Experimental Design and Modeling to Improve HPLC Method Performance for Small Molecules

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Disclaimer:
This presentation reflects the views of the author and should not be construed to represent FDA's views or policies.

Overview

- Analytical procedures (APs) are a key part of the control strategy for a product.
- Many factors can influence analytical results.
- "Enhanced approaches for development of analytical procedures" (a.k.a. AQbD) can improve method robustness and understanding.
- Terminology
  - Analytical Target Profile
  - Method Operable Design Region

Analytical Target Profile

- The ATP is a prospective summary of measurement requirements that ensure a procedure is “fit for purpose”
  - ATP may be method independent
- Regulatory considerations for implementing ATP
  - Not all methods with same ATP are inter-changeable
    - E.g. from HPLC to NIR
  - Can use comparability protocols

Method Operable Design Region

- Analytical method design space
  - Typically Design of Experiments is used to find ranges for instrument operating parameters and understand sources of variation.
  - Method performance criteria are response factors.
  - Can be conducted together with method validation.
- Considerations for implementing MODR
  - Availability of adequate data to support proposed MODR
  - Assess validation criteria across MODR
  - Confirm system suitability throughout MODR

Current Status

- FDA has approved some NDA applications applying QbD approach to analytical procedures.
- Regulatory flexibility has been granted for movements within the defined MODR.
  - Movements within an approved “Analytical Design Space” are not considered a change in method.

How do we evaluate Enhanced APs at the CDER Division of Pharmaceutical Analysis ?

- DPA Program: Verification of methods submitted in new drug applications
  - Limited experience for Enhanced APs
  - Focus on model equations to select experiments
  - Select conditions for evaluation at MODR extrema based on the sign (+ or -) of the model coefficients
- DPA Research: Develop an Enhanced AP to expand our understanding.

FDA-EMA Collaborative Research on QbD for Analytical Methods

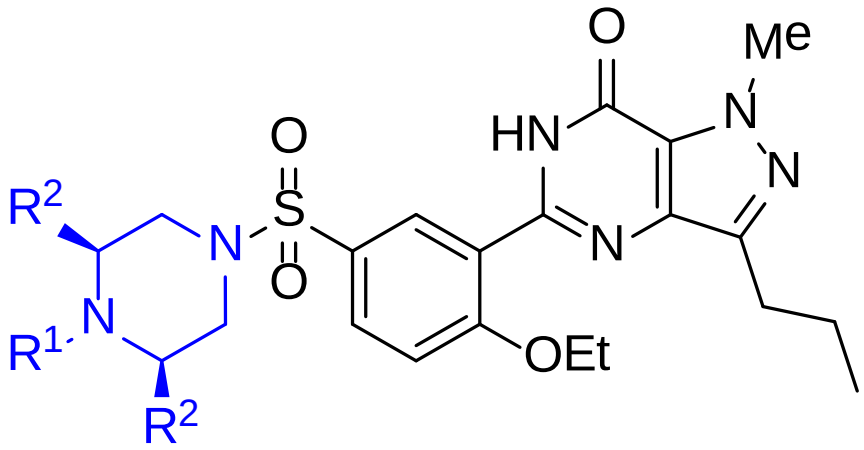
- Joint research with FDA's laboratory/review divisions and EMA
  - Initiated in January, 2013
- Goal of this project is to:

  - Develop analytical methods (e.g. HPLC) based on QbD paradigm.
  - Define protocols for method transfer.
  - Establish methodology for validation of MODR upon site transfer.
  - Define review criteria for evaluation of QbD based analytical methods.

Initial Research Problem Statement

- CDER/DPA will develop an analytical procedure using the QbD paradigm, to be transferred 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



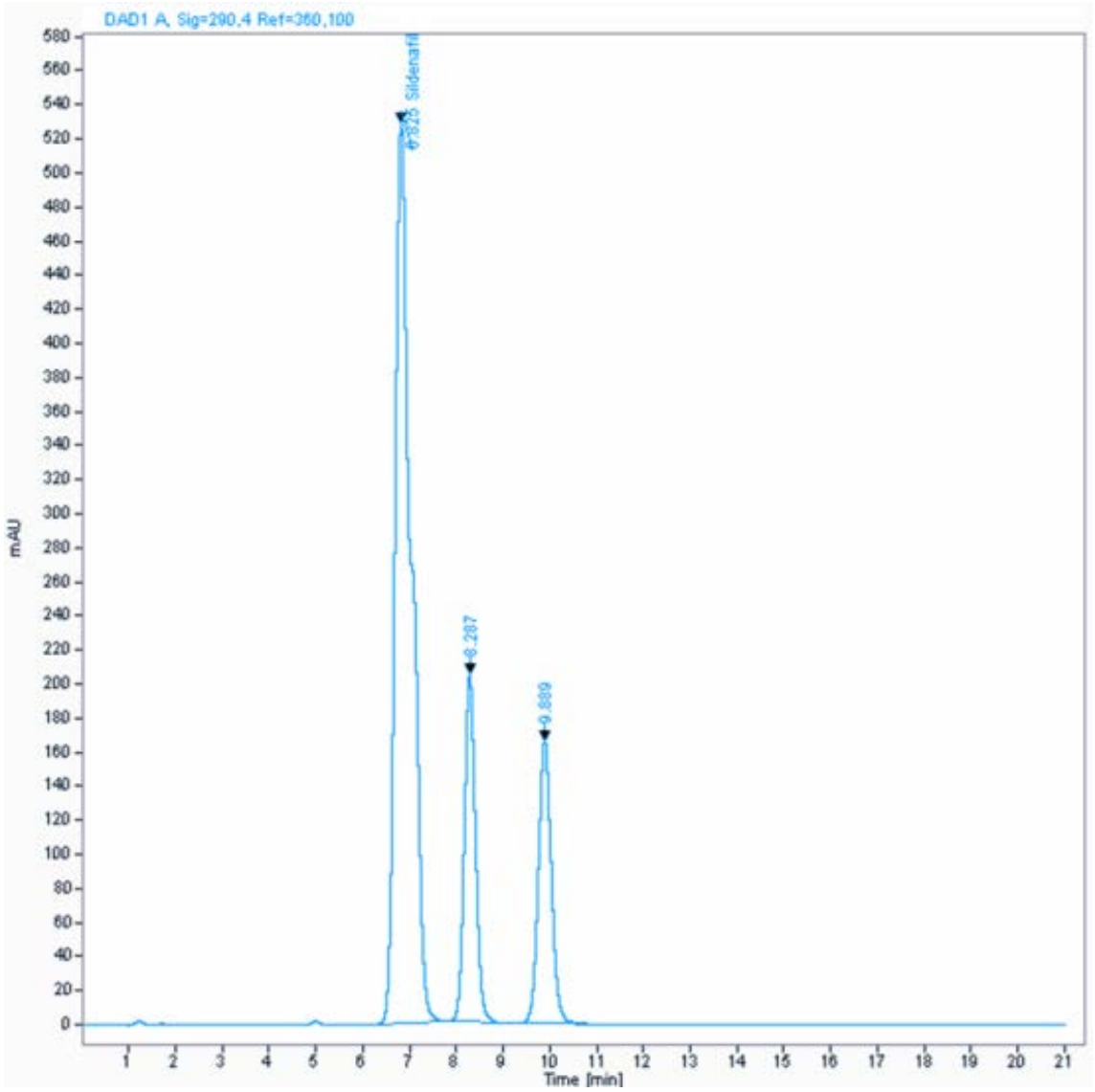
R^1 = Me; R^2 = H Sildenafil
R^1 = CH_2CH_3; R^2 = H homosildenafil
R^1 = CH_2CH_2OH; R^2 = H Hydroxyhomosildenafil
R^1 = H; R^2 = H *N*-desmethylsildenafil
R^1 = H; R^2 = CH_3 *N*-desmethylsildenafil
R^1 = cyclopentyl; R^2 = H Cyclopentynafil

