Screening and Optimization Designs to Improve Method Performance and Robustness

John F. Kauffman, Ph.D.

Daniel J. Mans, Ph.D.

FDA Division of Pharmaceutical Analysis

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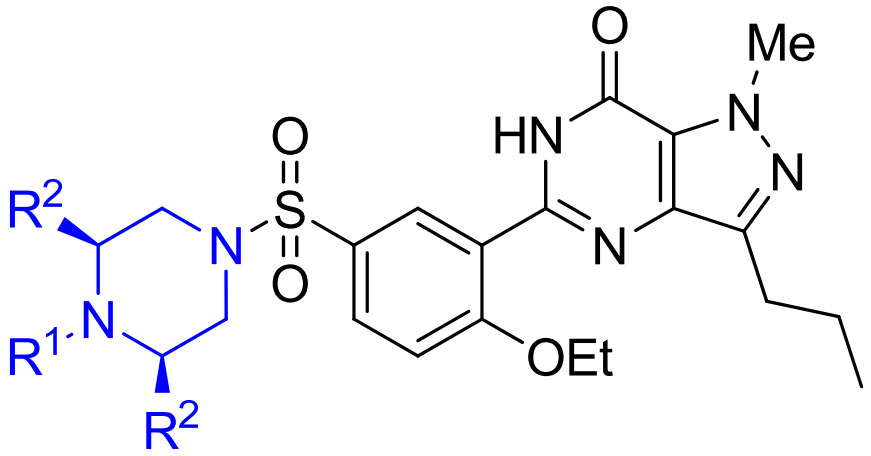
Research Problem Statement

- FDA will develop a method using the QbD paradigm, and transfer the method to an EMA lab.
  - Begin with a harmonized compendial method and apply QbD concepts to improve the method
  - Method: HPLC analysis of sildenafil and analogues of sildenafil

Sildenafil and some Analogues



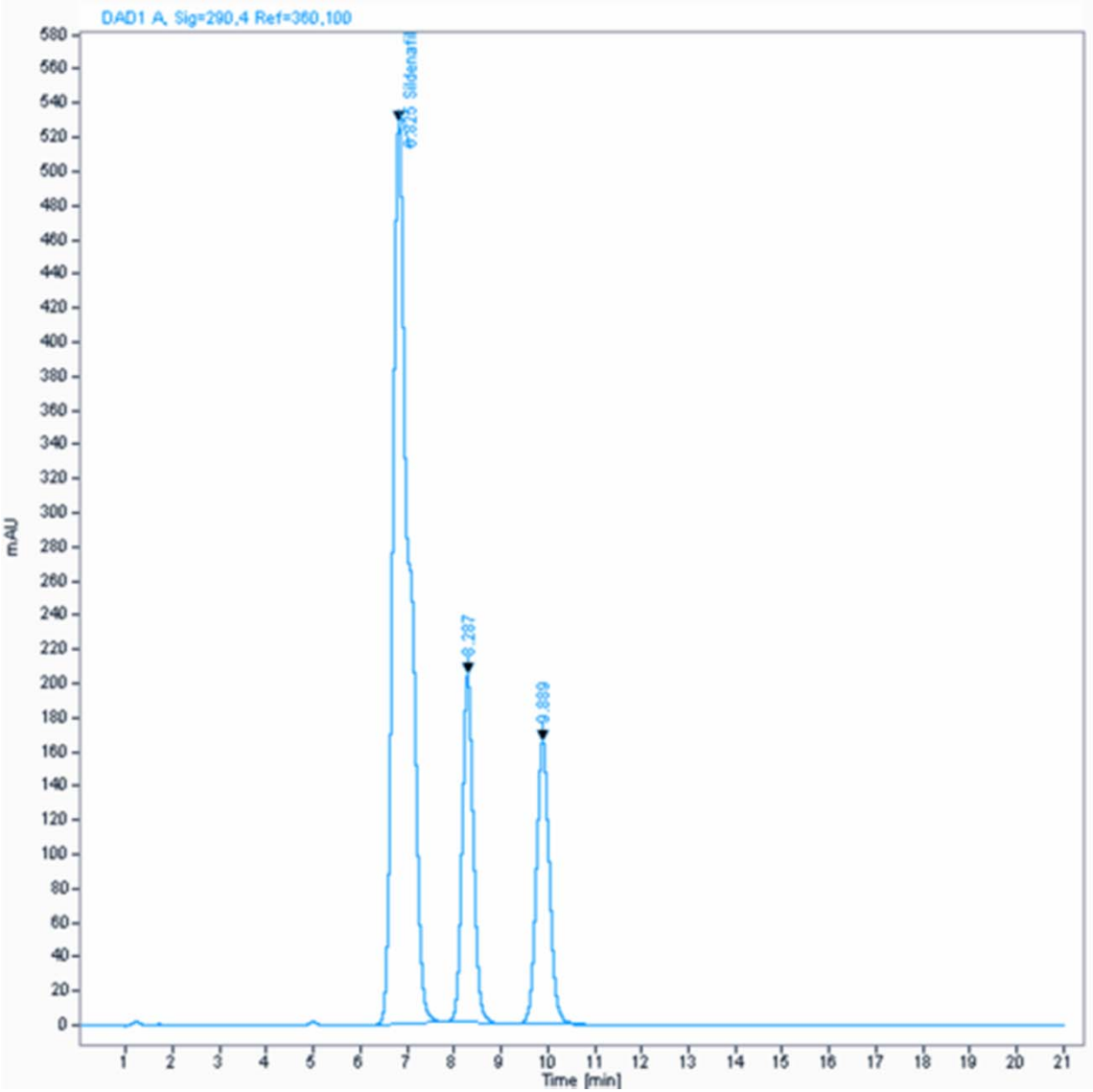
R^1 = Me; R^2 = H Sildenafil
R^1 = CH_2CH_3; R^2 = H homosildenafil
R^1 = CH_2CH_2OH; R^2 = H Hydroxyhomosildenafil
R^1 = H; R^2 = H *N*-desmethylsildenafil
R^1 = H; R^2 = CH_3 *N*-desmethylsildenafil
R^1 = cyclopentyl; R^2 = H Cyclopentynafil

*Pre-existing analogue library prepared for rapid screening surveillance program; Harmonized Method exists

Example ATP

- The method will separate 6 compounds with high specificity (HPLC resolution ≥ 1.5)
- Quantify each compound at levels from 25 ug to 100 mg per gram of finished product.
  - Multiple dilutions may be required
- Repeatability: $\leq 2\%$ over six replicates
- Accuracy: within $\pm 15\%$ of the true value at 25 ug and within $\pm 2\%$ of the true value at 100 mg, with 95% confidence.

Starting Point: USP Method for Sildenafil



- Isocratic: 57/28/15 Buffer/Methanol/CH_3CN

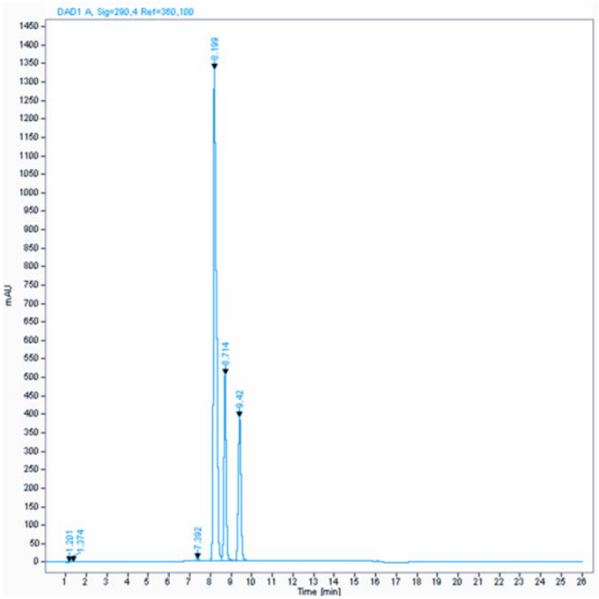
(Buffer = Phosphoric acid, pH 3 with triethylamine)

