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アプリケーションノート

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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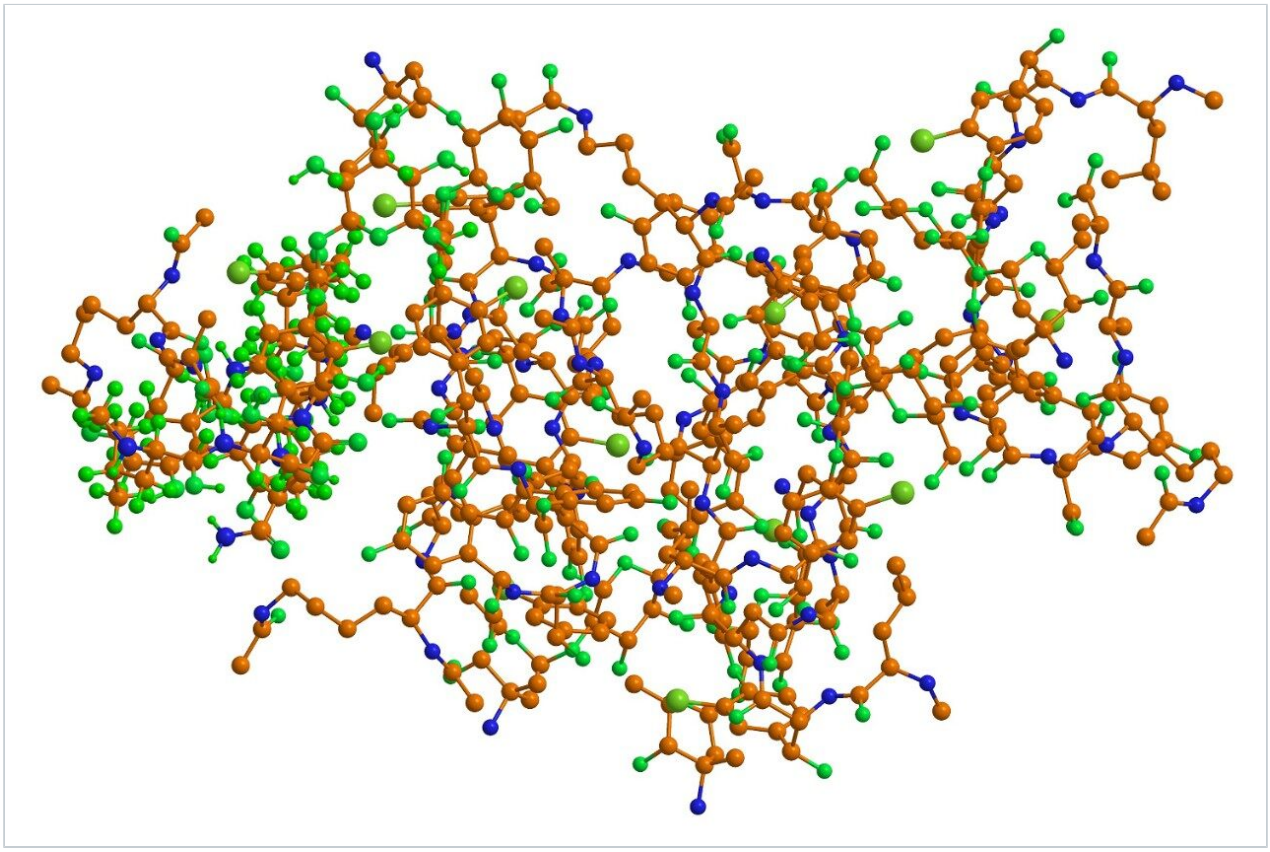


Abstract

This paper describes a novel method development approach using Quality by Design (QbD) with Design of Experiments to develop a UPLC method for separating 39 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.

Benefits

Using specialized software in conjunction with UPLC Technology, an optimized QbD method for the impurities in vancomycin can be developed that will be robust for method validation and transfer.

Introduction

Analytical methods are developed at various stages of the drug develoment process for samples of varying complexity. Due to the inherent nature of the method development process, redundant efforts take place across an organization, resulting in a very costly and time-consuming activities. 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 methicillinresistant (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-µm ACQUITY UPLC Column chromatography 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 separating 39 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

Analytical instrumentation

The vancomycin studies described here were carried out using an automated integrated system consisting of Fusion Method Development Software, Empower 2 Chromatography Data Software (CDS), and an ACQUITY UPLC System with PDA, Column Manager, and Solvent Select Valve allowing for the screening of up to four different column chemistries, six different aqueous buffers/pHs, and two different organic mobile phases in one run.