*Pre-existing analogue library prepared for rapid screening surveillance program; harmonized compendial method exists for sildenafil

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: ≤ 2% over six replicates
- Accuracy: within ± 15% of the true value at 25 ug and within ± 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

A Systematic QbD Approach

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

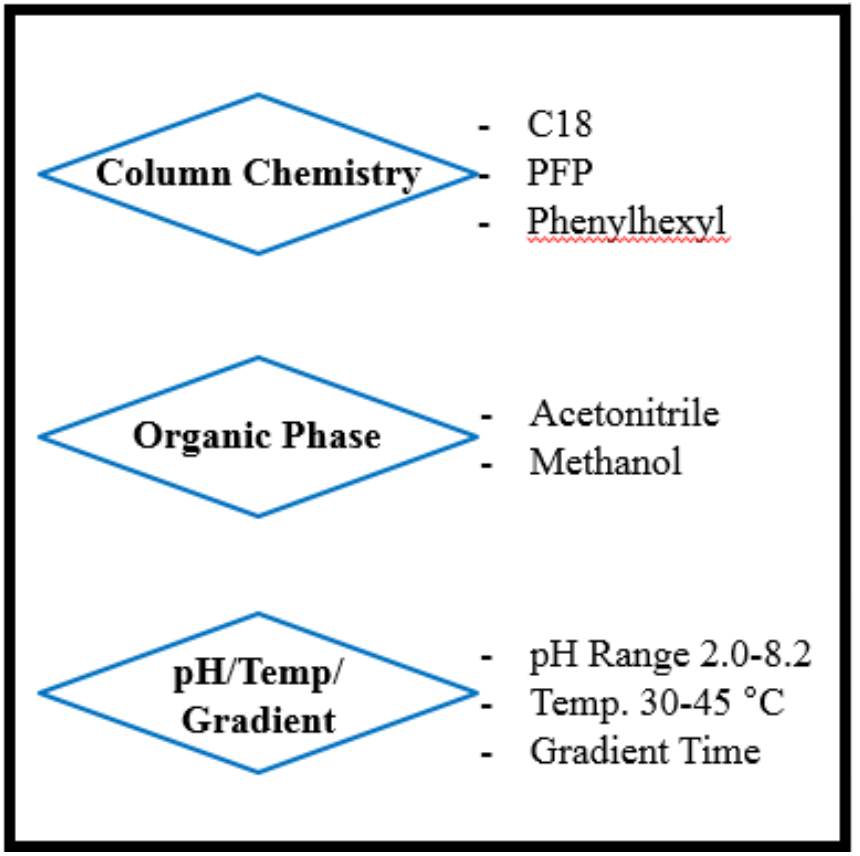
- Implemented using S-Matrix Fusion QbD Software

Three Step DOE

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

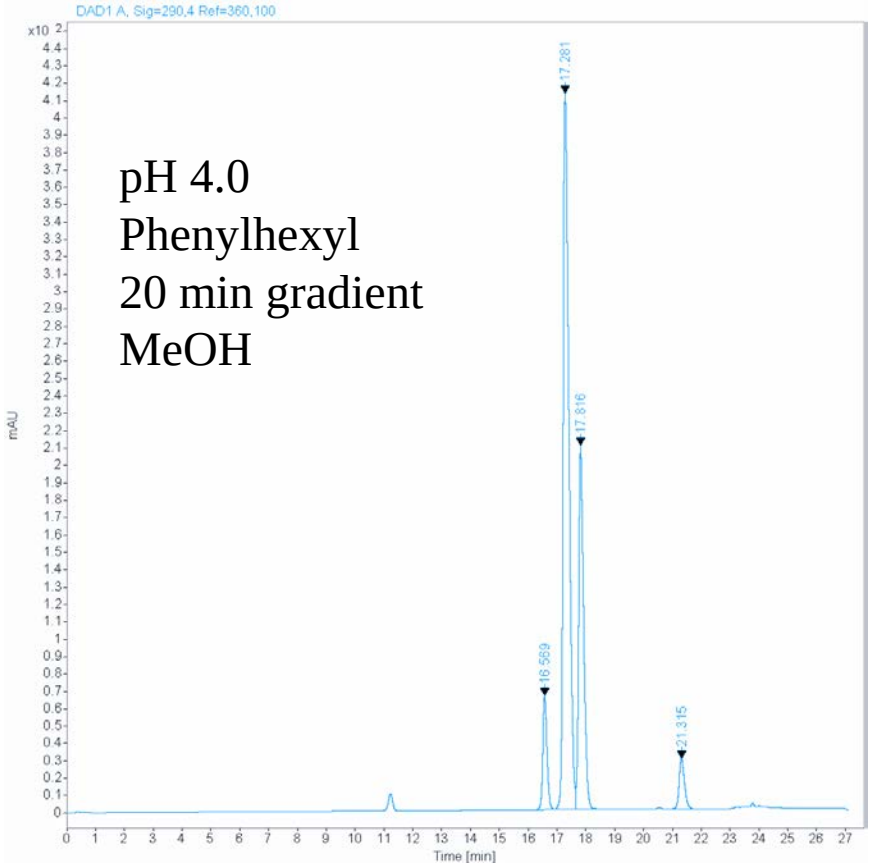
Screen 1: Best Column (37 Experiments)

- 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)

