Analytical Quality by Design Based Method Development for the Analysis of Cold and Cough Formulations

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Introduction

HPLC is commonly used for analyzing cold and cough syrups because it can separate and quantify APIs and excipients with high sensitivity and selectivity. [1-3] Currently, the industry uses separate chromatographic methods to analyze each API in pharmaceutical formulations. While effective, this approach can be very time consuming and can generate large amounts of hazardous waste from organic solvents. To make such analyses more efficient, one solution is to use a single chromatographic method to analyze multiple APIs in various pharmaceutical formulations.

Analytical Quality by Design (AQbD) is a systematic approach to analytical method development that aims to ensure quality by identifying and controlling sources of variability throughout the method's lifecycle. AQbD principles are becoming increasingly important in the pharmaceutical industry, where regulatory agencies such as the USP and ICH are emphasizing the need for quality assurance and control. AQbD involves the use of statistical tools, design of experiments (DOE), risk assessments, and knowledge management to enhance method robustness, reliability, and consistency. By incorporating AQbD principles, analysts can reduce the time and cost of method development while improving the overall quality of analytical results.

This work utilized Fusion QbD™ software to develop a method for separating six APIs in a standard mixture and was tested on four different cough and cold formulations from the.

Experimental

System

The experiments were performed using an Arc™ Premier System. Fusion QbD method development software was used as an AQbD software in this study. Four 2.1×100 mm with 2.7 µm particle size columns was used for this study. These columns are: A CORTECS™ Premier C18 Column, a CORTECS Premier C18⁺ Column, a CORTECS Premier T3 Column, and a CORTECS Premier Phenyl Column.

Sample Preparation

To create a standard solution with a concentration of 1 µg/mL, phenylephrine, acetaminophen, doxylamine succinate, guaifenesin, dextromethorphan, and diphenhydramine were dissolved in DI water. Chemical structures of these compounds are depicted in Figure 1.

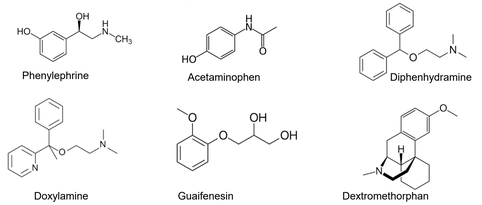


Figure 1: Chemical structures of six APIs that are commonly present in cold/cough medications and were used in this study.

Results

Method Development Workflow

The study employed a four-stage method development workflow, which is briefly outlined in Figure 2. Further information about each of these stages is presented in the results section.

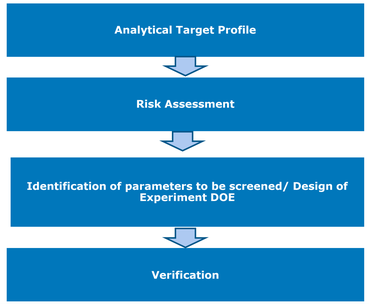


Figure 2: A workflow illustrates the numerous stages entailed in the process of this AQbD method development.

Analytical Target Profile

The Analytical Target Profile (ATP) is a crucial element of the Analytical Quality by Design (AQbD) approach for developing analytical methods. It encompasses a specific set of measurable characteristics that an analytical method must meet to ensure it is suitable for its intended purpose. The ATP consists of critical analyte attributes and performance characteristics, including but not limited to bias, precision, specificity, limit of detection, limit of quantitation, linearity, range, ruggedness, and robustness. By providing guidance for the development and optimization of the analytical method, the ATP ensures that the method meets predefined criteria, resulting in a fit-for-purpose method.

In this particular study, the ATP aimed to develop a method capable of separating APIs from excipients present in different cold/cough syrup formulations. The study began by selecting an appropriate technology, which was an Arc Premier UHPLC System equipped with a Quaternary Solvent Manager (QSM), a Column Manager (CM), and a solvent select valve to enable automated exploration of a wide range of conditions. A QDa™ Mass Detector was also employed to assist with method development and peak identification.