Data management

Fusion Method Development Software (S-Matrix Corporation, Eureka, CA) is a Quality by Design based LC Method Development software package with built-in robustness metrics. Fusion includes a built-in interface with the Empower 2 CDS Software that controls the ACQUITY UPLC System. Using the chromatographic results collected from Empower 2 CDS, Fusion manages complex statistics and models for method optimization. Fusion builds experiments, analyzes data, and presents results as visual and numerical method predictions.

Results and Discussion

Phase 1: Rapid screening

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 in Figure 1’ s tables.

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

Reservoir Assignments

Reservoir A1-1	Level
pH	3.0
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	---

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

Figure 1. Screened variables and ranges.

Overlay graphics

The experimental design is run and data processed on the chromatographic system and the results are imported back into Fusion. The software predicts the optimum LC method after modeling all significant effects – linear, interaction, and complex – on each critical method performance characteristic. The unshaded (white) area of the overlay graph shown for the BEH C_8 column with methanol as the organic mobile phase (Figure 2) highlights the experimental region where the mean performance goals are obtained.

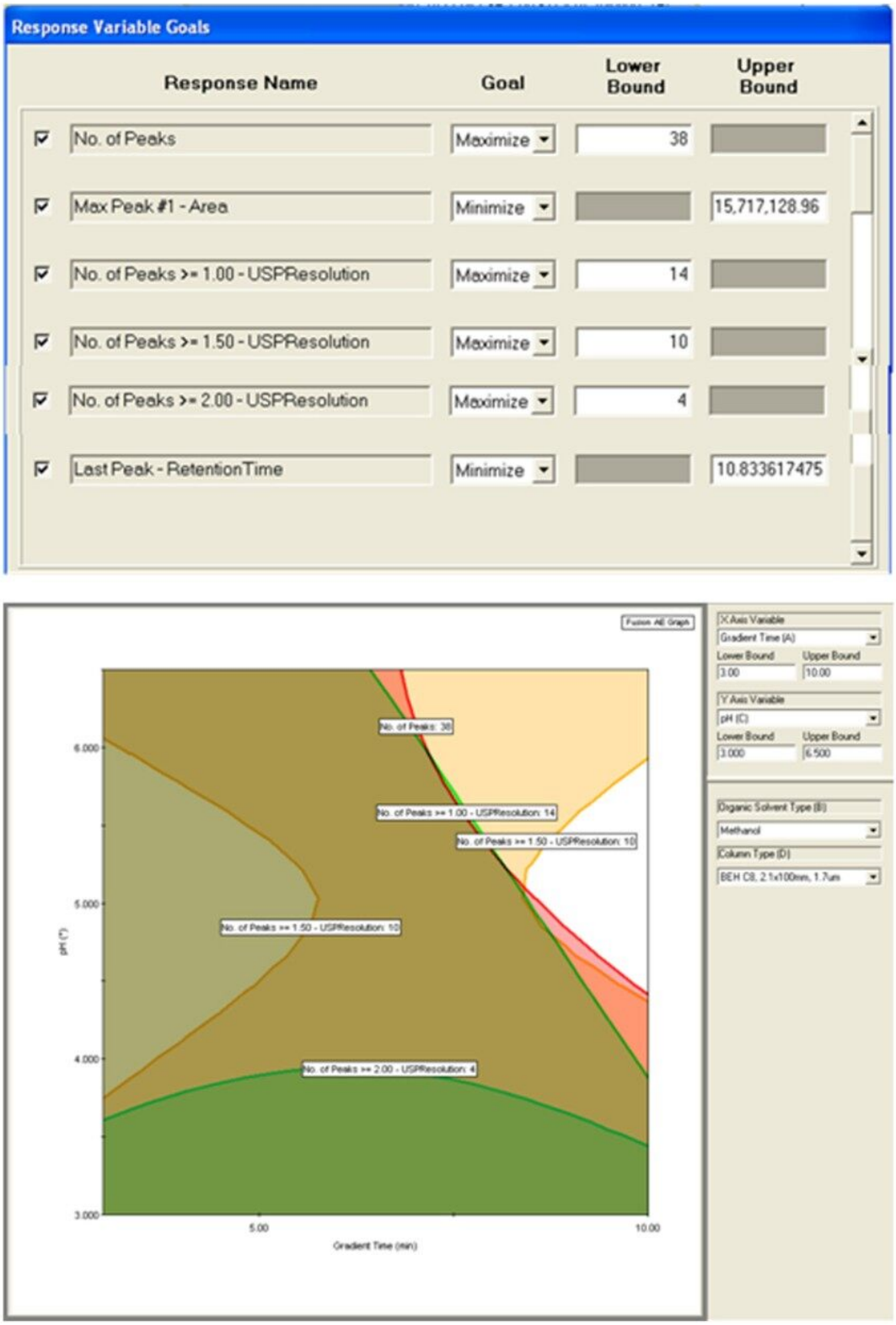
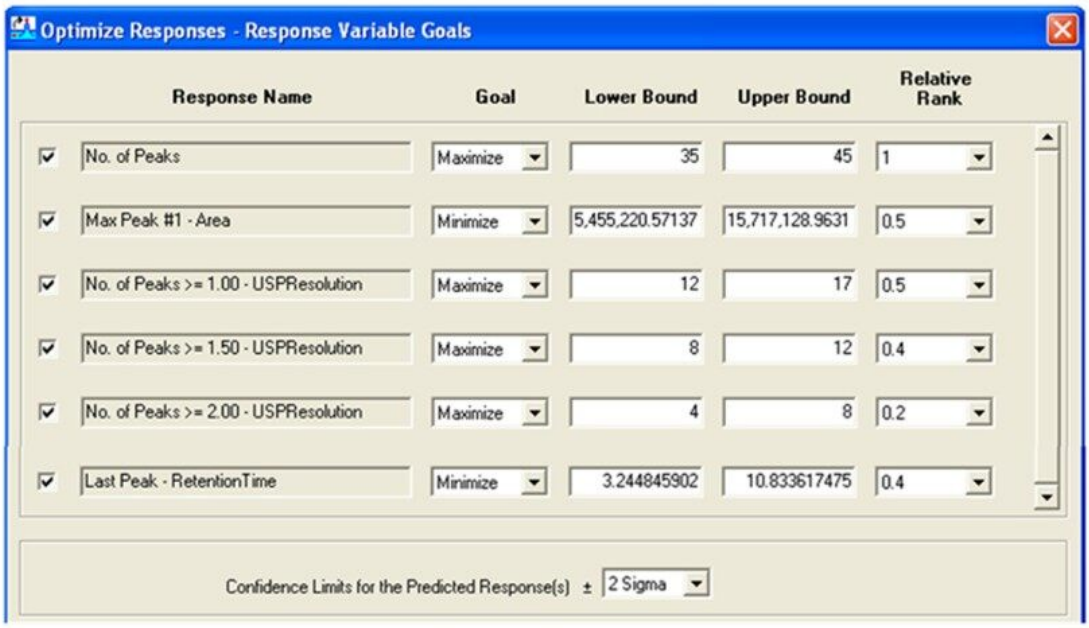