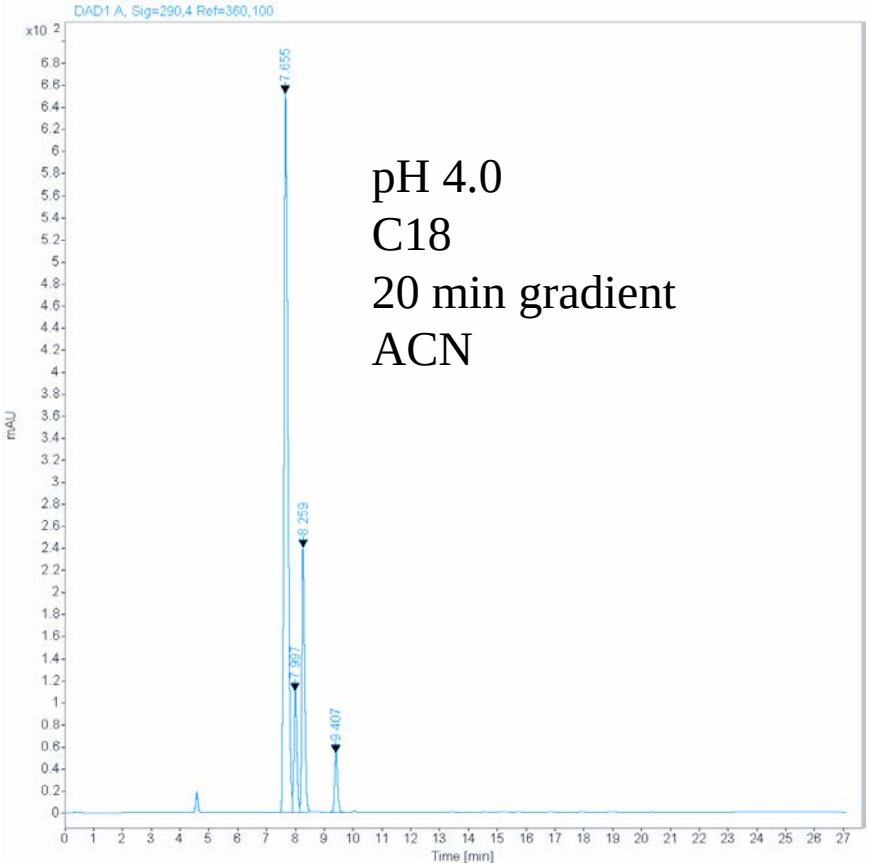


Column Screening: A Few Examples

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



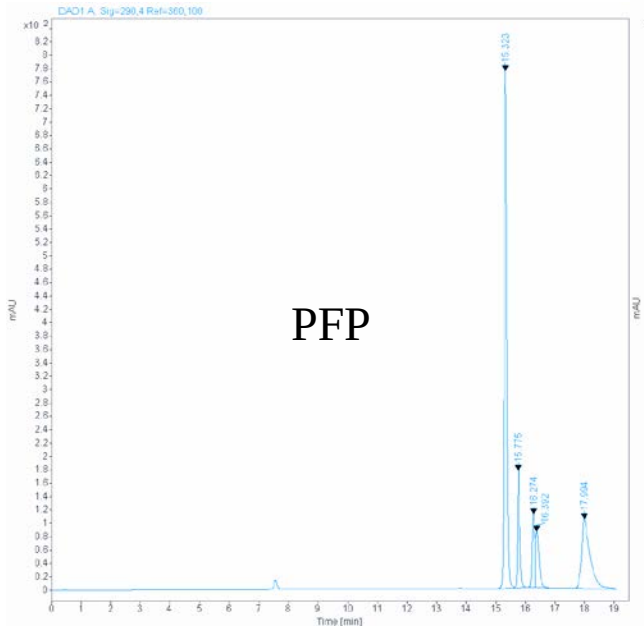
pH 4.0
Phenylhexyl
20 min gradient
MeOH

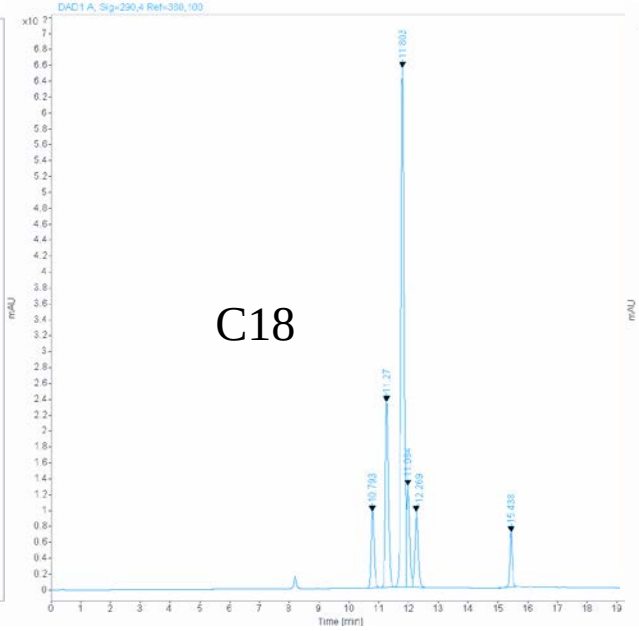


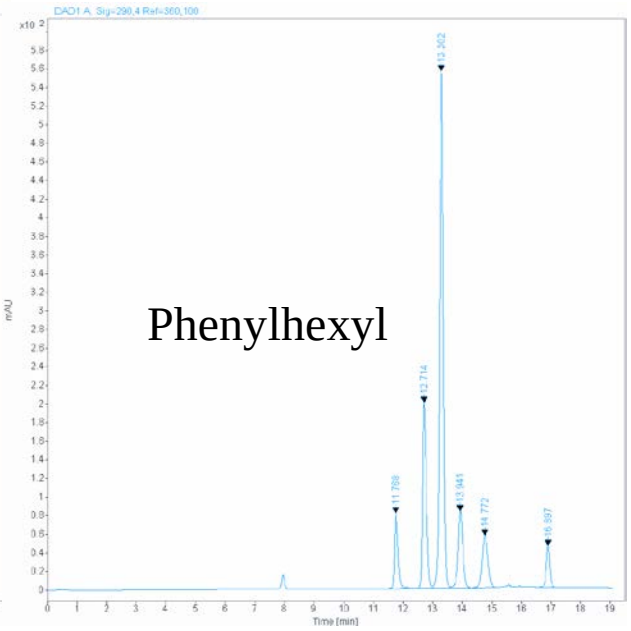
pH 4.0
C18
20 min gradient