- C18 column
- 30 °C

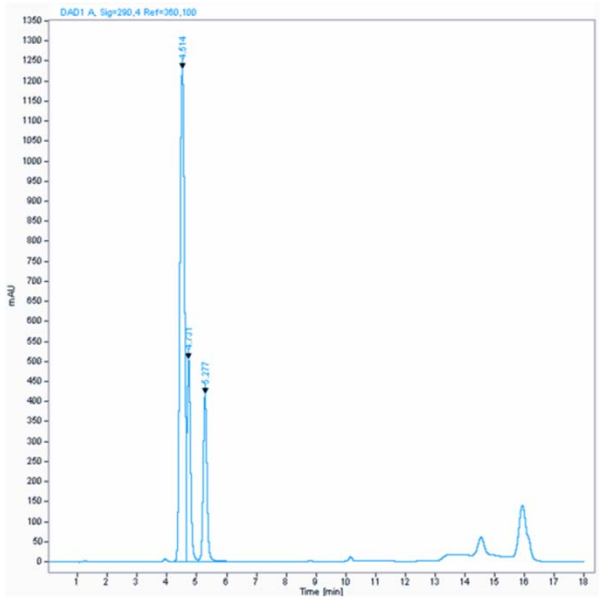
- Poorly separated: 6 compounds → 3 peaks

Initial Studies: Mobile Phase Evaluation

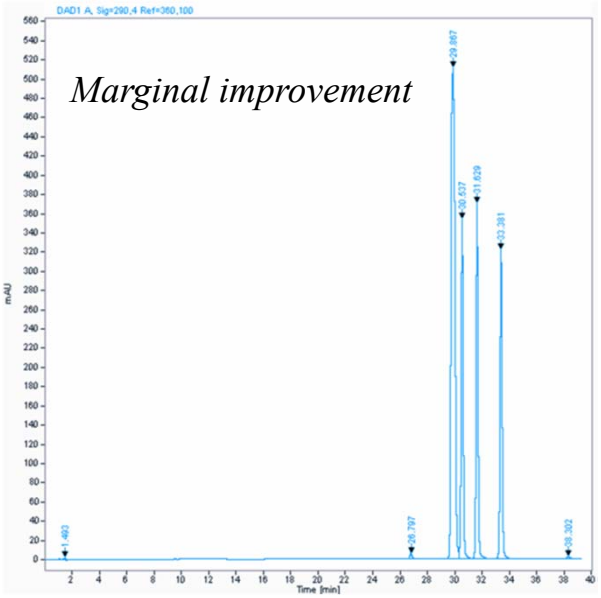
- Change from Isocratic to Gradient (A=Buffer, B=MeOH/CH_3CN)? Remove CH_3CN? Remove Methanol?



A=Buffer
B=MeOH/CH_3CN (25/17)



A=Buffer
B=MeOH



A=Buffer
B=ACN

Summary and Conclusion of Initial Screen

- 6 columns screened (4 C18, 2 PFP): Results did not conform with theoretical expectations
- Varied combinations of mobile phases and gradient times
- Began to investigate pH effects: 4.5 vs. 3.0
  → affords separation of the 6 components but
  does not meet criteria of the ATP

❖ Time consuming and tedious one-variable-at-a-time conventional approach. Difficult to keep track of numerous generated method files.

A Systematic QbD Approach

- Develop screening designs to evaluate diverse method options
- Use DOE methodology to predict optimal conditions
- Use statistical analysis to determine ranges of acceptable operating parameters - Robustness

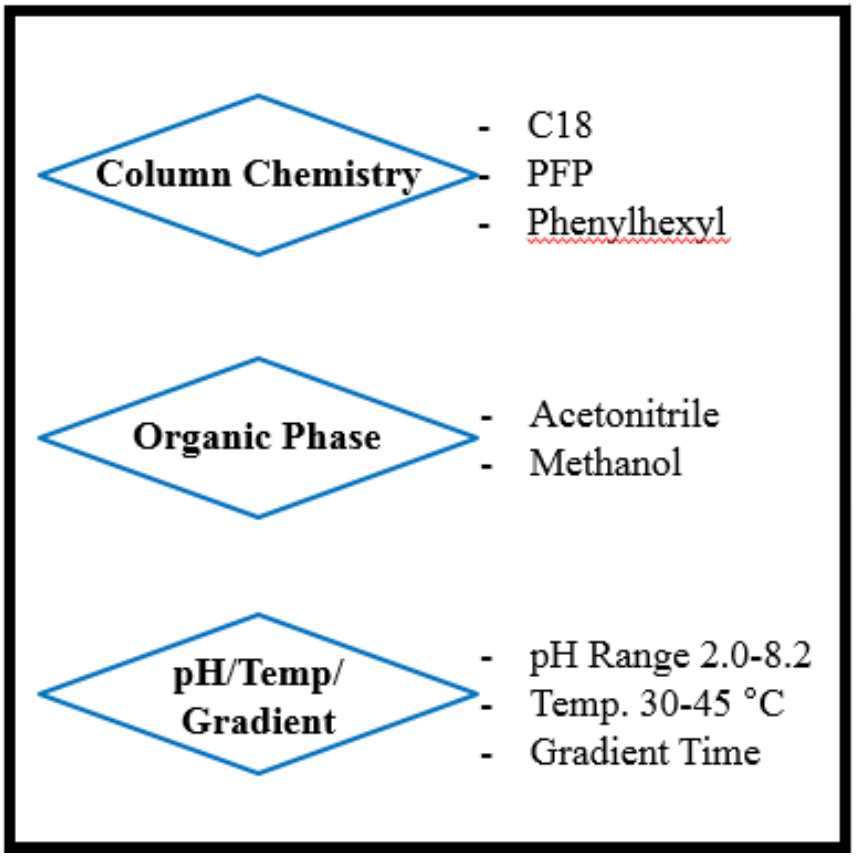
- Implemented using S-Matrix Fusion QbD Software

Three Screening Designs

- 1. Broad screen of 3 columns, 2 organic phases, pH and gradient time. (37 experiments)
  - Purpose: Identify the best column, pH range
- 2. Fix column and screen 2 organic phases, most promising pH range, gradient time (19 experiments)
  - Purpose: Select most promising organic phase, further narrow pH range
- 3. Fix column and organic phase, screen pH, gradient time, column temperature (16 experiments)
  - Purpose: Final method, operable design region

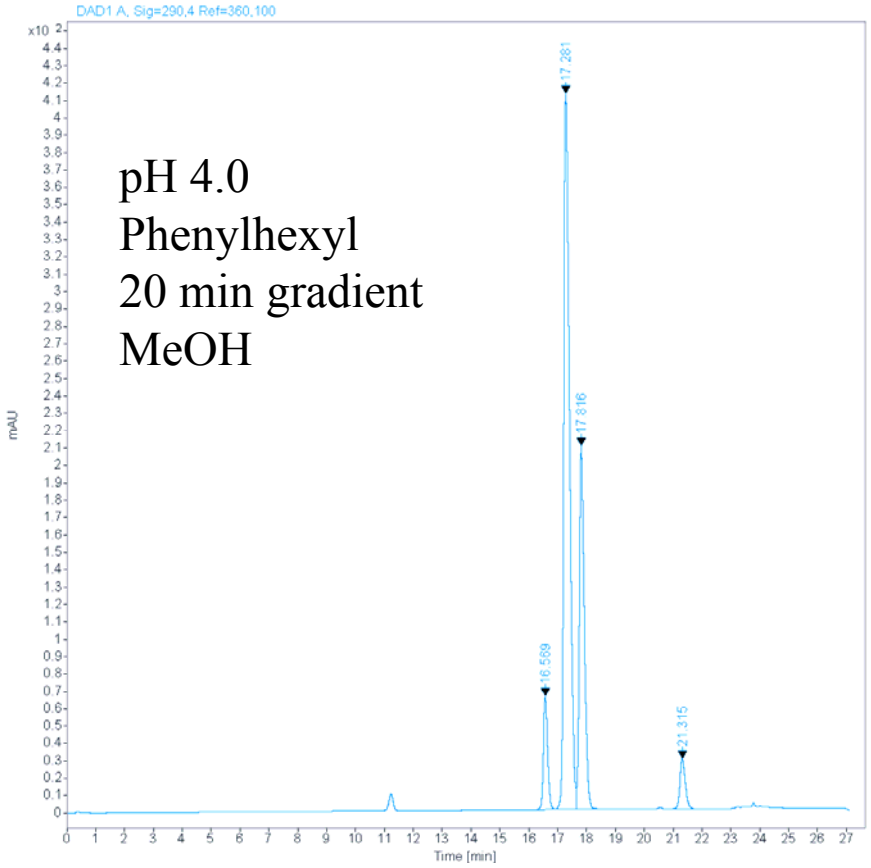
Screen 1: Best Column (37 Experiments)