Risk Assessment

During this phase of the study, we thoroughly evaluated high-risk parameters that could potentially impact the quality of the data generated by the method, as well as its ability to achieve its goals. Our evaluation is based on sound chromatographic principles, prior knowledge, and expertise.
To illustrate all of the method parameters and their respective impact, we have included a fishbone diagram in Figure 3.

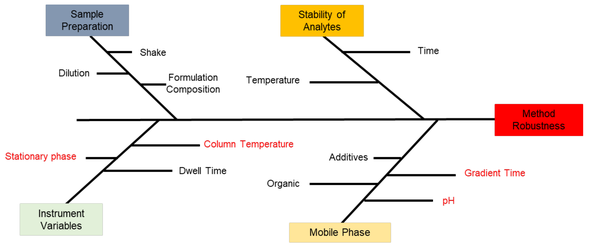


Figure 3: Ishikawa fishbone diagram of risk assessment. Red Text denotes a high-risk variable.

Identification of Parameters/DOE

At this phase of the study, a DOE was devised based on the critical method attributes and risk factors identified during the risk assessment, utilizing Fusion QbD software. The objective was to thoroughly explore the impacts of column chemistry, gradient time, and temperature on the process. A 1 µg/mL placebo was used for the DOE experiments. The data were processed in Empower™ Software and imported into Fusion QbD software to find the best overall answer (BOA) where all the performance goals are met. The performance goals that were set in this screening stage included the maximum number of peaks that were baseline resolved and the maximum number of peaks with a USP tailing factor of ≤1.5 in each chromatogram. These goals were set after visual examination of the screening experiment chromatograms. Processing the data revealed that the best combination of conditions predicted to achieve the set performance criteria were a CORTECS Premier T3 Column, a temperature of 45° C and gradient time of 9 minutes. In Figure 4 the chromatogram corresponding to the run with method conditions closest to those predicted as best overall by the software is shown.

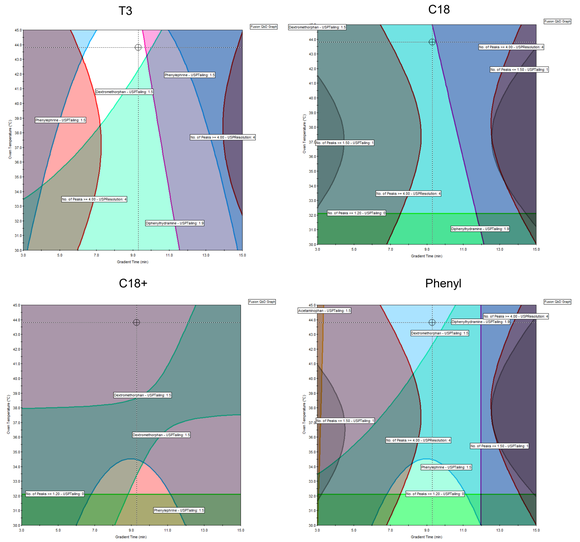
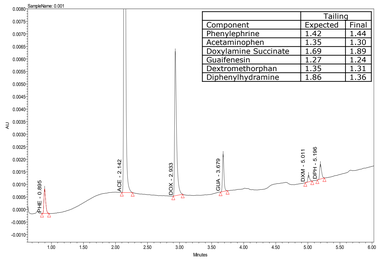


Figure 4: Acceptable performance regions of each column based on the gradient time and temperature. These were chosen as a high resolution was needed to separate APIs from excipients but the higher the resolution generally the worse the peak shape. The white color represents the design space where a minimum resolution of 4 between all peaks was achieved while maintaining a USP tailing of less than 1.5 for each peak. The crosshair pointer location on the corresponding graphs represent the Working Point conditions.

Verification

In order to verify the results as predicted by the software, it was important to compare these results with actual runs of the analytes. To do this, multiple verification experiments were run under the final conditions that were obtained from the BOA for the standard solution. These experiments showed that the predicted performance agreed well with the observed performance as shown in Figure 5. Further, the methods precision in terms of retention time and peak area was also evaluated and the results are summarized in Table 1.