ACN

Column Screening: A Few Examples

- Constant: pH 5.0, MeOH, 12 min gradient

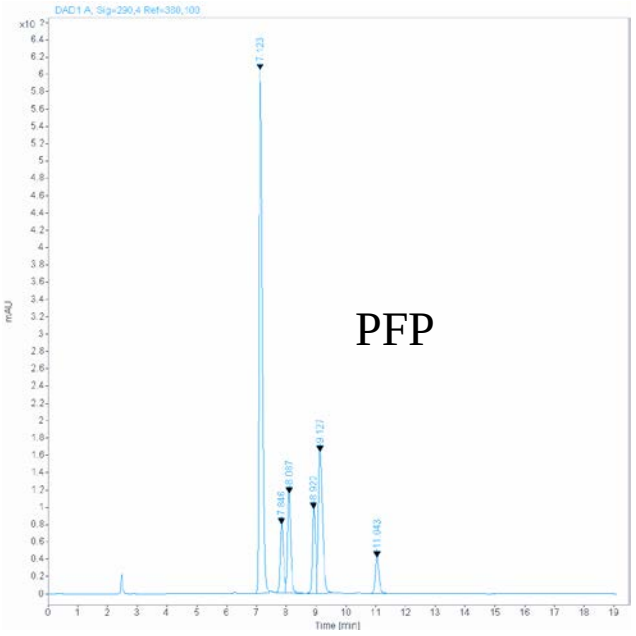


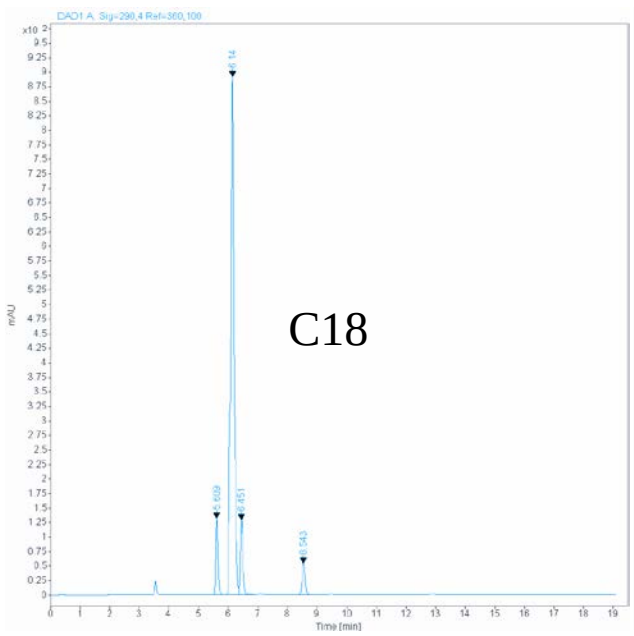


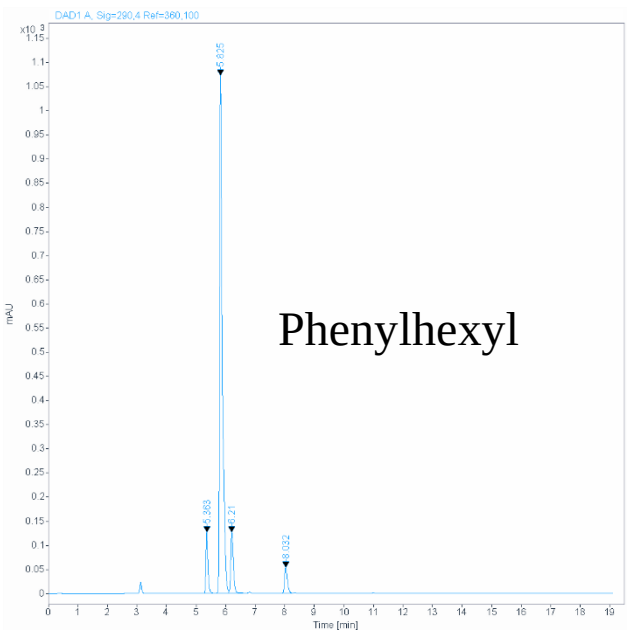


Column Screening: A Few Examples

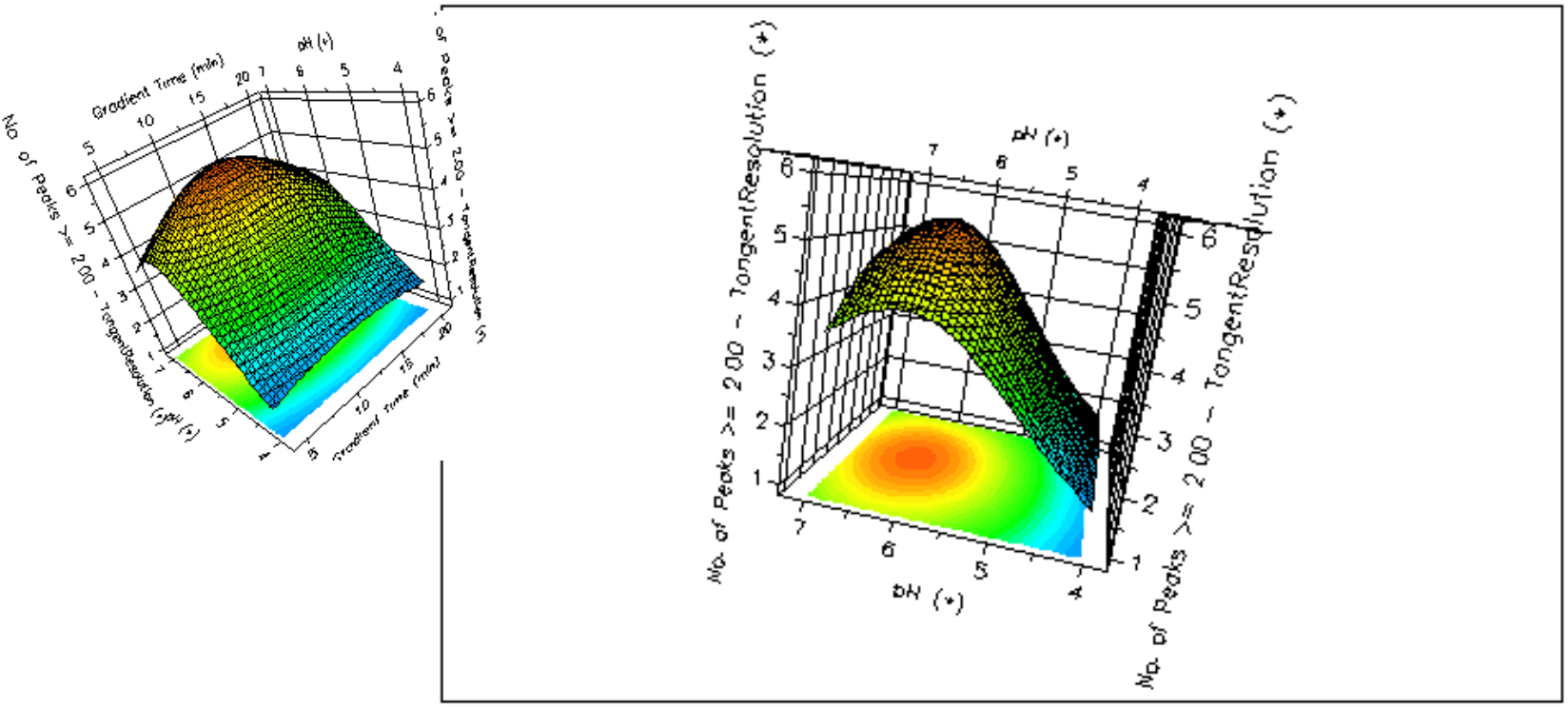
- Constant: pH 5.0, ACN, 12 min gradient





