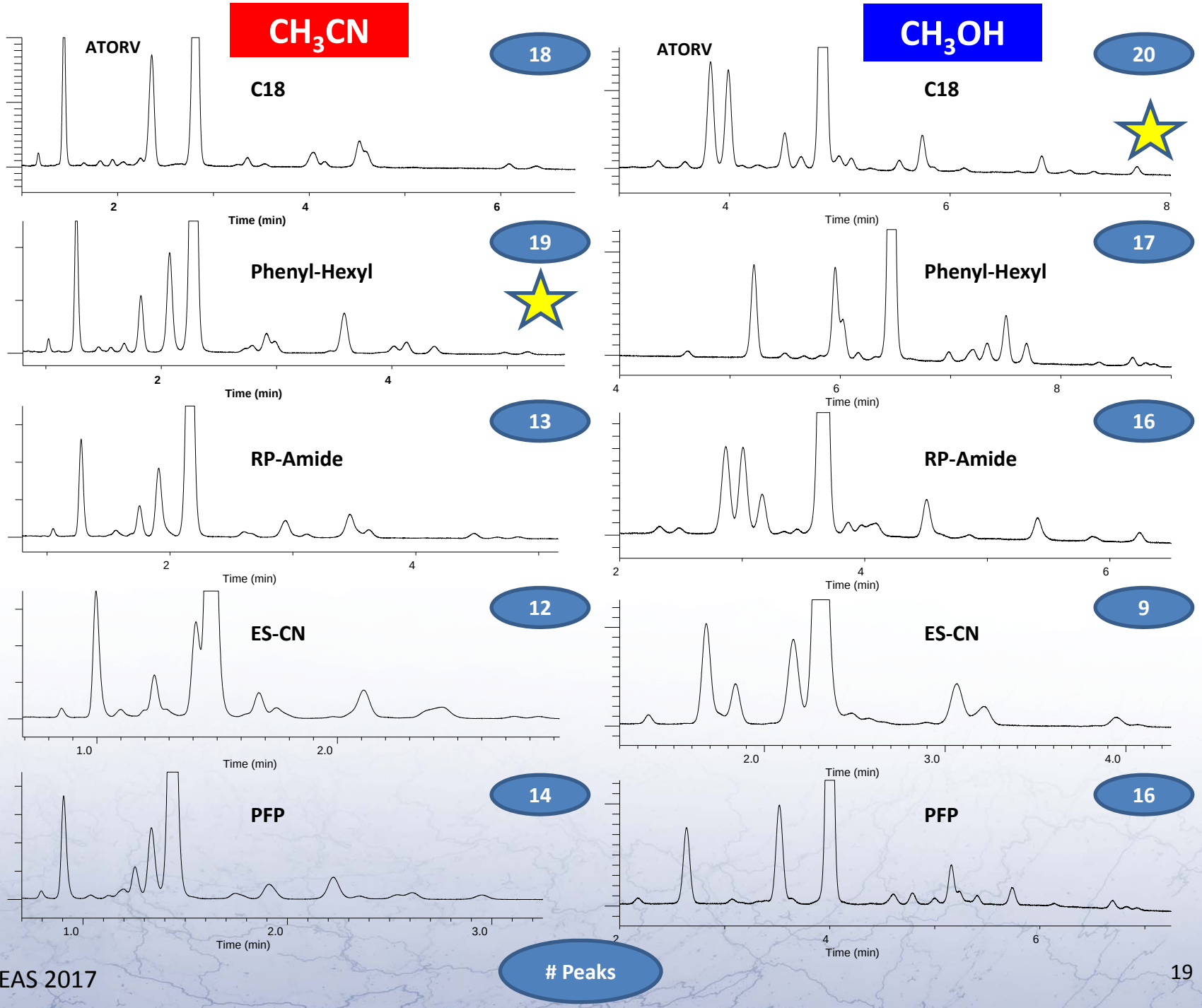
Initial screen with HALO C18



Raise starting % organic modifier

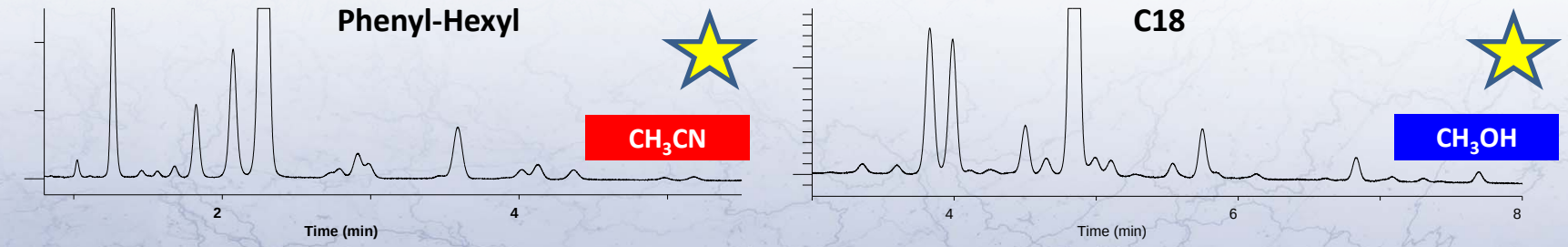


Truncate both ends of gradient to allow fairer comparison of phases


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



SUMMARY AND CONCLUSIONS

- 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

KEY FOR GRADING CHROMATOGRAMS

Means that latter 5 larger peaks are separated to a reasonable degree prior to optimization

