

Quality by Design Systematic Chromatographic Method Development

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ABSTRACT

This presentation reports a quality by design systematic approach to chromatographic method development. In this approach, first the requirement for a method is defined. Based on the critical material, process and product attributes, understand what the method has to measure and the performance requirement for the method. Then, previous knowledge is reviewed and documented to identify initial conditions. A design space is then identified and a screening study is then conducted with the resulting data analyzed to construct a resolution response surface to identify potential optimum chromatographic conditions. The predicted optimum conditions are examined and further fine tuned prior to GMP validation. In addition, proper documentation is emphasized to build a body of knowledge relevant to the developed method for future reference, troubleshooting and transfer.

INTRODUCTION

HPLC method development for the analysis of drug substances and drug products is one of the major challenges an analytical chemist faces everyday. It is a task that requires much expertise and can be extremely time-consuming. In spite of advances in chromatographic theory and the development of computer software that facilitates this process, HPLC method development is still based mainly on "trial-and-error."

The quality of an analytical method is defined by the design of the method, not by the outcome of method validation. By utilizing a quality by design approach, higher quality methods are developed in significantly less time than traditional "trial and error" methods.

METHOD

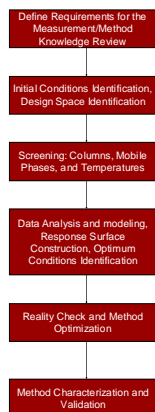
The systematic approach to chromatographic method development is designed to gain scientific knowledge while developing methods that are fit for their intended purpose. By understanding the underlying chemistry during development, potential issues are minimized. The approach is summarized in the adjacent flow chart on the left.

Critical Factors for Success:

Samples: Samples should include API, API precursors, Mother Liquors, forced degradation samples, reference substances, drug products, stability samples, excipients, and leachables and extractables (if applicable). The goal is to have the most complex sample to ensure method specificity.

Peak Tracking: Proper peak tracking is essential to the success of systematic HPLC method development. Different techniques were used to ensure all peaks were tracked correctly from one system to another. The most reliable means of peak tracking is mass spectrometry; however, UV spectra and peak area can also be effective.

System Dwell Volume Measurement: The system dwell volume can dramatically impact gradient methods. Therefore, it is important to accurately measure and account for differing system dwell volumes to ensure successful gradient method prediction and transfer.



Documentation: Proper documentation is essential during chromatographic method development and the associated method development technical report should include several key attributes.

- The requirements of the measurement/method or target analytical/method profile.
- Design of the chromatographic method, and rationale of the design.
- An Impurity Grid in which the origin, identification, and notebook reference of each impurity is documented along with the rationale for tracking or not tracking in the final method characterization.

Requirements for the method:

The method will be used for assay and impurity measurement for both drug substance and DPI drug product. For the assay the accuracy requirement is 98.0-102.0%, and for impurity the accuracy is 90-110%. LOQ should be NMT 0.05% of nominal API concentration.

Design Space:

The major elements of the design space in this study include column, mobile phase, mobile phase gradient, and column temperature. Other parameters should also be investigated, such as detection wavelength, injection volume, etc. Sample preparation was relatively straight forward for this study and is not discussed in this presentation.

Column Selection: It was imperative during the screening process to use columns with a wide range of selectivity and not simply of different manufacturers of similar materials (e.g., Multiple C-18 columns). In this study various C18, C8, cyano, phenyl, and pentafluorophenyl columns were investigated.

Mobile Phase Selection: Mobile phase pH was first investigated and concluded that a low pH (pH 2) was most appropriate. Different types of organic solvent was then tested.

Screening Gradients: Screening gradients should be chosen that encompass the full useful range of the column with significantly different gradient times and column temperatures (Design Space). The goal is to maximize retention differences to gain understanding of how each compound interacts with the given stationary phase and mobile phase.