- Columns: analytical columns of same ID and length from same supplier
- Mobile Phase
  - MeOH and ACN
  - 10 mM buffer @ pH 4.0, 5.0, 6.0, 7.0, 8.2
- Gradient Time: 4-20 minutes (10-55% organic)
- Fixed column temperature (30 °C)

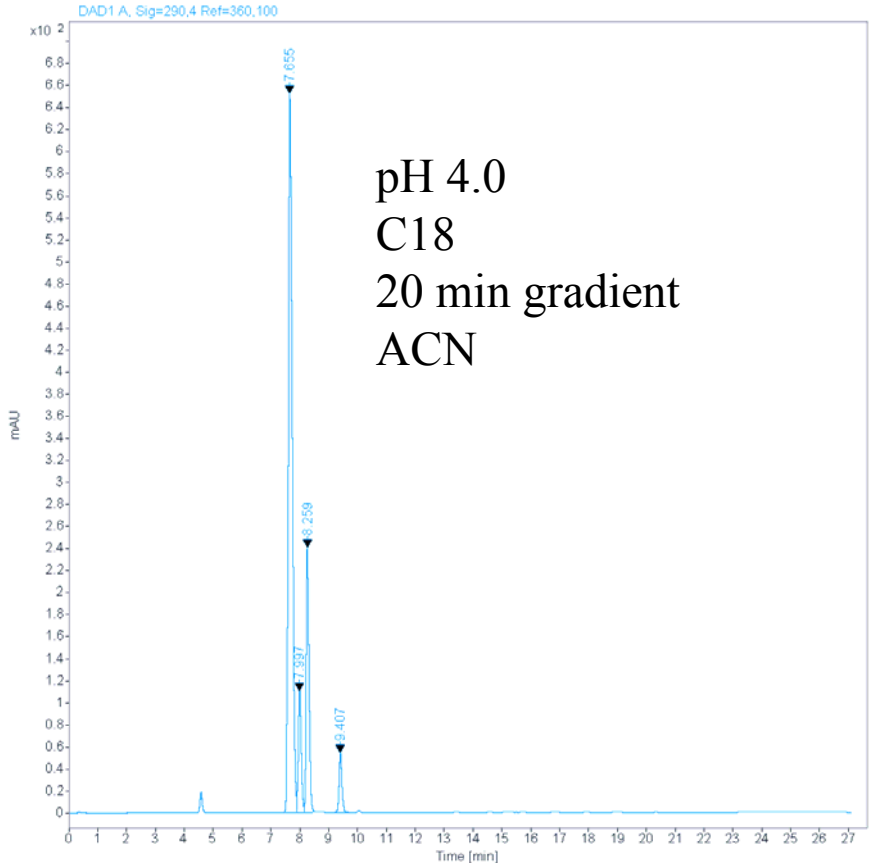


Column Screening: A Few Examples

- Low pHs (3.0, 4.0) gave the least # peaks (recall USP pH 3.0)



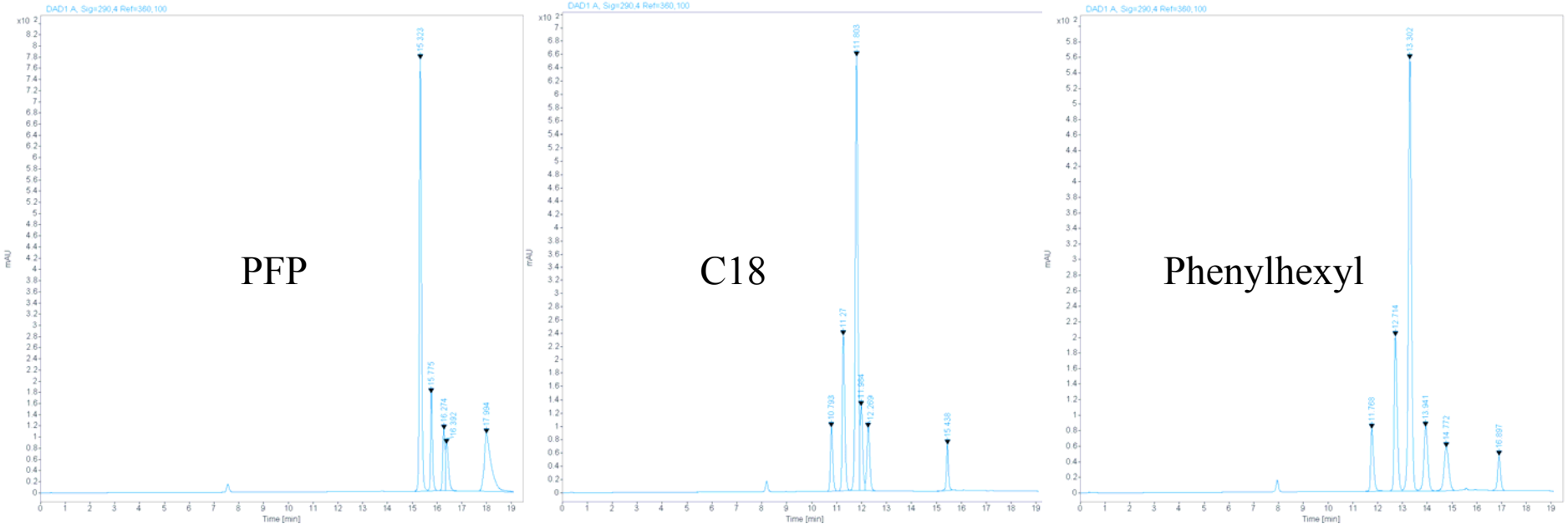
pH 4.0
Phenylhexyl
20 min gradient
MeOH



pH 4.0
C18
20 min gradient
ACN

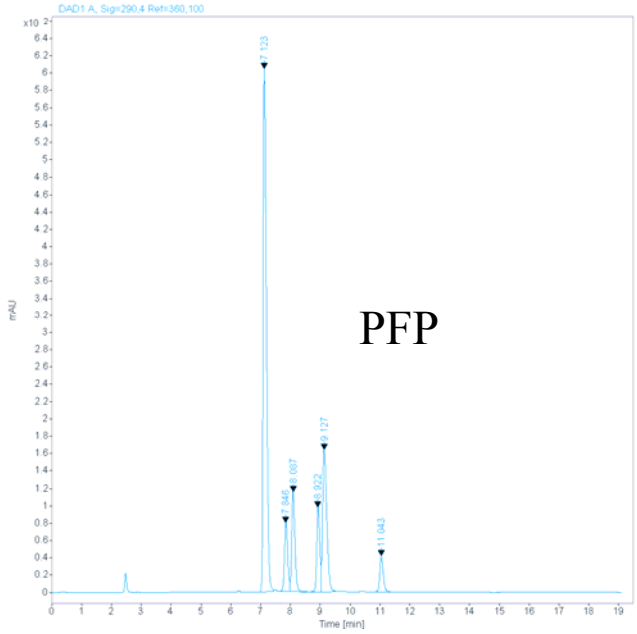
Column Screening: A Few Examples

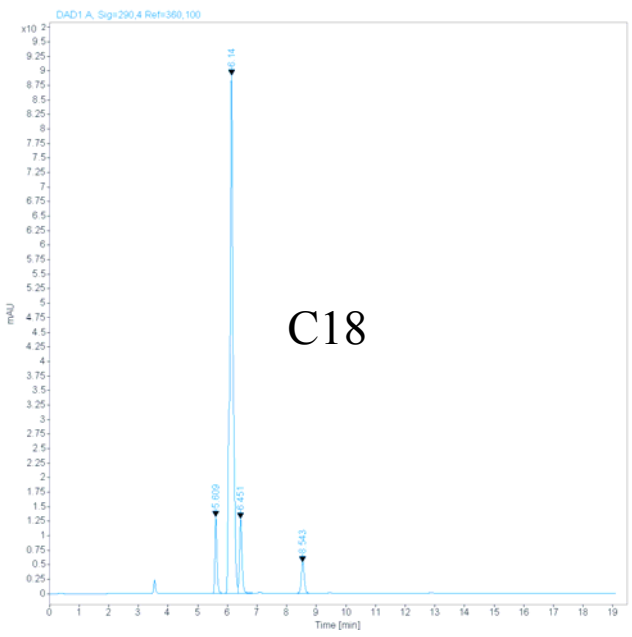
- Constant: pH 5.0, MeOH, 12 min gradient