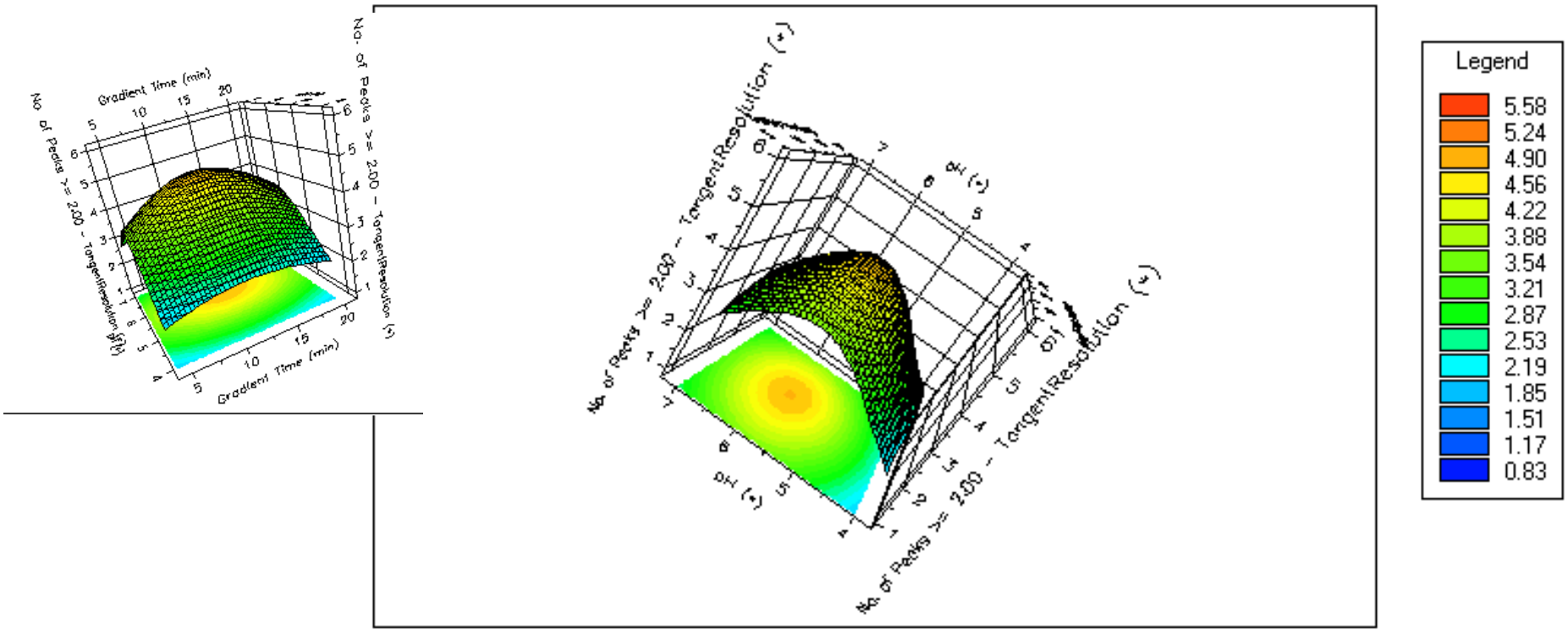


Number of peaks with resolution ≥ 2: ACN Phenylhexyl



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

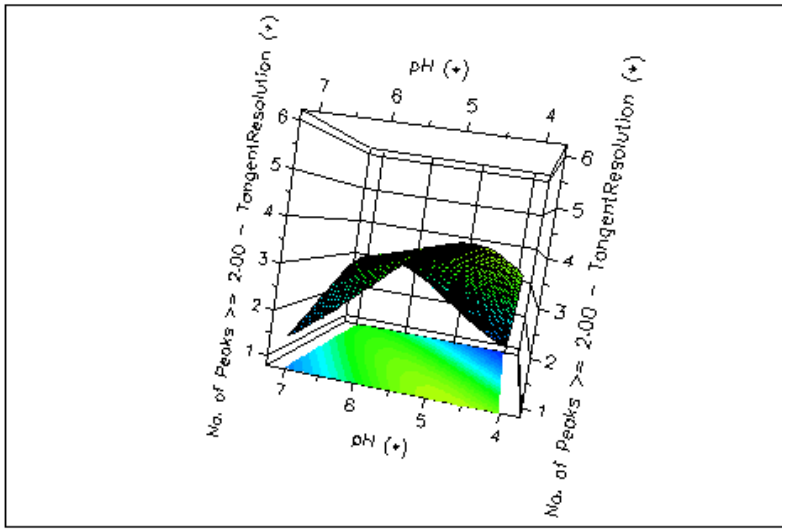
Number of peaks with resolution ≥ 2: MeOH Phenylhexyl



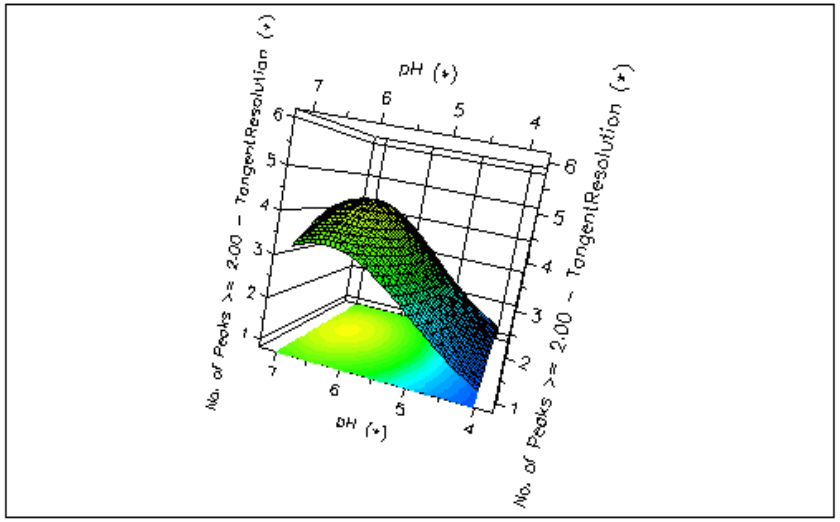
Modeling predicts pH 5.5-6.0 optimal for MeOH with 10-17 min gradient times

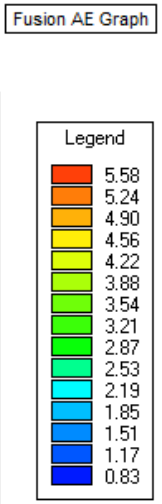
By comparison PFP and C18 have about 4 peaks with resolution ≥ 2.00