Signifies the # of peaks in the 1st or 2nd half of the separation

Signifies the total # of peaks in the separation

Remind symbols verbally

METHOD DEVELOPMENT: TIME CONSIDERATIONS

Columns: 3 x 50 mm, 2.7 µm
Flow Rate: 0.6 mL/min
Temperature: 30 °C
Initial Gradient: 5–90% organic/buffer
Gradient Time: 10 min
Initial Hold: 0 initial, later 1 min

Agilent 1200 binary 600 bar system
Delay volume: 0.74 mL (from DryLab runs)
Hold 1 min at %B initial x 0.6 mL/min = 0.6 mL
Effective delay volume: 1.34 mL

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

<u>Column Treatment and Handling</u> (no col. switching valve)

1. 5 min: Flush column with 50:50 organic/buffer
2. 5–7 min: Flush column with 90:10 organic/buffer
3. 5 – 7 min: Equilibrate column with 2:90 organic/buffer
4. 2 replicate gradient runs
5. End of use: Flush with 50:50 organic/water, 7 min
6. End of day: Flush with 100% organic (CH_3CN), 7 min

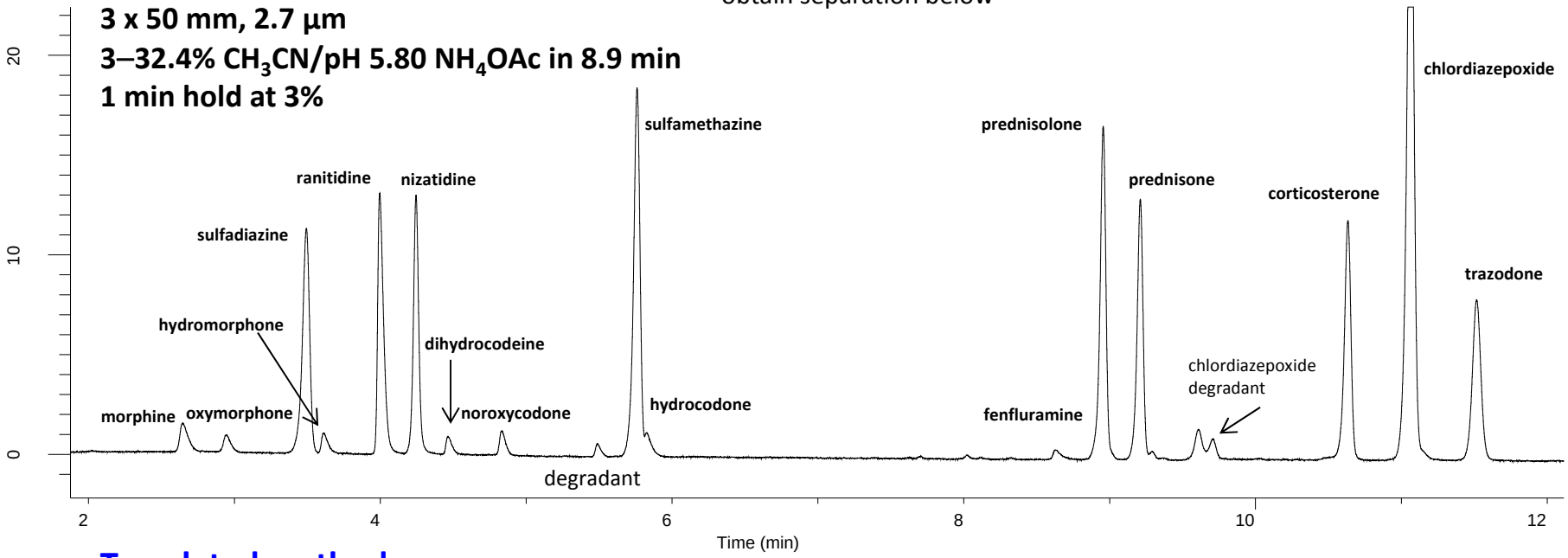
Carried out 10 and 30 min gradients and used DryLab 4 to obtain separation below

