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

John F. Kauffman, Ph.D.
Daniel J. Mans, Ph.D.
FDA Division of Pharmaceutical Analysis
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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

R¹ = Me; R² = H   Sildenafil
R¹ = CH₂CH₃; R² = H   homosildenafil
R¹ = CH₂CH₂OH; R² = H   Hydroxyhomosildenafil
R¹ = H; R² = H   N-desmethylsildenafil
R¹ = H; R² = CH₃   N-desmethylysildenafil
R¹ = cyclopentyl; R² = 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/CH₃CN (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

PFP

C18

Phenylhexyl
Column Screening: A Few Examples

- Constant: pH 5.0, ACN, 12 min gradient

PFP

C18

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 $\geq 2.00$

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 $\geq 2$: ACN  Phenylhexyl
Number of peaks with resolution $\geq 2$: MeOH  Phenylhexyl
Screen 2 Results:
Optimal Conditions for ACN and MeOH

- 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

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

\( \sigma = \) response standard deviation

• Monte Carlo simulation using model equation estimates \( \sigma \) 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 $6.3 \pm 0.1$, Gradient $18.5 \pm 0.5$ min, Temp $42 \pm 2$ °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°C)
  - 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
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