Figure 2. Processed data are imported to Fusion, where an overlay graph illustrates in white the region where the mean performance goals are achieved.

Optimization

The Automated Optimizer wizard defines the LC method performance goals and ranks them 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 (Figure 3). These conditions are used for the next stage, Method Optimization.



Optimizer Answer #1: 34 of 34

Study Variable Data

Study Variable Name	Optimizer Answer Level Setting
Gradient Time	10.00
Organic Solvent Type	Methanol
pH	5.543
Column Type	BEH C8, 2.1x100mm, 1.7um

Best conditions from rapid screen runs

Predicted Response Data

Response Variable Name	Target	Optimizer Answer Predicted Response	-2 Sigma Confidence Limit	+2 Sigma Confidence Limit	Relative Rank
No. of Peaks	Maximize	42.21	36.54	47.87	1.0
Max Peak #1 - Area	Minimize	10,802,293.47829680000	7,112,353.74702277000	14,492,233.20957080000	0.5
No. of Peaks >= 1.00 - USPResolution	Maximize	16.36	11.55	21.17	0.5
No. of Peaks >= 1.50 - USPResolution	Maximize	10.95	8.65	13.26	0.4
No. of Peaks >= 2.00 - USPResolution	Maximize	8.78	5.57	12.70	0.2
Last Peak - RetentionTime	Minimize	9.11778162928	8.39564415224	9.90124127730	0.4

Figure 3. Fusion's Automated Optimizer facilitates determination of the LC method that meets all performance goals.

Phase 2: Method optimization

Experiment design

Phase 2 experiments use the column (ACQUITY UPLC BEH C_8, 2.1 x 100 mm, 1.7 µm) 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 is created using pump flow rate, gradient time, final percent organic, and column temperature as final optimization variables in the ranges shown (Figure 4).