MeOH PFP



MeOH C18



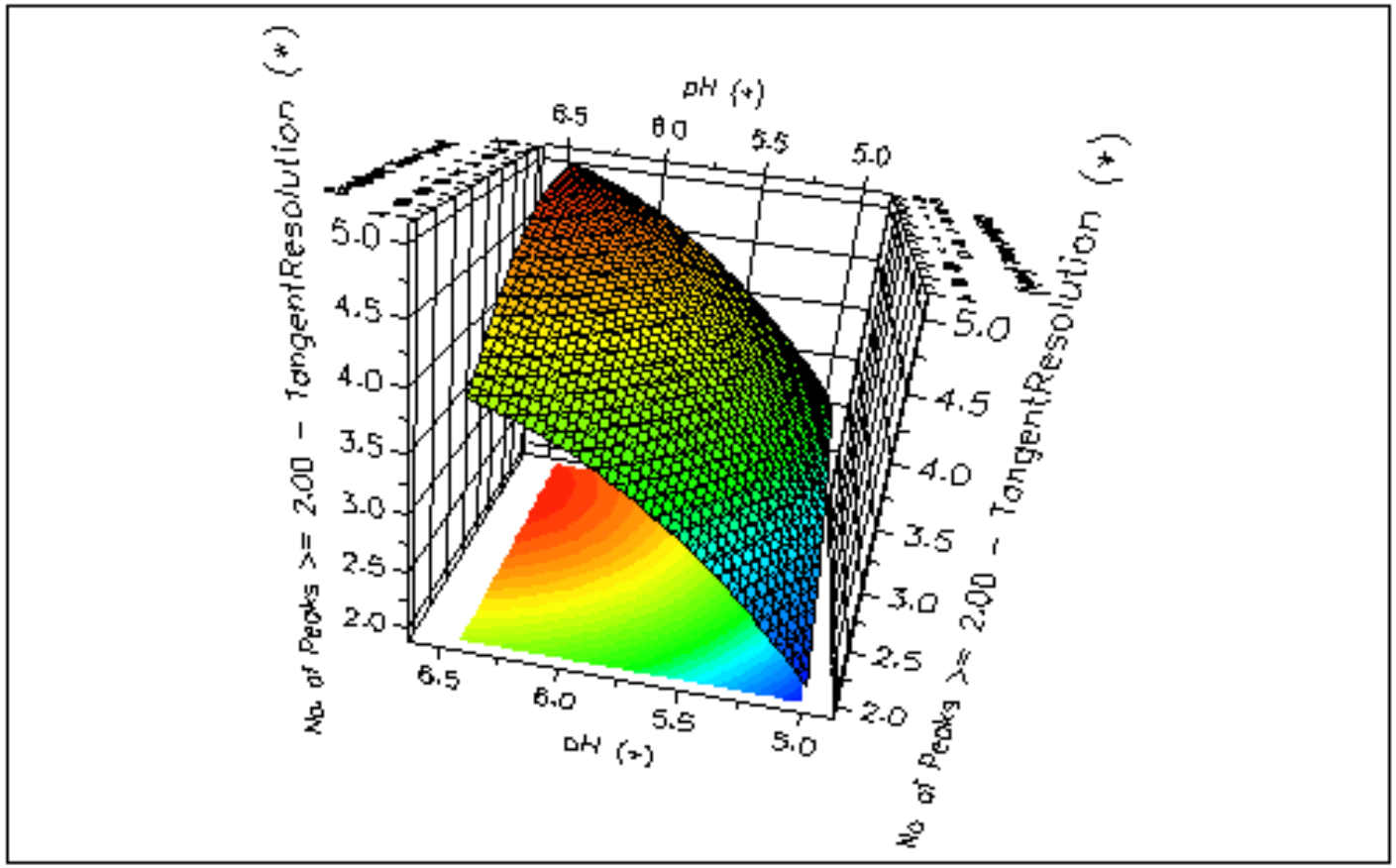


Best Overall Answer: Phenylhexyl

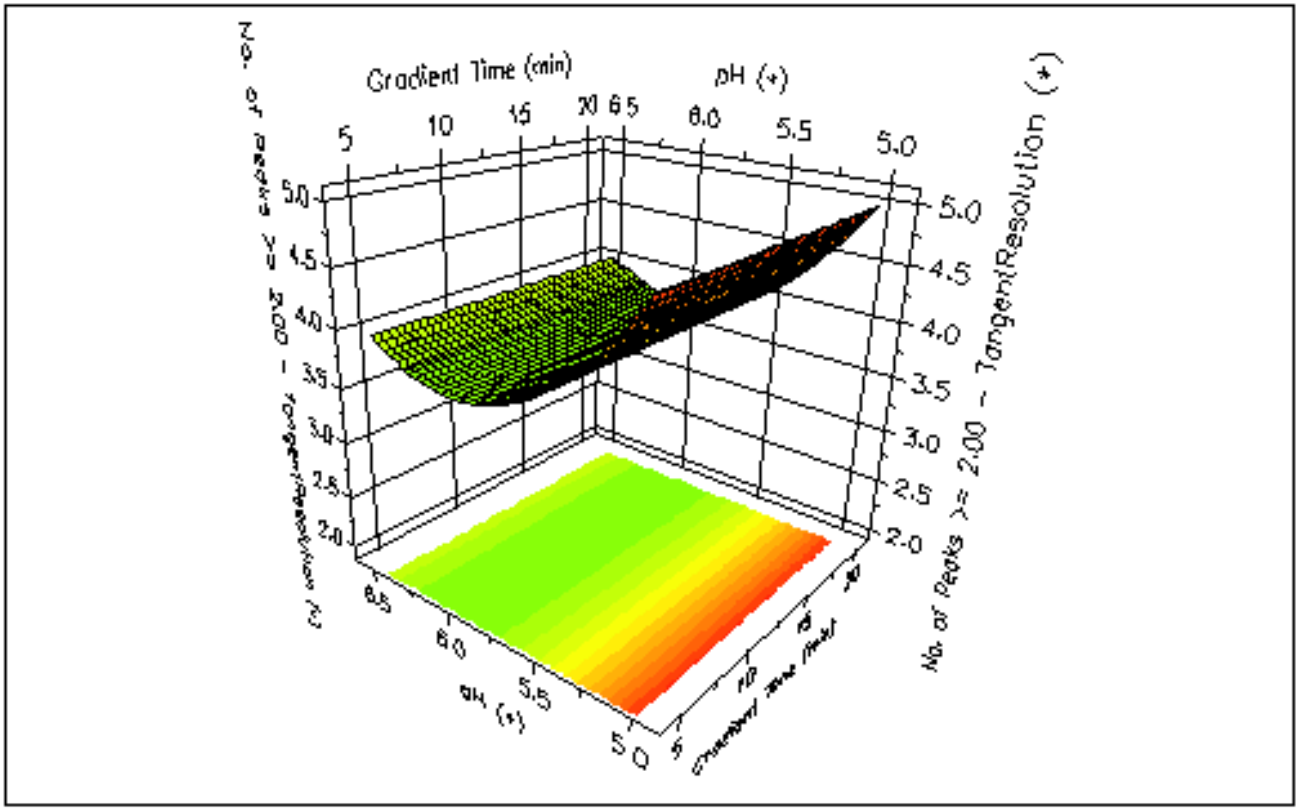
Screen 2 (19 Experiments)

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

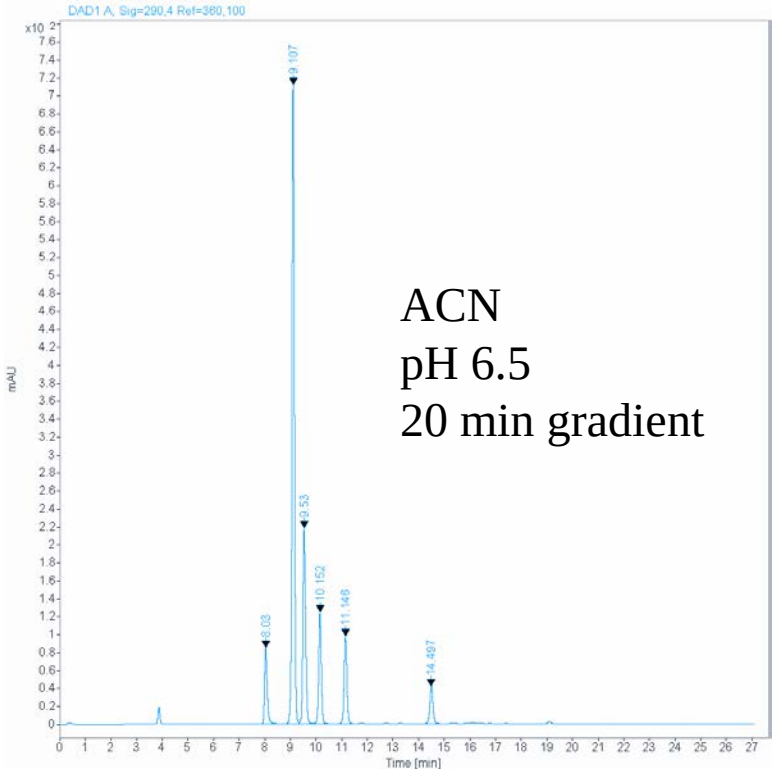
Number of peaks with resolution ≥ 2: ACN Phenylhexyl