HPLC Screening Gradients				
Gradient	Gradient #1	Gradient #2	Gradient #3	Gradient #4
Gradient Time (h)	48 min	27 min	22 min	22 min
Column Temp	40°C	25°C	40°C	25°C
Mobile Phase (Beginning)	5% Water, 95% ACN, 0.05% TFA	10% Water, 90% ACN, 0.05% TFA	15% Water, 85% ACN, 0.05% TFA	20% Water, 80% ACN, 0.05% TFA
Mobile Phase (End)	5% Water, 95% ACN, 0.05% TFA	5% Water, 95% ACN, 0.05% TFA	5% Water, 95% ACN, 0.05% TFA	5% Water, 95% ACN, 0.05% TFA

Impurity Grid (example)				
Identity	Source	Track?	Rationale	
Counter Ion	Counter-ion	Yes		
Imp 1	Impurity	No	Not a potential process related impurity. The peak may be from starting material. It is controlled by the starting material.	
Imp 2	Degradation	Yes	It is the primary acid and base degradation product.	
Imp 3	Hydrolytic Degradation	Yes	Hydrolytic degradation product.	
Imp 4	Starting Material	No	Early Synthetic Step / Never found by MS in API and degradation samples.	
Main Band	API	Yes		

HPLC Column Screened (Example)
1) VMC ProPack C18 4.6mm x 150mm (4.6mm x 150mm, 5µm particle)
 Rationale: The VMC ProPack C18 column was selected due to its high degree of end-capping and previous successful experiences with VMC ODS based columns.
 Results: The column provided high efficiency; however, sufficient separation was not possible between the API and two impurity peaks.
2) Waters xBridge™ Phenyl 4.6mm x 150mm (4.6mm x 150mm, 3.5µm particle)
 Rationale: The xBridge™ Phenyl column was selected due to its unique silica surface presented with xBridge™ columns that offer a high degree of surface protection and hydrophobicity.
 Results: This column was found to not be suitable for separation of the active and counter-ion. Sufficient separation was not possible between the API and two impurity peaks.
3) Cytel Pentafuorophenyl 4.6mm x 150mm (4.6mm x 150mm, 3µm particle)
 Rationale: The pentafluorophenyl column provided an opportunity unique to fluoro containing analytes. The selectivity is altered by interactions with fluoro groups. These interactions could be used to potentially enhance the retention characteristics of the analytes.
 Results: The retention properties of API were drastically changed when using the pentafluorophenyl column, however resolution was still present between the API and degradation. This was an expected result as most of the potential degradation products contain fluoro. The column was not further evaluated.
4) Spectra Separation LC-C18 4.6mm x 150mm (4.6mm x 150mm, 5µm particle)
 Rationale: The silica column was selected due to its robust ability to handle both a neutral phase and a reversed phase column. By operating the column in reversed phase column mode, separation of the two degradation products can be achieved from the solvent front while maintaining ion-pair analysis. Sufficient separation was not possible.
 Results: The column was not considered as retaining a very poor analysis. The column was not used to resolve the API from the critical process impurities and the forced degradation product. The column was selected for the detailed methodology for analysis of impurities.
5) Waters xBridge™ Phenyl 4.6mm x 250mm (4.6mm x 250mm, 3.5µm particle)
 Rationale: The xBridge™ Phenyl column was selected for two primary reasons. The xBridge™ silica surface offers a wide pH range for operation (pH 1-12). In addition, phenyl columns provide reversed phase character with an additional separation mechanism that operates through aromatic ring interactions. The column was screened as an alternative to C18 columns which had all failed up to the point to separate the API from two critical potential impurities.
 Results: The xBridge™ Phenyl column was the only column found to successfully separate the API and the counter ion peaks from all the impurities studied. The column was selected for the final assay and impurity method and will be discussed in more detail in subsequent sections.

RESULTS

Presented here is an assay and impurity method development experiment to demonstrate the the systematic approach to method development.

Impurities Grid: A table of potential impurities was constructed that contains all LC-MS identified impurities either known or unknown. A total of 23 compounds were uniquely identified by their molecular weight and listed with reference to the notebook page of their initial identification and labeled by their source. The rationale for tracking or not tracking an impurity as part of the final method is documented for future reference.