Fusion Software creates the experimental design and exports it to Empower 2, automatically creating all the necessary instrument methods, method sets, and sample sets. The experimental design is run and data processed on the chromatographic system and the results are imported back into Fusion.

In addition to the data analysis for method optimization, Fusion applies 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 min
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	5.0

Figure 4. Fusion determines optimal method conditions and exports this information back to Empower 2 to be run a

Multiple response surface plots

Optimization results

Column:	ACQUITY UPLC BEH C_8 Column, 2.1 x 100 mm, 1.7 µm
Mobile phase A:	10 mM Ammonium Acetate, pH 5.0
Mobile phase B:	Methanol
Flow rate:	0.427 mL/min
Gradient:	5% to 29.66% Methanol in 8.85 min
Column temp.:	46.3 °C

This method was exported to Empower 2 and the vancomycin sample was run to evaluate the prediction accuracy. The chromatogram in Figure 9 shows the separation of vancomycin impurities obtained with the optimized method.

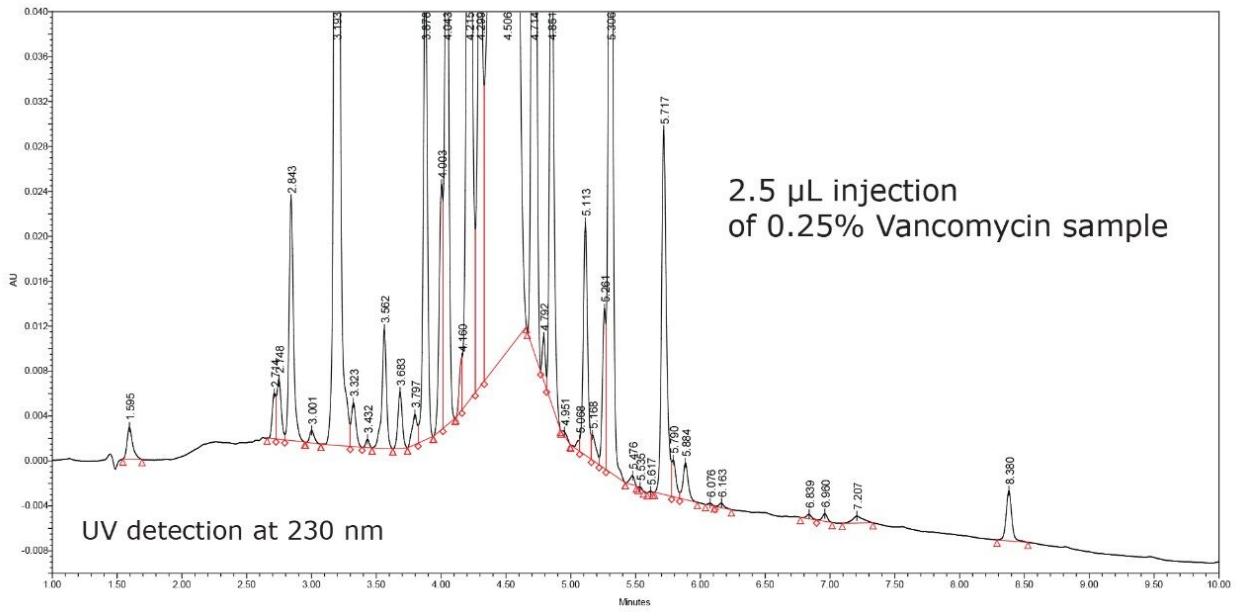


Figure 9. Confirmation run of the final UPLC method recommended by Fusion Software, where the number of impurities observed increased from 26 to 39.

The experimental results compare favorably with Fusion Software's predictions.

Response variable	Predicted response	Experimental 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 QbD-based Fusion Software 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 Method Development Software, used with the ACQUITY UPLC System, generated an optimized method for the analysis of vancomycin and its impurities in two business days.
- The use of UPLC data managed and processed by Fusion and Empower 2 software established a valid design space with both mean performance (set point optimization) and robustness (operating space).
- The QbD method's resolution improved from 26 peaks in previous method to 39 peaks.
- Integrated robustness calculations ensure a reproducible method, which increases confidence in the ability to validate and transfer that method.

Featured Products

ACQUITY UPLC System <https://www.waters.com/514207>

Empower 3 Chromatography Data Software <https://www.waters.com/513188>

ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

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