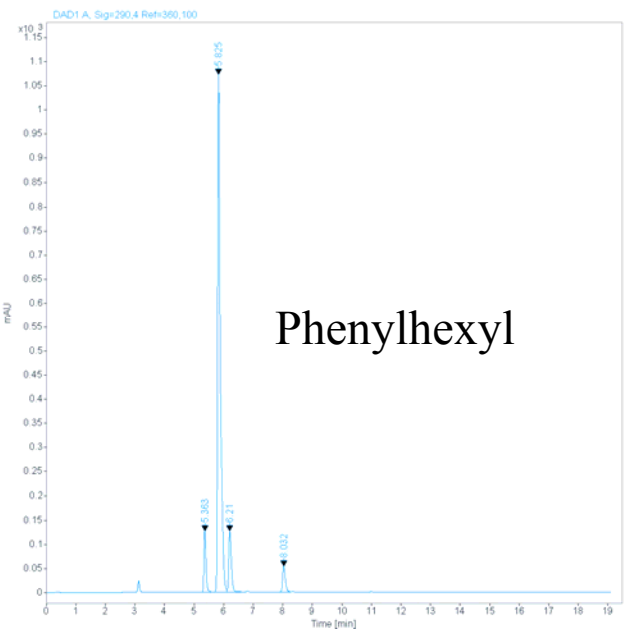


Column Screening: A Few Examples

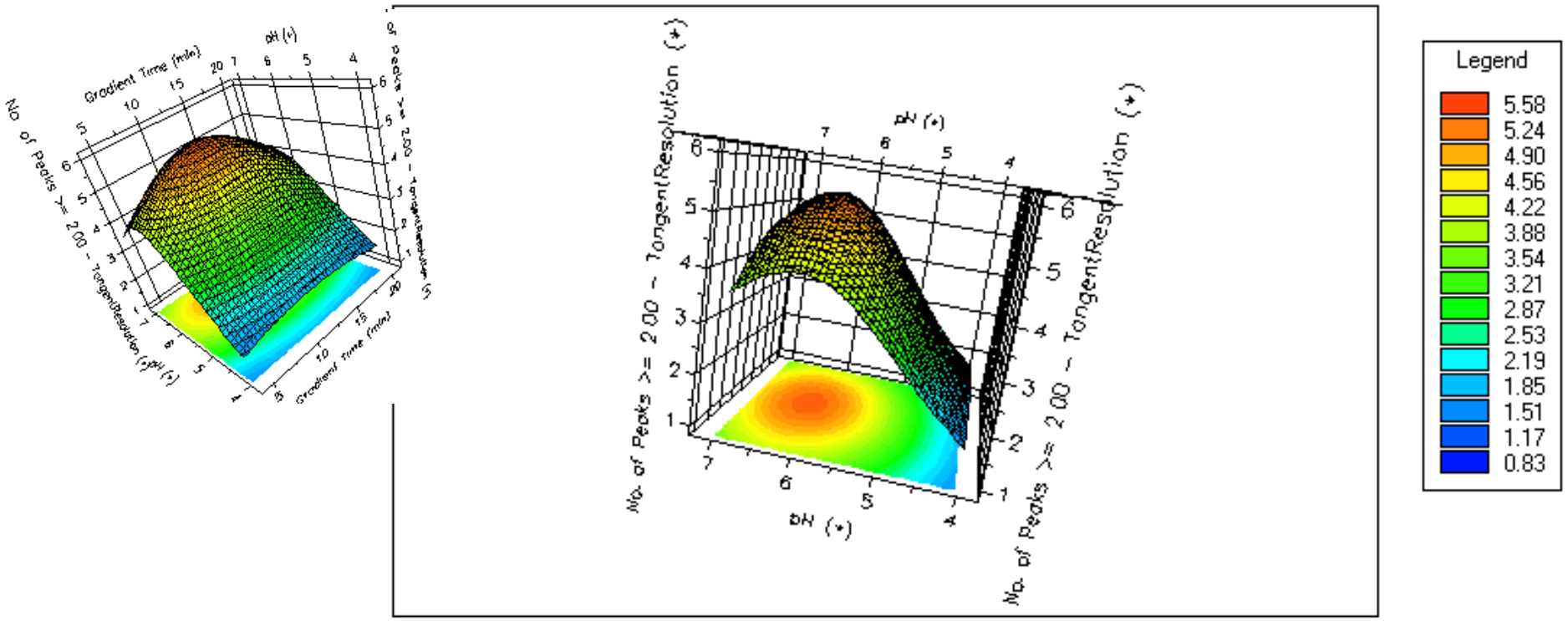
- Constant: pH 5.0, ACN, 12 min gradient





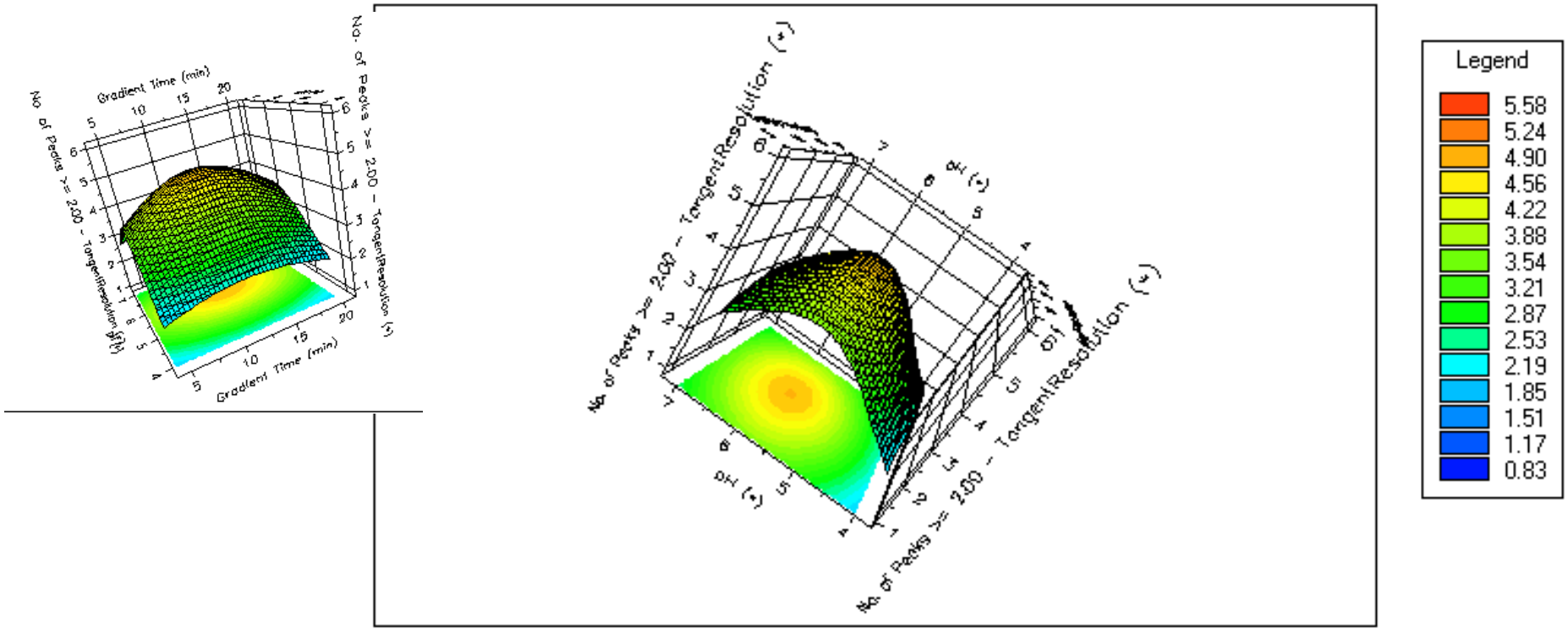


Number of peaks with resolution ≥ 2: ACN Phenylhexyl



Modeling predicts pH ~6-6.5 optimal for ACN with 10-17 min gradient times (using the resolution ≥ 2.00 metric)