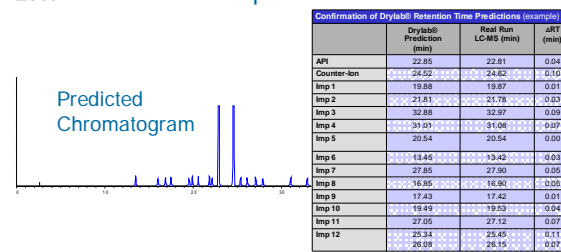
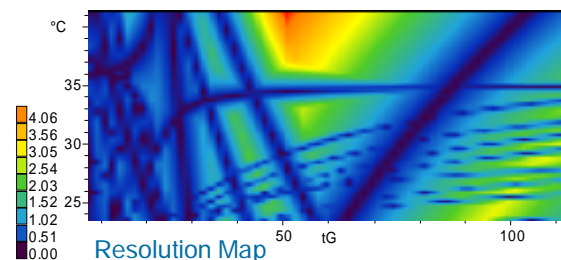
Data Analysis and Modeling: The data generated from the screening are analyzed using DryLab. The columns that showed the greatest promise in the column screening study were analyzed by LC-MS to positively identify the peaks for proper peak tracking. The compounds that were selected for tracking were used to model the chromatography in the DryLab prediction software.

Construction of Response Surface - Resolution Map: A three dimensional resolution map is constructed that demonstrates locations of maximum resolution between critical pairs. The resolution map for the xBridge Phenyl column is shown on the right demonstrating a wide range of potential conditions for the final method.

Critical Parameters Identification: From the screening data it was concluded that column temperature, mobile phase composition and gradient slope are the critical method parameters. Dwell volume is not a critical parameter for this method.

Predictive LC Chromatogram: Once the gradient is optimized DryLab will output a theoretical chromatogram based on its prediction. The example for the xBridge Phenyl column is shown below.

Optimization and Critical Parameter Confirmation: The conditions from the DryLab prediction were verified by LC-MS. Peaks were identified by their molecular weight with retention times recorded for comparison to the DryLab predictions. The real run values of retention time were predicted within 0.1 minute. A robustness study was conducted to confirm the critical parameters.



Confirmation of DryLab Retention Time Predictions (example)				
API	DryLab Prediction (min)	Real Run LC-MS (min)	API	API
	22.85	22.81	0.04	
Counter-ion	24.52	24.82	0.10	
Imp 1	19.88	19.87	0.01	
Imp 2	21.81	21.78	0.03	
Imp 3	32.88	32.97	0.08	
Imp 4	31.01	31.08	0.07	
Imp 5	20.54	20.54	0.00	
Imp 6	13.45	13.42	0.03	
Imp 7	27.85	27.90	0.05	
Imp 8	16.85	16.80	0.05	
Imp 9	17.43	17.42	0.01	
Imp 10	18.49	18.53	0.04	
Imp 11	27.05	27.12	0.07	
Imp 12	25.34	25.45	0.11	
	28.88	28.15	-0.07	

Primary HPLC Method Conditions:

- Column: Waters xBridge™ Phenyl, 3.5 µm particle size, 25 cm x 4.6 mm ID
- Mobile Phase: Solvent A: Water (0.05% TFA v/v) Solvent B: ACN (0.05% TFA v/v)
- Gradient: Linear, from 5%B to 75% in 35 minutes.
- Flow Rate: 1.0 ± 0.1 mL/minute
- Detector: 246 ± 2 nm
- Column Temperature: Isothermal at 40°C ± 2°C
- Injection Volume: 10 ± 5 µL

Method Validation: Upon finalizing the method a set of DOE characterization experiments were conducted to verify the method was robust and would pass validation. Critical parameters are confirmed. The method was successfully transferred to the GMP labs and validated without requiring further modifications.

CONCLUSIONS

This work demonstrated the quality by design systematic approach to chromatographic method development. The advantages of the strategy include:

- A better understanding of the factors influencing chromatographic separation;
- Methods developed are more robust;
- The potential for simultaneous development of multiple methods including orthogonal impurity methods, assay method, dissolution method, cleaning validation method, etc;
- Easier transfer of the project/knowledge through proper documentation;
- Greater confidence in the ability of the methods to meet their intended purposes;
- Potential regulatory advantages?