Phenyl-Hexyl

3 x 50 mm, 2.7 µm

3–32.4% CH_3CN/pH 5.80 NH_4OAc in 8.9 min

1 min hold at 3%

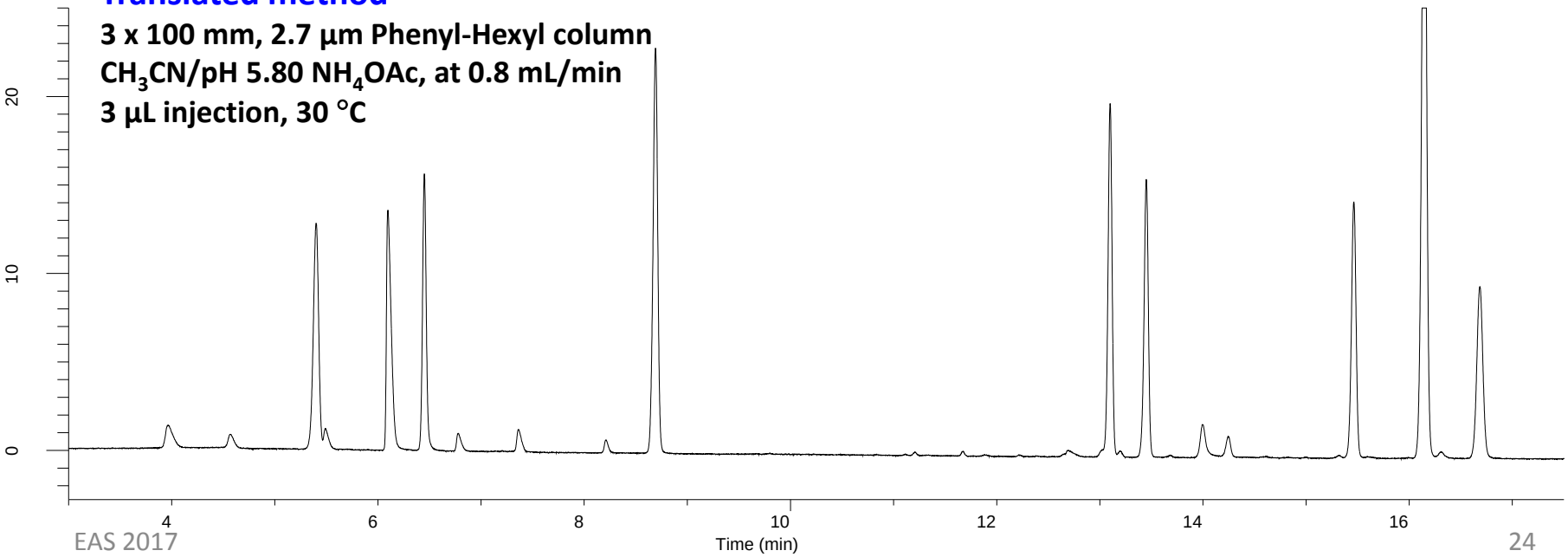


Translated method

3 x 100 mm, 2.7 µm Phenyl-Hexyl column

CH_3CN/pH 5.80 NH_4OAc, at 0.8 mL/min

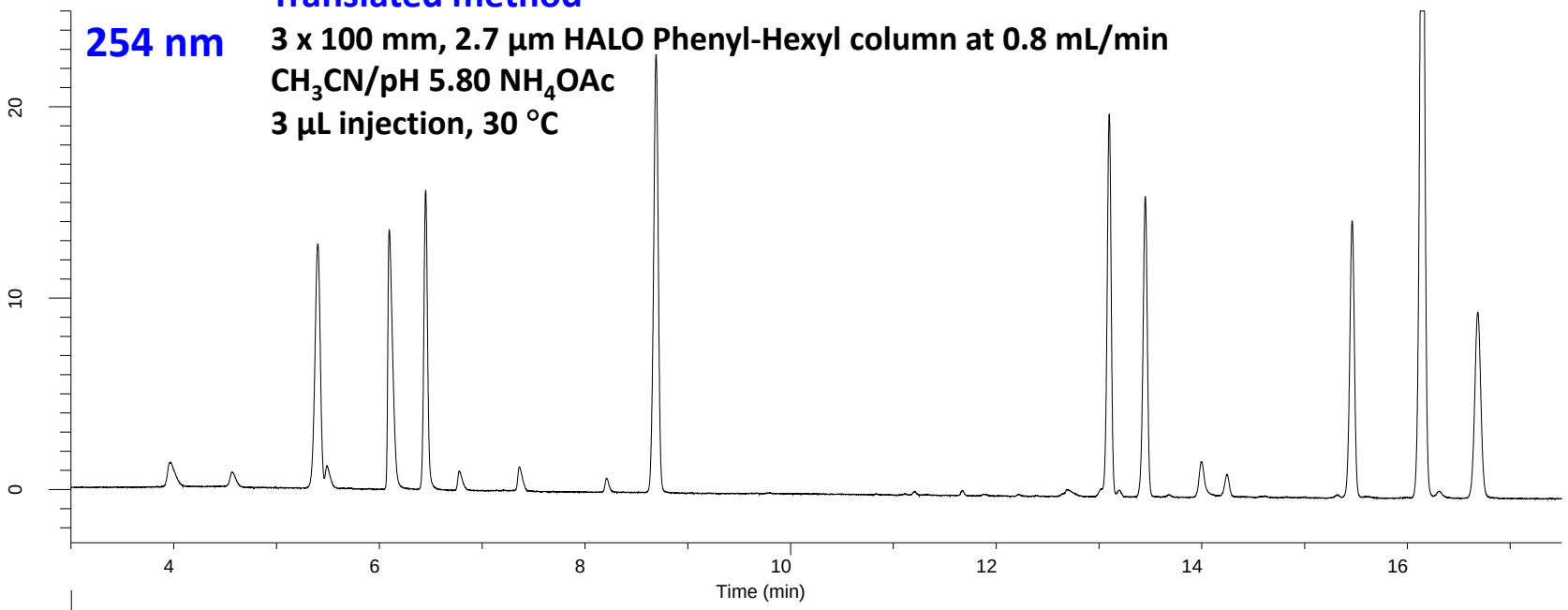