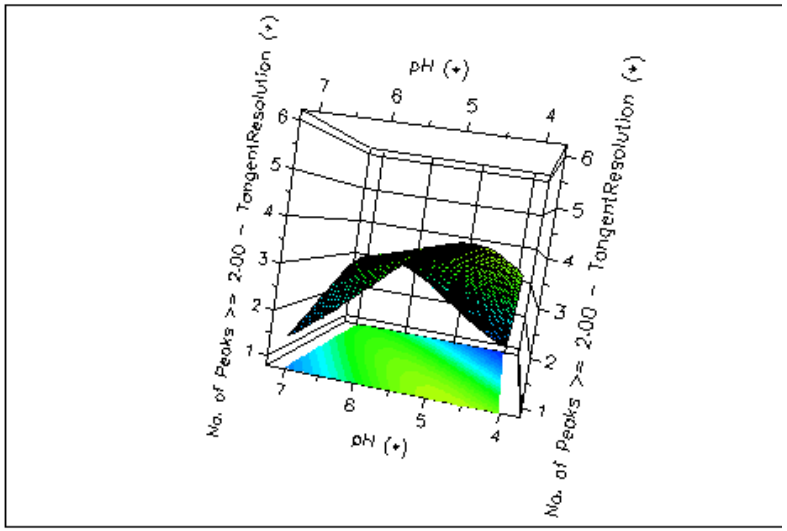
Number of peaks with resolution ≥ 2: MeOH Phenylhexyl



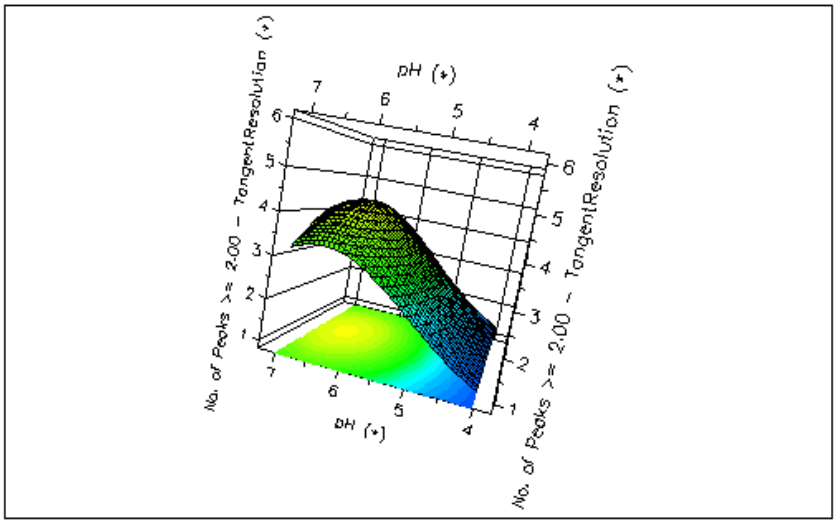
Modeling predicts pH 5.5-6.0 optimal for MeOH with 10-17 min gradient times

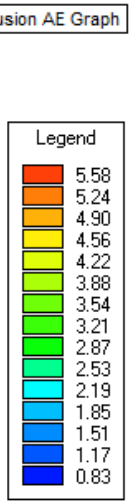
By comparison PFP and C18 have about 4 peaks with resolution ≥ 2.00