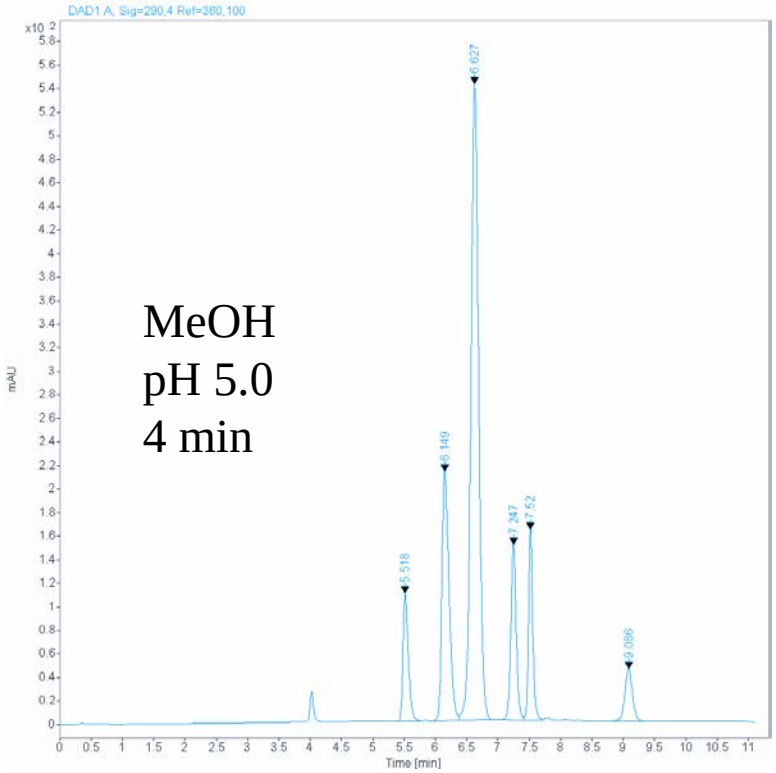
Number of peaks with resolution ≥ 2: MeOH Phenylhexyl



Screen 2 Results:
Optimal Conditions for ACN and MeOH



ACN
pH 6.5
20 min gradient



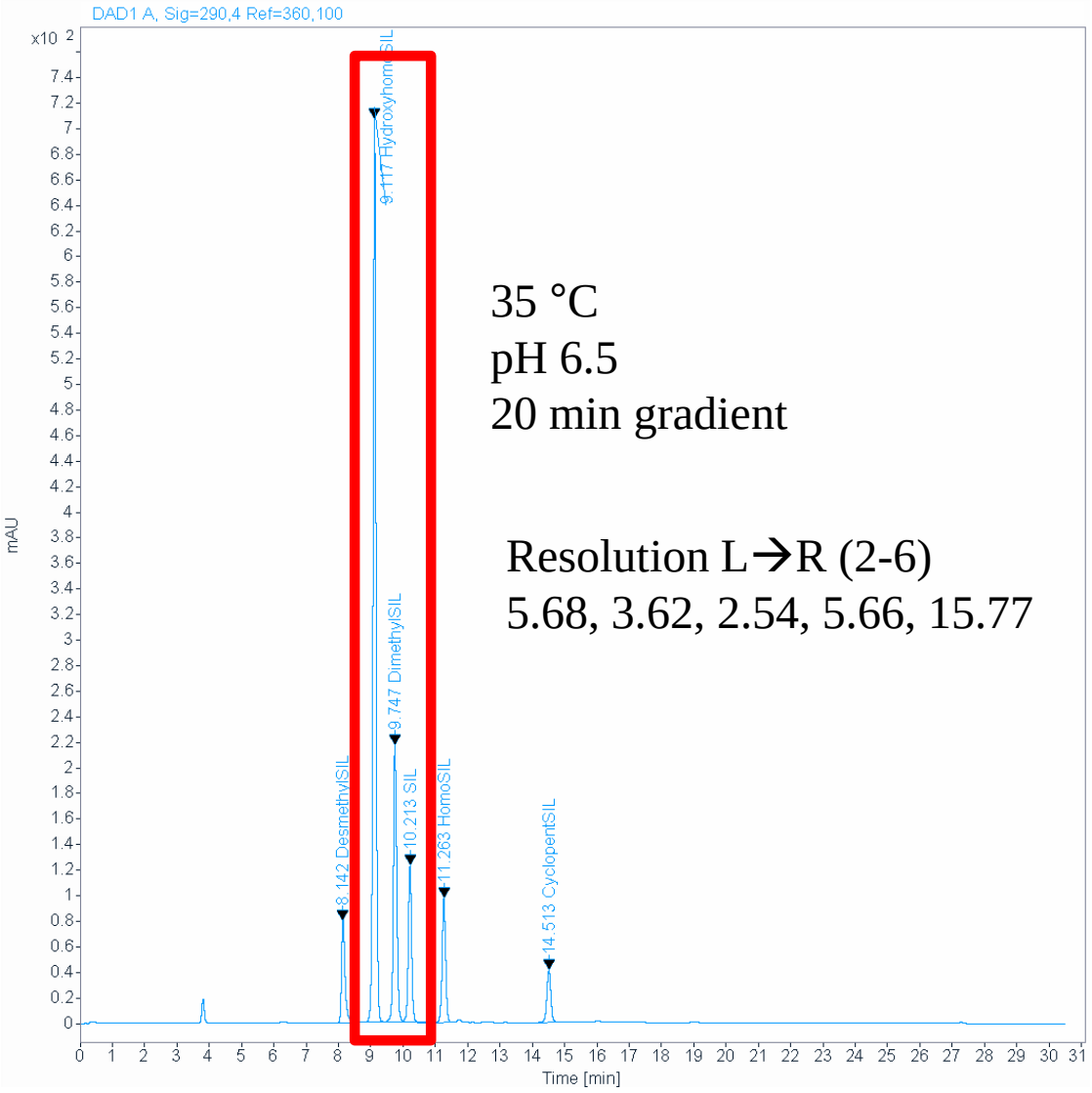
MeOH
pH 5.0
4 min

- 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)

Screen 3 Results: Optimal Conditions for ACN



35 °C
pH 6.5
20 min gradient

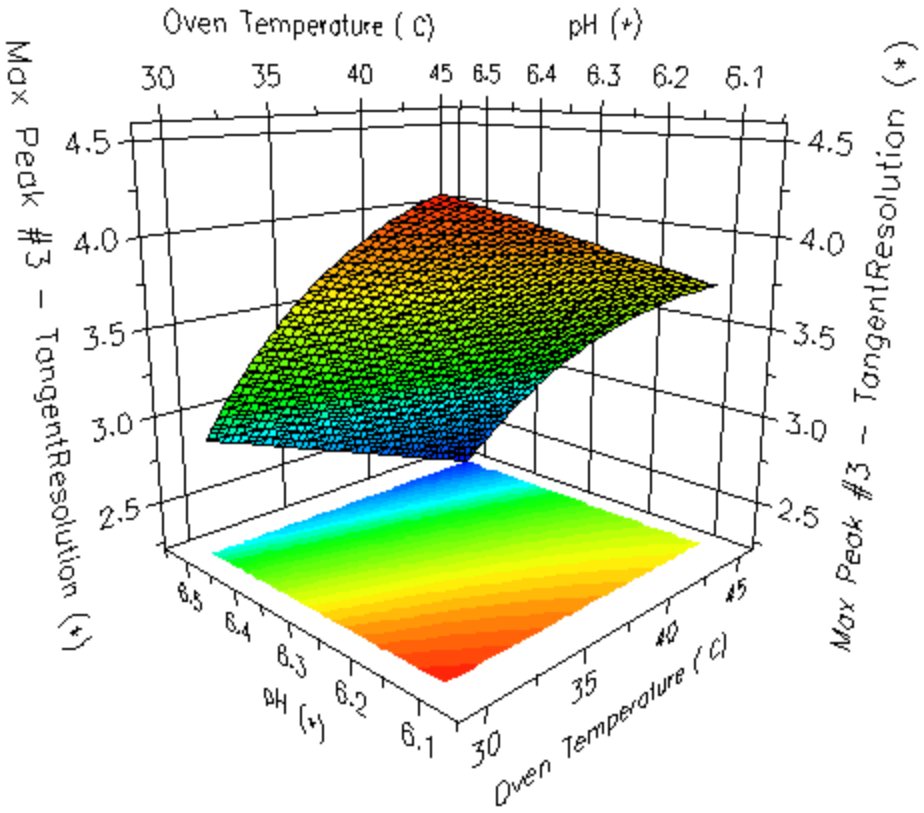
Resolution L→R (2-6)
5.68, 3.62, 2.54, 5.66, 15.77