3 µL injection, 30 °C



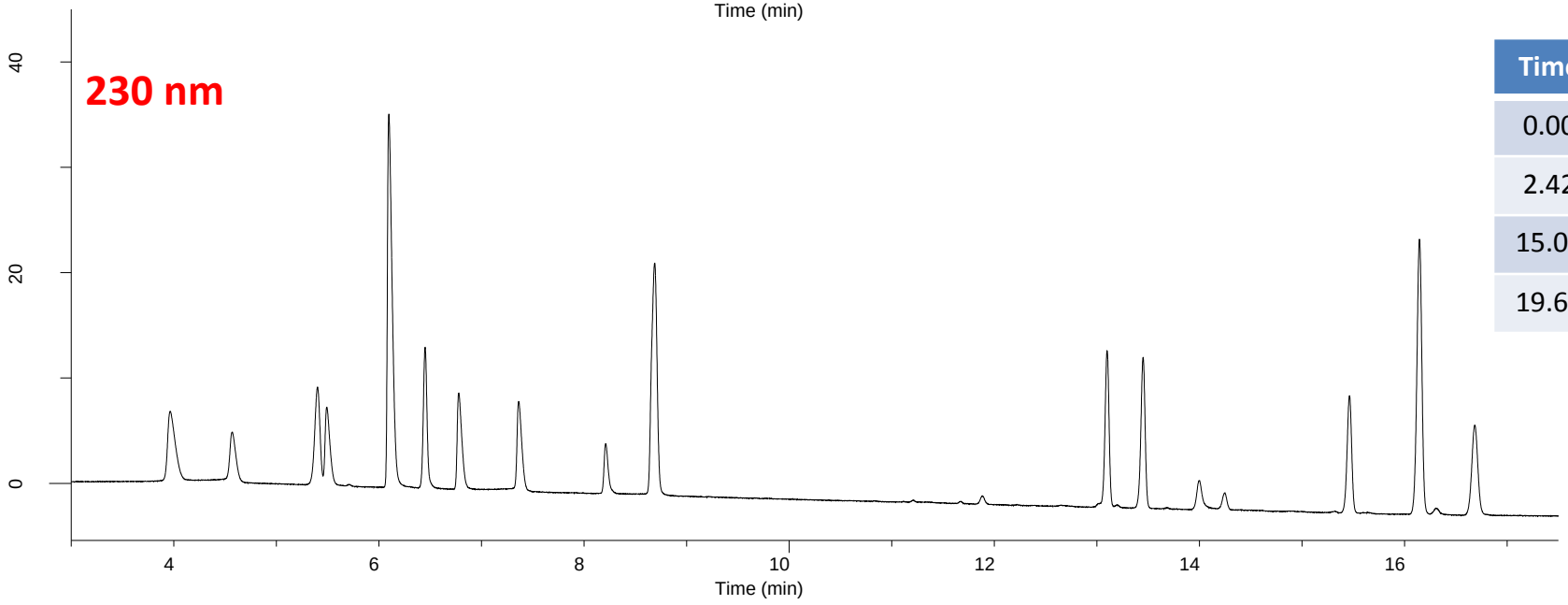
Translated method

254 nm

3 x 100 mm, 2.7 µm HALO Phenyl-Hexyl column at 0.8 mL/min
CH_3CN/pH 5.80 NH_4OAc
3 µL injection, 30 °C



230 nm



Time	%B
0.00	3
2.42	3
15.02	32.4
19.67	32.4