MeOH PFP



MeOH C18



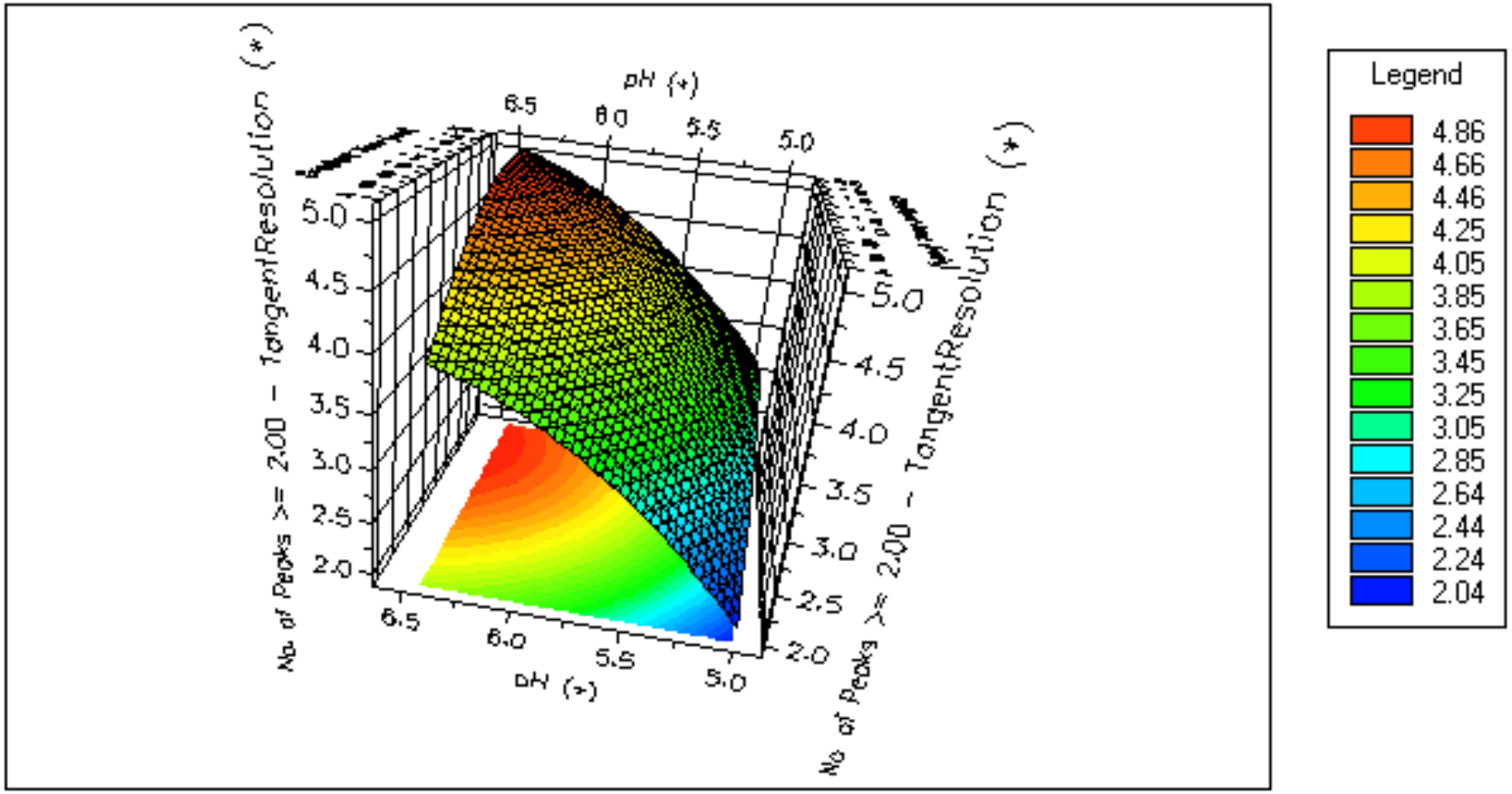


Best Overall Answer: Phenylhexyl

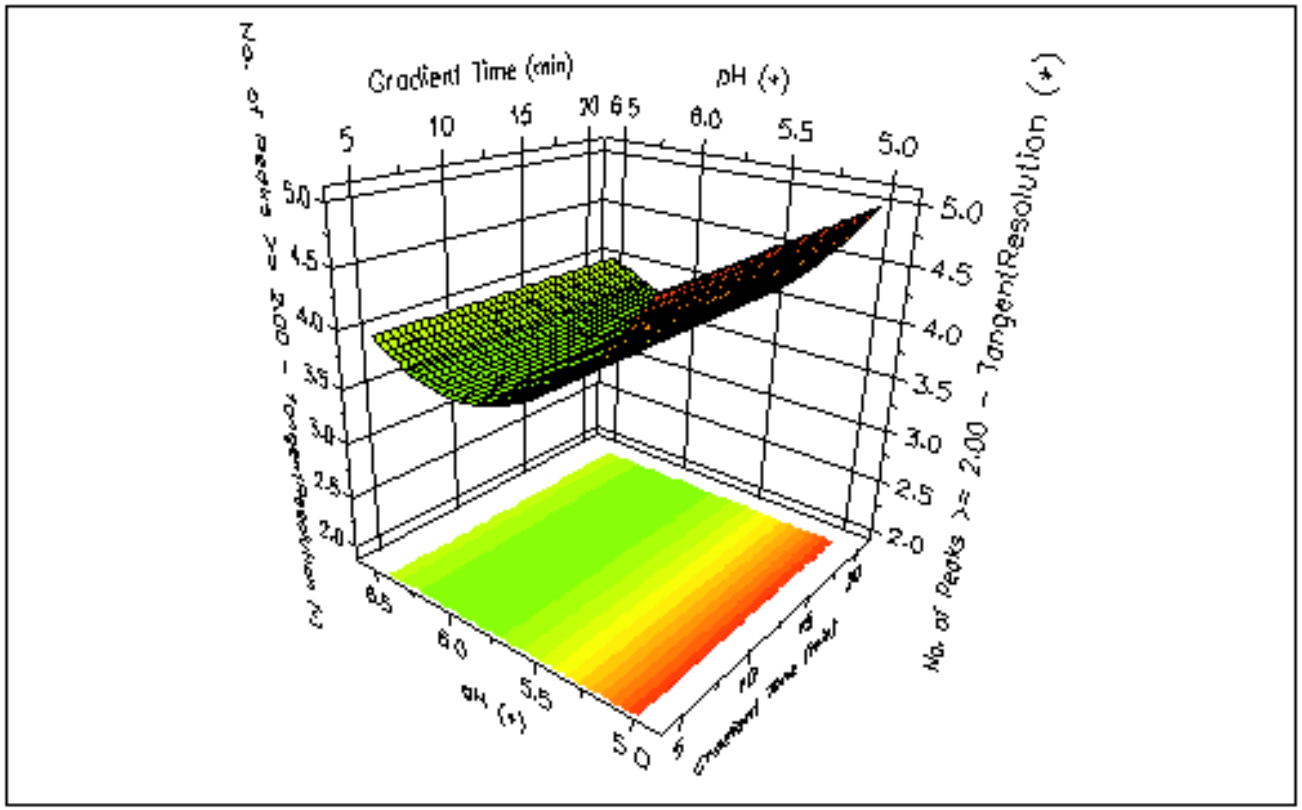
Screen 2 (19 Experiments)

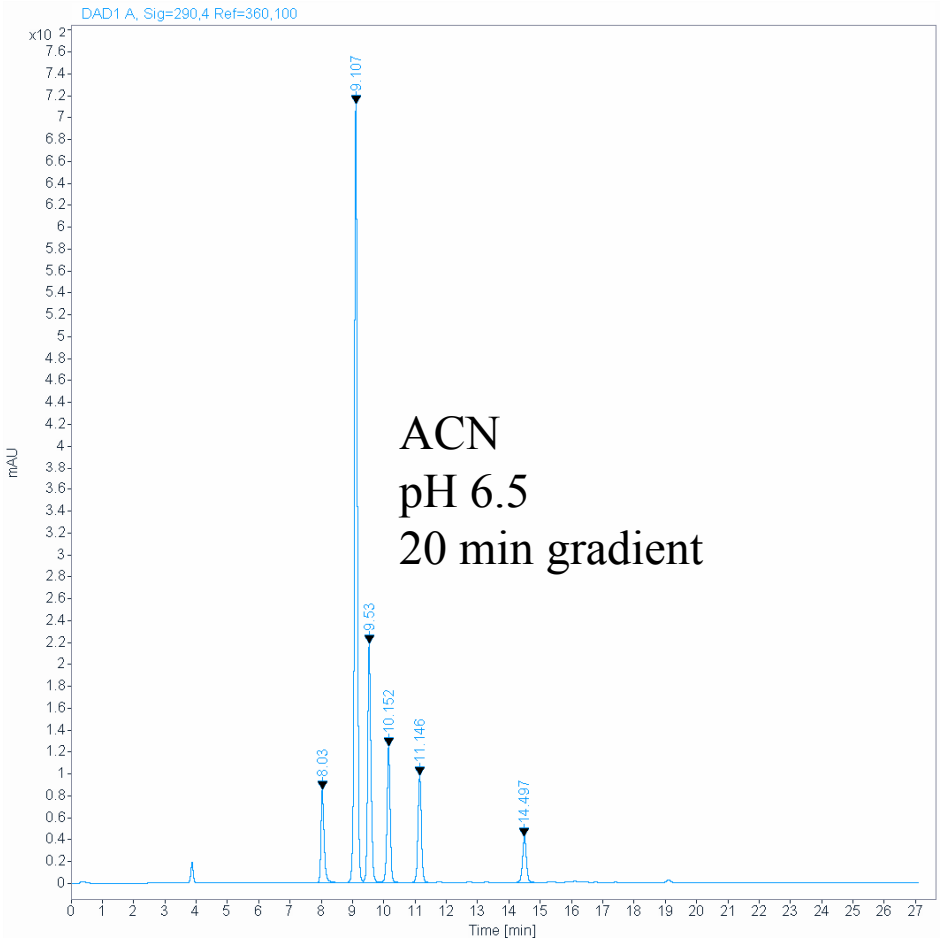
- Phenylhexyl column
- pH 5.0, 5.5, 6.0, 6.5
- ACN vs. MeOH
- Gradient Time: 4-20 minutes (10-55% organic gradient)

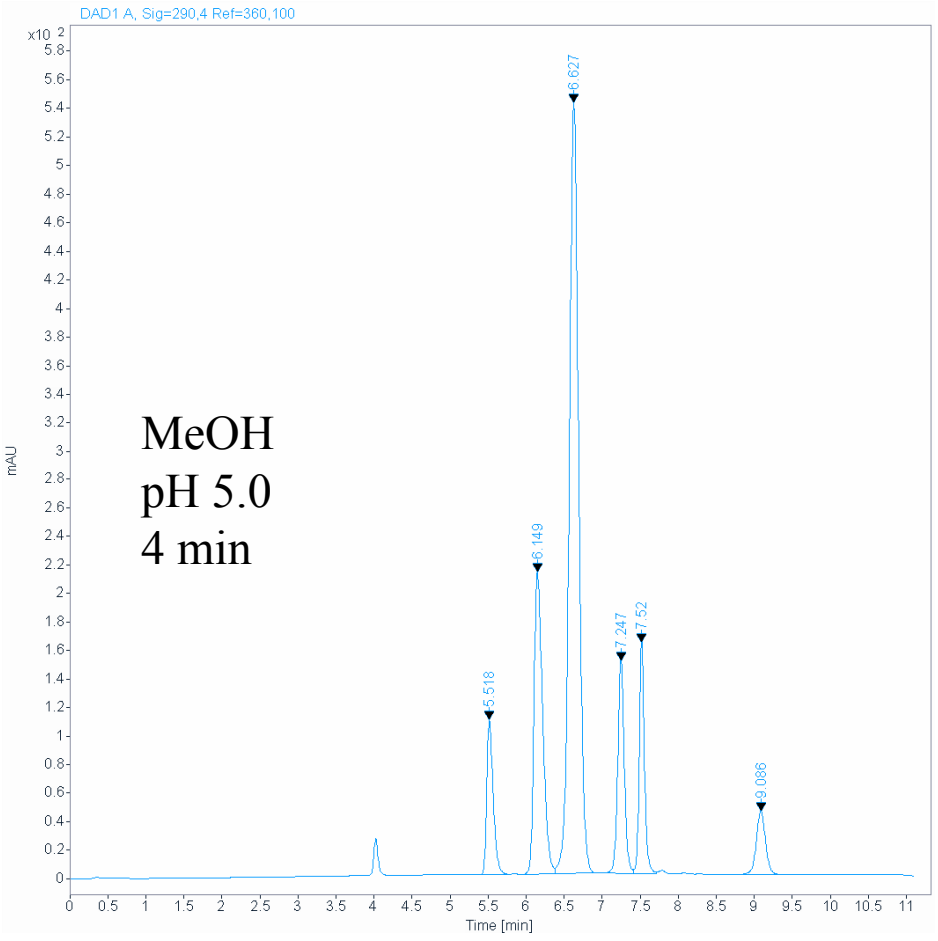
Number of peaks with resolution ≥ 2: ACN Phenylhexyl



