A QBD WITH DESIGN OF EXPERIMENTS APPROACH TO THE DEVELOPMENT OF A CHROMATOGRAPHIC METHOD FOR THE SEPARATION OF IMPURITIES IN VANCOMYCIN

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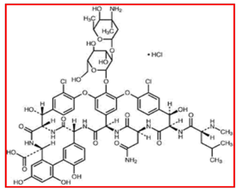
INTRODUCTION

Throughout the drug development process, methods are developed at various stages, often consisting of samples that vary in complexity. Due to the inherent nature of this process, redundant efforts take place across an organization, resulting in a very costly and time consuming process. If we can streamline the process by which we develop methods, products can be brought to market faster and in a more cost effective manner.

Many different approaches are typically used to develop chromatographic methods today including trial and error, method/column scouting, and software approaches such as first principles approaches and simplex optimization procedures. All these approaches suffer from the inability to determine complex interactions effects between method variables or measurably consider method robustness during the method development process.

Vancomycin is a tricyclic glycopeptide antibiotic derived from Amycolatopasis orientalis (formerly Nocardia orientalis) and is indicated for the treatment of serious or severe infections caused by susceptible strains of methicillin-resistant (beta-lactam-resistant) staphylococci. Vancomycin is a large molecule (MW 1485.71 daltons) and contains many impurities that are difficult if not impossible to separate. Traditional HPLC gradient methods have shown the ability to separate out as many as 13 of these impurities while the use of sub-2 micron LC column chromatography (UPLC/UHPLC) has demonstrated the separation of as many as 26 impurities.



This paper describes a novel method development approach using Quality by Design (QbD) with Design of Experiments to develop a UPLC® method for impurities in Vancomycin resulting in an optimally performing analytical method while simultaneously applying robustness limits to ensure success in final method validation and ultimately in method transfer.

EXPERIMENTAL

The vancomycin studies described here were carried out using an automated integrated system consisting of Fusion AE Method Development Software, Empower 2 chromatography data system, and an ACQUITY UPLC® system with PDA, Column Manager, and Solvent Select Valve allowing for the screening of up to 4 different column chemistries, 6 different aqueous buffers/pH's, and 2 different organic mobile phases in one run. Fusion Method Development Software (S-Matrix Corporation, Eureka, CA) is a Quality-by-Design based LC Method Development software with built-in robustness metrics. Fusion AE interfaces with the Empower 2 CDS that controls the ACQUITY UPLC. Using the chromatographic results from the Empower 2 CDS, Fusion AE manages complex statistics and models for method optimization. Fusion AE builds experiments, analyzes data, and presents results as visual and numerical method predictions.

Fusion AE MDS, Empower 2 CDS, and ACQUITY UPLC System used for method development.

PHASE 1—RAPID SCREENING

Vancomycin Rapid Screen - Experiment Design

The first phase of the method development involves the screening of the major effectors of selectivity, primarily the column chemistry, buffer pH, and organic mobile phase. The variables and ranges screened along with the constant conditions are listed here.

Column Assignments

Column Valve Position	Column Level
ValvePosition_1	BEH C18, 2.1x100mm, 1.7um
ValvePosition_2	BEH RP18, 2.1x100mm, 1.7um
ValvePosition_3	BEH Phenyl, 2.1x100mm, 1.7um
ValvePosition_4	BEH C8, 2.1x100mm, 1.7um

Experiment Constants

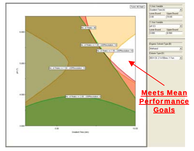
Constant Name	Constant Value
Sample Concentration	0.25
Pump Flow Rate	0.450
Injection Volume	2.5
Oven Temperature	45.0
Wavelength	254
Equilibration Time	10.0
Equilibration % Organic	2.0
Initial Hold Time	1.0
Initial Hold % Organic	2.0
Final Hold Time	2.0
Final Hold % Organic	40.0
Ramp Up to Wash Time	0.1
Column Wash Time	2.0
Column Wash % Organic	95.0
Ramp Down from Wash Time	0.1
Re-equilibration Time	1.0
Re-equilibration % Organic	95.0

Reservoir Assignments

Reservoir A1-1	Level
pH	5.0
Reservoir A1-2	Level
pH	5.0
Reservoir A1-3	Level
pH	6.5
Reservoir A2	Level
Aqueous Solution	---
Reservoir B1	Level
Acetonitrile	---
Reservoir B2	Level
Methanol	---

Vancomycin Rapid Screen - Overlay Graphics

The experimental design was run and data processed on the chromatographic system and the results were imported back into Fusion AE. The software predicts the optimum LC method after modeling all significant effects - linear, interaction, and complex - on each critical method performance characteristic. The overlay graph shown for the BEH C8 column with methanol as the organic mobile phase highlights the experimental region where the mean performance goals were obtained (unshaded area).



Vancomycin Rapid Screen - Optimization

The Automated Optimizer wizard defines the LC method performance goals and ranks in order of importance. The software searches for the LC method that meets all the performance goals simultaneously. The best result(s) are reported along with predicted results for an experimental run. These conditions were used for the next stage - Method Optimization.