Component	Tailing Expected	Tailing Final
Phenylephrine	1.42	1.44
Acetaminophen	1.35	1.30
Doxylamine Succinate	1.69	1.89
Guaifenesin	1.27	1.24
Dextromethorphan	1.35	1.31
Diphenylhydramine	1.86	1.36

Figure 5: Chromatogram and tailing results of a 1 µg/mL placebo containing the 6 APIs.

Table 1: Reproducibility of 1 µg/mL of a standard solution containing the six APIs with the final method.

Relative Standard Deviation of six Replicate Injections		
Component	**Retention Time**	**Area**
Phenylephrine	0.40 %	0.4%
Acetaminophen	0.09%	0.2%
Doxylamine succinate	0.09%	0.7%
Guaifenesin	0.05%	0.6%
Dextromethorphan	0.05%	0.4%
Diphenhydramine	0.04%	0.4%

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Method Application: Spiking with Various Levels of Potassium Sorbate

In this phase of the study, our objective was to evaluate the performance of the developed method in analyzing actual pharmaceutical formulations of cold/cough syrup. Additionally, we sought to assess the method's robustness by determining its ability to accurately detect the presence of varying concentrations of one of the excipients in the samples. Results of these experiments are demonstrated in Figures 6.

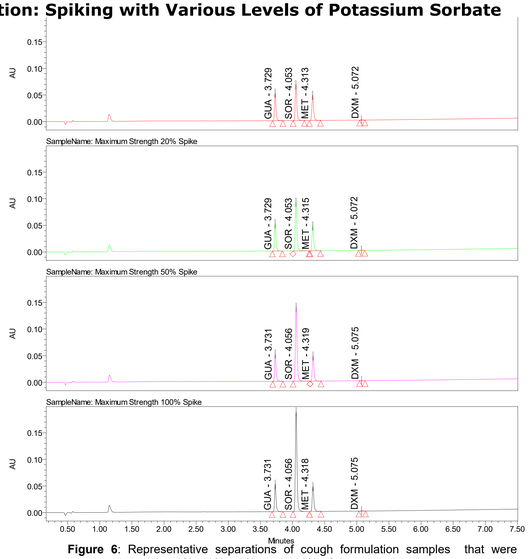

Figure 6: Representative separations of cough formulation samples that were prepared by adding 0%, 20%, 50%, and 100% of the original sorbate present in the cough formulation as potassium sorbate spike in a 1000-fold diluted cough formulation.

Method Application: Pharmaceutical Formulations and Peak Purity

In this experiment the method was tested on four widely used cold/cough formulations. These formulations were all spiked with potassium sorbate. All samples were found to have acceptable resolution and chromatographic characteristics. For example, peak purity was also examined in this experiment and the analysis of the syrup sample shows that the purity angle of each API is below the threshold angle, demonstrating spectral homogeneity
(Figure 7)

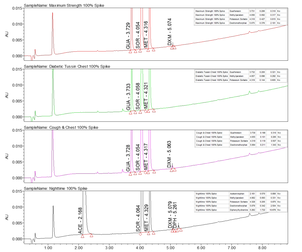

Figure 7: Representative separations of four cough formulations with peak purity evaluations.

Conclusions

- An analytical method that can analyze various cold/cough formulations with multiple active pharmaceutical ingredients (APIs) and varying excipient levels was developed using the AQbD principles.
- Employing the AQbD principles in analytical method development helps obtaining robust and reproducible method.
- The use of Fusion QbD in conjunction with Empower Software and Waters systems is very beneficial for automating the whole method development process.

References

[1] T. Dongala, N. K. Katari, A. K. Palakurthi and S. B. Jonnalagadda Biomed Chromatogr 2019 Vol. 33 Issue 11 Pages e4641
[2] Alkhateeb F, Cleary R, Rainville P. Maziarz M, Rainville P. Robust and Rapid Method Development for Analysis of Active Pharmaceutical Ingredients in Multi-Component Cold and Flu Medication. Waters Application Note: 720006287.
[3] Maziarz M, Rainville P. Robust and Rapid Method Development for Analysis of Active Pharmaceutical Ingredients in Multi-Component Cold and Flu Medication. Waters Application Note: 720006523.

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