Number of peaks with resolution ≥ 2: MeOH Phenylhexyl







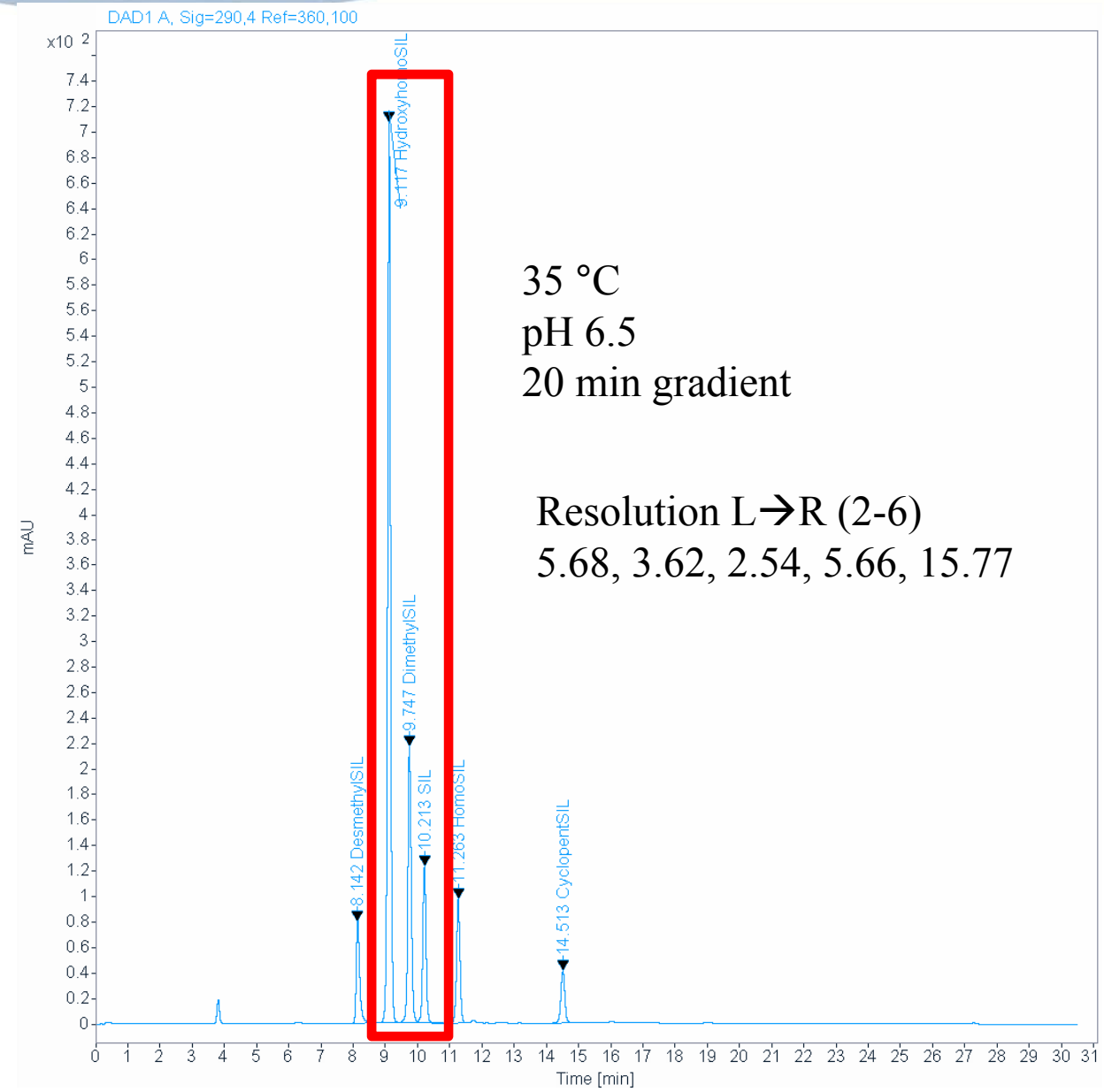
- Phenylhexyl elution order of Peaks 2 & 3 (L→R) changes between MeOH and ACN
- Peak Areas also change
- Both solvents viable for the ATP, ACN chosen for # plates, sharpness of peaks, and slightly better resolution

Screen 3 (16 Experiments)

- Phenylhexyl & ACN constant
- pH 5.90, 6.10, 6.30, 6.50
- Column temp 30, 35, 40, 45 °C
- Gradient Time: 10-20 minutes (10-55% organic gradient)

Sample of Screen 3 Experiments

Run No.	Sequence No.	Gradient Time	Oven Temperature	pH	No. of Peaks
Condition Column - 1	1	2	30	5.9	
1	1	20	30	5.9	5
2	1	10	30	5.9	6
3	1	10	30	5.9	6
Condition Column - 2	1	2	35	6.11	
4	1	15	35	6.11	6
5	1	15	35	6.11	6
6	1	17.5	35	6.11	6
7	1	12.5	40	6.11	6
Condition Column - 3	1	2	45	5.9	
8	1	15	45	5.9	5
Condition Column - 4	1	2	45	6.11	
9	1	20	45	6.11	6
10	1	10	45	6.11	6
Condition Column - 5	1	2	45	6.11	
Condition Column - 6	2	2	30	6.51	
11	2	15	30	6.51	6



35 °C
pH 6.5
20 min gradient

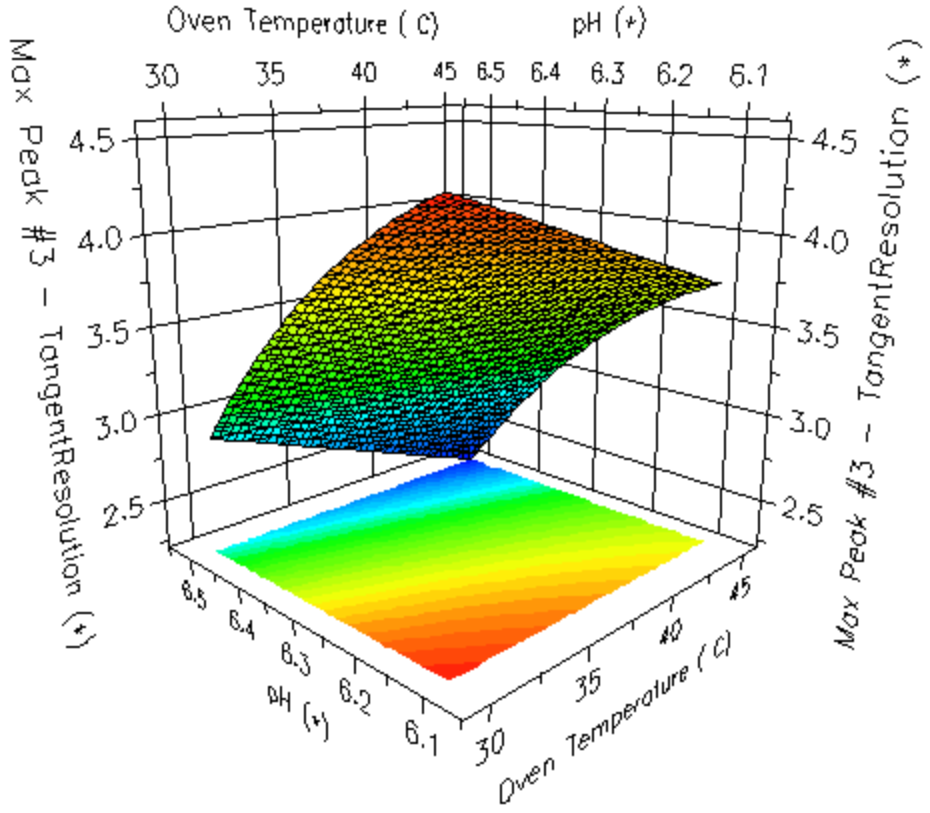
Resolution L→R (2-6)
5.68, 3.62, 2.54, 5.66, 15.77

Example of a Resolution Model Eqn.