PHASE 2—METHOD OPTIMIZATION

Vancomycin Method Optimization - Experiment Design

Phase 2 experiments use the column (BEH C8, 2.1x100mm, 1.7µm) and mobile phase (pH 5.0 buffer, methanol B solvent) results from Phase 1 plus additional variables with tighter ranges to determine the optimum LC method. The experimental design was created using pump flow rate, gradient time, final % organic, and column temperature as final optimization variables in the ranges shown. Fusion AE created the experimental design and exported it to Empower 2, creating all the necessary instrument methods, method sets, and sample sets. The experimental design was run and data processed on the chromatographic system and the results were imported back into Fusion AE. In addition to the data analysis for method optimization, Fusion AE applied a combination of Monte Carlo Simulation and Process Capability statistics to evaluate method robustness without running additional experiments.

Design Variables

Variable	Range
Pump Flow Rate	0.25 - 0.45 ml/min
Gradient Time	6.0 - 10.0 minutes
Final % Organic	25% - 40% B
Column Temperature	35° - 60° C

Reservoir Assignments

Reservoir A1-1	Level
pH	5
Reservoir A2	Level
Aqueous Solution	---
Reservoir B2	Level
Methanol	---

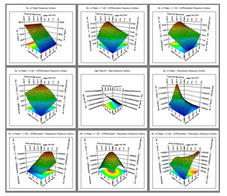
Experiment Constants

Constant Name	Constant Value
Column Type	BEH C8 100mm
Injection Volume	2.5
Wavelength	254
pH	5.0
Initial % Aqueous	95
Initial % Organic	5
Equilibration Time	10.0
Equilibration % Organic	5.0
Initial Hold Time	1.0
Final Hold Time	2.0
Ramp Up to Wash Time	0.1
Column Wash Time	2.0
Column Wash % Organic	95.0
Ramp Down from Wash Time	0.1
Re-equilibration Time	1.0
Re-equilibration % Organic	6.0

Vancomycin Method Optimization - Multiple Response Interactions

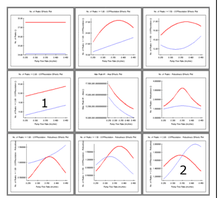
Multiple Response Surface Plots

Visualizing the results with 3D Response Surface Plots demonstrates the combined effects of variables on key chromatographic responses such as resolution, peak tailing, and retention time. Colors represent the magnitude of interaction and the curvature indicates the type of interaction.



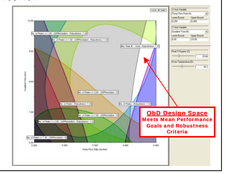
Multiple Response Effects Plots

Another option for visualizing the interactions between variables is through Multiple Response Effects Plots. These plots clearly identify simple linear additive effects (1), complex interactions of variables (2), and other types of response effects.

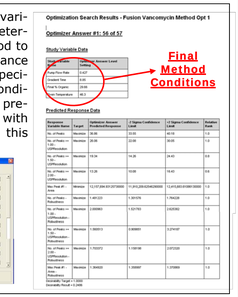


Vancomycin Method Optimization - Final Results

An overlay graph was created using a number of critical chromatographic responses. Of primary importance for the vancomycin separation is maximizing the number of peaks observed and the number of peaks exceeding different levels of resolution while minimizing the area of the Vancomycin peak (equates to separating out the most impurities). The overlay graph shows the QbD Design Space (unshaded region) where the method meets the mean performance goals and robustness criteria.



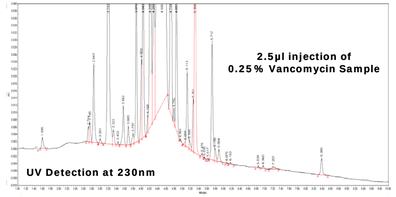
Using ranked response variables, the Optimizer determines the optimum method to best meet the performance and robustness goals specified. The final method conditions are listed along with predicted response results with confidence limits for this method.



OPTIMIZATION RESULTS

The optimum method determined by the Fusion AE Optimizer was
- BEH C8 Column, 2.1x100mm, 1.7µm
- 10mM Ammonium Acetate, pH 5.0
- Methanol - Mobile Phase B
- 0.427 ml/min Flow Rate
- 5% - 29.66% Methanol in 8.85 minutes
- Column Temperature - 46.3° C

This method was exported to Empower 2 and the vancomycin sample was run to evaluate the prediction accuracy. The chromatogram below shows the vancomycin impurities separation obtained with the optimized method.



The experimental results compare favorably with the Fusion AE predictions.

Response Variable	Predicted Response	Exper. Response
# of Peaks	36.9 Peaks	39 Peaks
# of Peaks ≥1.0 Rs	26.1 Peaks	27 Peaks
# of Peaks ≥1.5 Rs	19.3 Peaks	18 Peaks
# of Peaks ≥2.0 Rs	13.3 Peaks	12 Peaks

The Fusion AE method improved the separation of impurities in vancomycin from 26 obtained previously with UPLC methods developed manually to 39 impurities observed with the method shown.

CONCLUSION

- Fusion AE with ACQUITY UPLC Generated an Optimized Vancomycin Method in ~2 Days
  - Resolution improved from 26 peaks in previous method to 39 peaks
- Integrated Robustness Calculations Ensure Reproducible Method
  - Increased confidence in method passing validation and method transfer
- Establishes a valid design space with both mean performance (set point optimization) and robustness (operating space)

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