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

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

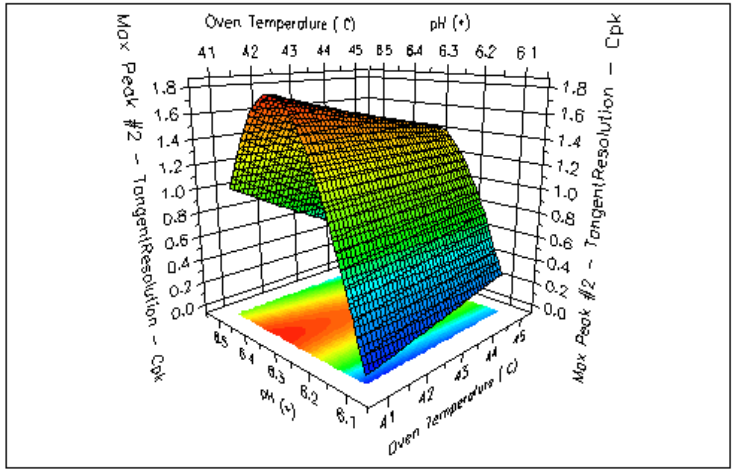
σ = response standard deviation

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

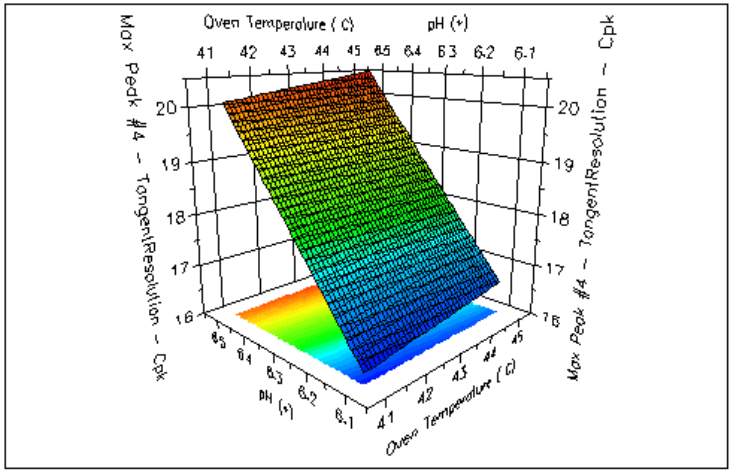
C_{pk} of Res_{1-2} : Range = 0 - 1.75, Robust region at surface ridge, sensitive to pH*Temp.

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

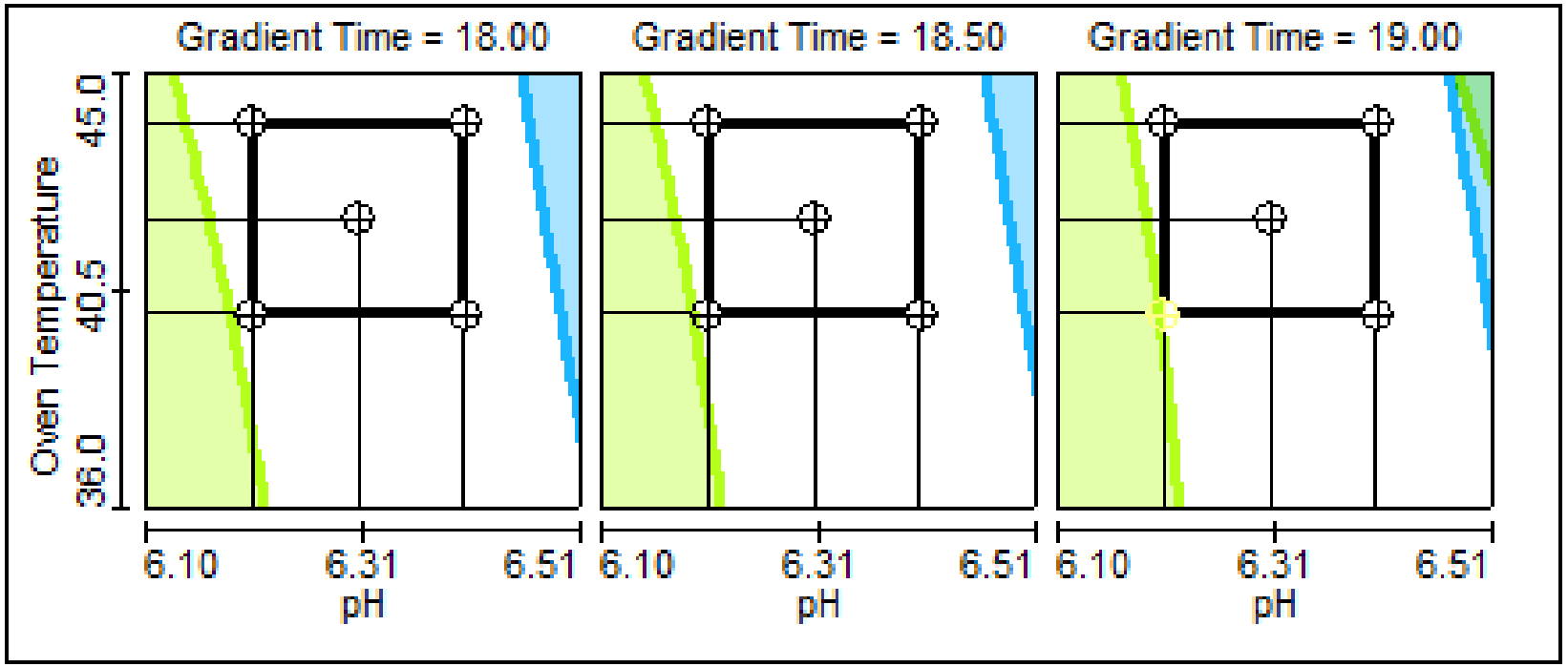
Peak 2 resolution Cpk: Gradient Time – 20 min.



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 6.3 ± 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

Peak 3 Resolution: How would we evaluate this MODR?

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

	+	+	–	–	–
To Check:		18(-1)	44(+1)	6.4 (+1)	6.4 (+1)

Prediction: R=1.3 Does not satisfy ATP

- Proposed Ranges
  - Gradient Time: 18-19 min (target 18.5)
  - Column Temperature: 40-44°C (Target 42°)
  - pH: 6.2-6.4 (Target 6.3)
  - (Values are mean centered and range scaled)

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

Conclusions

DOE methodology resulted in

- Significant improvement in the selectivity of the compendial method for separation of sildenafil analogues
- Improved method robustness
- Significant improvement in method understanding

Acknowledgements

- Sharmista Chatterjee, OPF: Reviewer support
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Thank You!