- Peak 3 resolution

$$R = 3.0607 + 0.4109(GT) - 0.3367(Temp) - 0.7772(pH) - 0.2013(pH)^2$$

Example of a Resolution Model Eqn. Predicted Response



Analysis of Robustness

- Method capability: Resolution criteria

$$C_{pk} = \frac{R - LSL_{ATP}}{3\sigma}$$

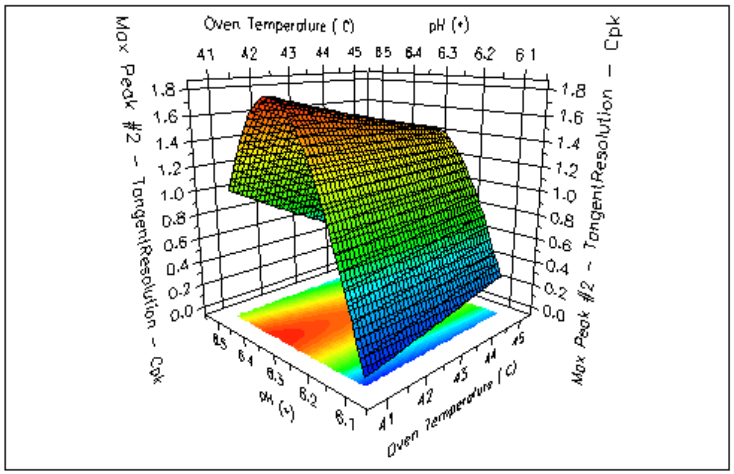
σ = response standard deviation

- Monte Carlo simulation using model equation estimates σ for specified response
  - pH ± 0.1, Temp ± 2°C, Gradient ± 0.25 min
  - Normally distributed
- Require $C_{pk} \geq 1.33 \rightarrow R - 1.5 \geq 4\sigma$.

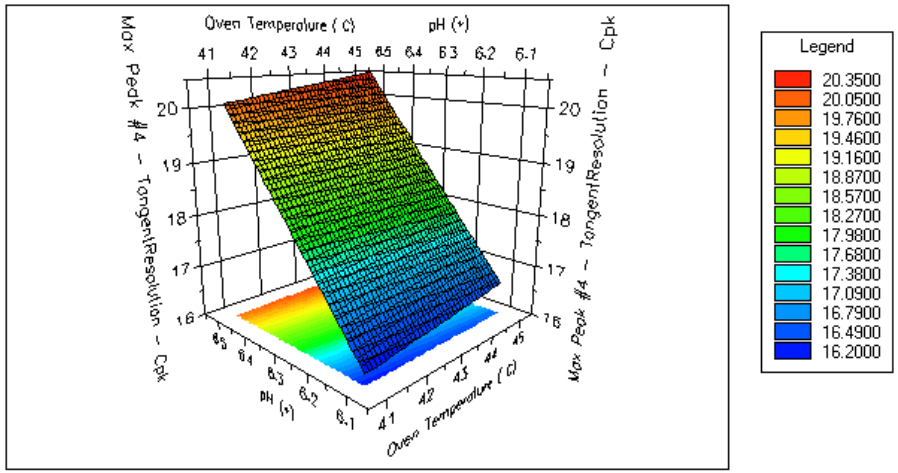
C_{pk} of Res_{1-2} : Range = 0 - 1.75, Robust region at surface ridge, sensitive to pH*Temp.

C_{pk} of Res_{3-4} : Range > 16, linear in pH but not Temp.

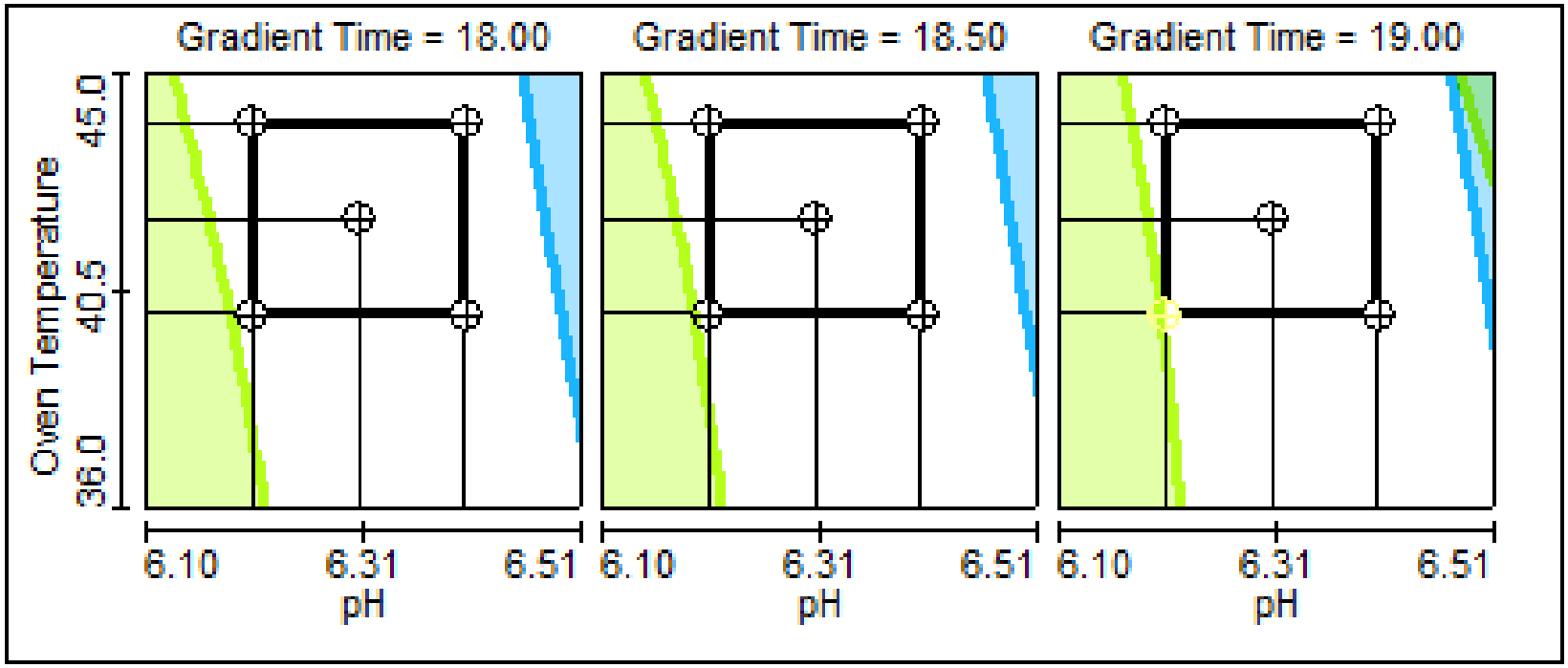
Peak 2 resolution Cpk: Gradient Time – 20 min.



Peak 4 resolution Cpk: Gradient Time – 20 min.



Method Robustness: Operable Region



- Corners: C_{pk} = 1.33 for Resolutions 2, 3 and 4
- Ranges: pH 6.30 ± 0.1, Gradient 18.5 ± 0.5 min, Temp 42 ± 2 °C

Optimal Conditions

- Phenylhexyl is the best column
  - Literature methods use C18
- Acetonitrile gives best peak shape and resolution.
  - MeOH/Phenylhexyl can support a method that meets the ATP. This is extremely useful information for method understanding
- Gradient time, pH, column temperature have been optimized

Future Work and Interesting Questions

- Method validation for quantitative work
- Further exploration of method robustness and ruggedness
- Designing methods and models that incorporate multiple columns and organic phases

Acknowledgements

- Sergey Arzhantsev: IT support
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Thank You!