Screening and Optimization Designs to Improve Method Performance and Robustness

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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

\[
\begin{align*}
R^1 &= \text{Me}; \quad R^2 = \text{H} \quad \text{Sildenafil} \\
R^1 &= \text{CH}_2\text{CH}_3; \quad R^2 = \text{H} \quad \text{homosildenafil} \\
R^1 &= \text{CH}_2\text{CH}_2\text{OH}; \quad R^2 = \text{H} \quad \text{Hydroxyhomosildenafil} \\
R^1 &= \text{H}; \quad R^2 = \text{H} \quad \text{N-desmethylsildenafil} \\
R^1 &= \text{H}; \quad R^2 = \text{CH}_3 \quad \text{N-desmethylsildenafil} \\
R^1 &= \text{cyclopentyl}; \quad R^2 = \text{H} \quad \text{Cyclopentynafil}
\end{align*}
\]

*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 $\geq 1.5$)
• 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$_3$CN (Buffer = Phosphoric acid, pH 3 with triethylamine)
- C18 column
- 30 °C
- Poorly separated: 6 compounds $\rightarrow$ 3 peaks
Initial Studies: Mobile Phase Evaluation

- Change from Isocratic to Gradient (A=Buffer, B=MeOH/CH₃CN)? Remove CH₃CN? Remove Methanol?

A=Buffer  B=MeOH/CH₃CN (25/17)  Marginal improvement

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

PFP

C18

Phenylhexyl
Column Screening: A Few Examples

- Constant: pH 5.0, ACN, 12 min gradient
Number of peaks with resolution $\geq 2$: ACN Phenylhexyl

Modeling predicts pH $\sim 6$-$6.5$ optimal for ACN with 10-17 min gradient times (using the resolution $\geq 2.00$ 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 $\geq 2.00$

**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 $\geq 2$: ACN Phenylhexyl
Number of peaks with resolution $\geq 2$: 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

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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
  \[ C_{pk} = \frac{R - LSL_{ATP}}{3\sigma} \]
  \( \sigma = \text{response standard deviation} \)

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

C\textsubscript{pk} of Res\textsubscript{3-4} : Range > 16, linear in pH but not Temp.
Method Robustness: Operable Region

- Corners: $C_{pk} = 1.33$ for Resolutions 2, 3 and 4
- Ranges: pH $6.30 \pm 0.1$, 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
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
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