ANALYTICAL QUALITY BY DESIGN FOR THE ANALYSIS OF FORMOTEROL, BUDESONIDE, AND ITS RELATED COMPOUNDS

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INTRODUCTION

The primary objective of this study is to implement the AQbD principles to develop an Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) method for the analysis of Formoterol, Budesonide, and its related compounds. Budesonide is a corticosteroid used for long-term treatment of asthma. Agonists such as Formoterol have also widely been used in the management of asthma and chronic obstructive pulmonary disease. Inhalation of the two pharmaceutical ingredients as one dose in combination inhalers has proved to be more clinically effective . This work focuses mainly on exploring the analytical potential of the AQbD approach of achieving a high-resolution chromatographic method for the analysis of Formoterol, Budesonide, and its related compounds

METHODS

Materials and standard preparations:
A test mixture that contains all APIs and impurities was prepared by diluting stock solutions of each one of the analytes in 70/30 (v/v) water/acetonitrile as sample solvent. The final concentration of each analyte in the test mixture were approximately: 0.4 mg mL-1 Budesonide, 0.15 mg mL-1 Formoterol, 0.005 mg mL-1 related compounds, E and L, and 0.01 mg mL-1 related compound G. Structures of these analytes are depicted in Figure 1.

Instrumental Analysis:
Data management:
Empower 3 Chromatographic Data System (Empower CDS) and Fusion QbD®
LC System: ACQUITY UPLC H Class Plus System with Quaternary Solvent Manager (QSM), Sample Manager (FTN), Column Manager (CM with 2 Auxiliary Column Managers), PDA Detector, QDa Mass Detector

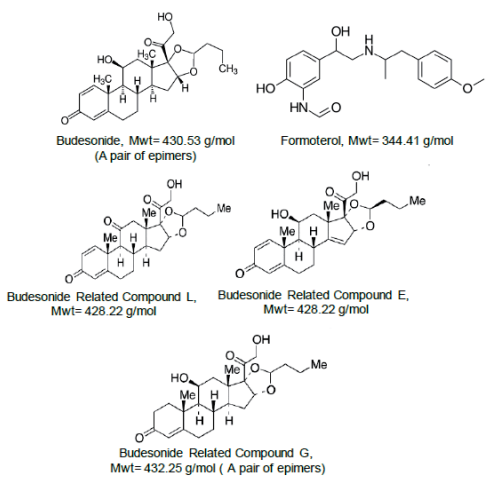


Figure 1. The chemical structure of Formoterol, Budesonide, and its related compounds.

RESULTS AND DISCUSSION

Fusion QbD Method Development Steps
Design of Experiment (DoE) is defined by the ICH as "a structured, organized method for determining the relationship between factors affecting a process and the output of that process". Fusion QbD is a software that uses the DoE approach to develop robust LC methods.
Figure 2 shows the general steps that are normally performed in Fusion QbD method development.

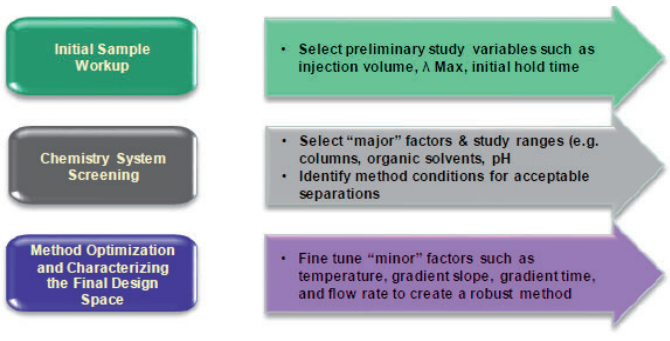


Figure 2. A schematic diagram of the general method development steps in Fusion QbD®

Initial Sample Workup

The main purpose of this step is to find initial chromatographic conditions that can be used as a starting point for the next screening step. These conditions should in general ensure that the analytes of interest are initially retained, elute before the end of the run, and are on scale using an appropriate wavelength. It should be noted here that the peaks are not expected to be well separated and with good shape at this point, they only need to be retained and integratable.

Chemistry System Screening

Five stationary phases were selected for study in this stage. These columns were a BEH C18 , BEH Shield RP C18, a CORTECS T3, a CORTECS Phenyl , and an HSS PFP. All are 5 cm × 2.1 mm × 1.x µm.
pH values ranging from 2 to 4.2 at half a unit interval were all explored. Results have shown that the BEH C18 is an appropriate choice as most of the analytes can be separated using this column as can be seen in Figure 3.

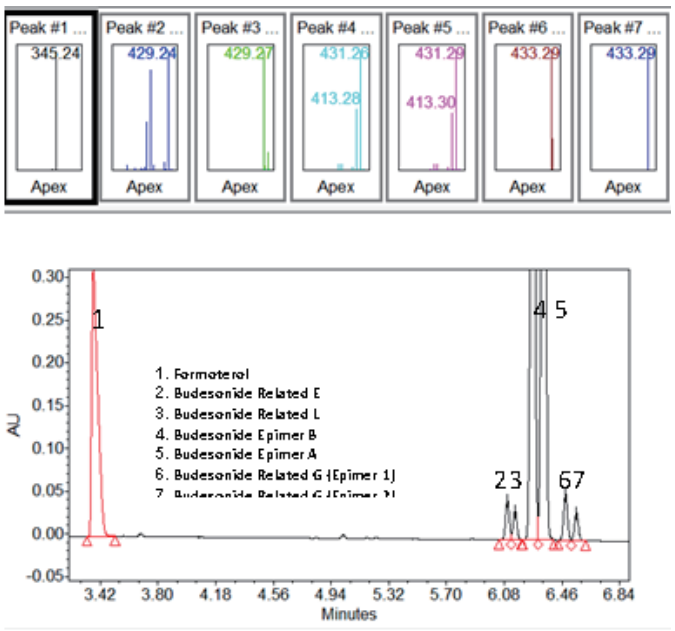


Figure 3. The "best looking" separation of Formoterol Budesonide, and its related compounds in the chemistry screening experiment.

Our first chemistry screening experiment was reasonably successful in separating all the analytes of interest. However, it was not able to achieve all the desired performance goals. Therefore, it was necessary to perform a second screening experiment to see if further improvements can be attained under different chromatographic conditions. In this experiment high pH values ranging between 6.7 to 10.7 were screened using the BEH C18 column. Results have shown that significant improvements in Formoterol peak shape can be obtained when high pH mobile phases are used as can be seen in Figure 4. For example, the tailing for the Formoterol peak was less than 1.3 for 10 out of the 15 high pH screening runs.

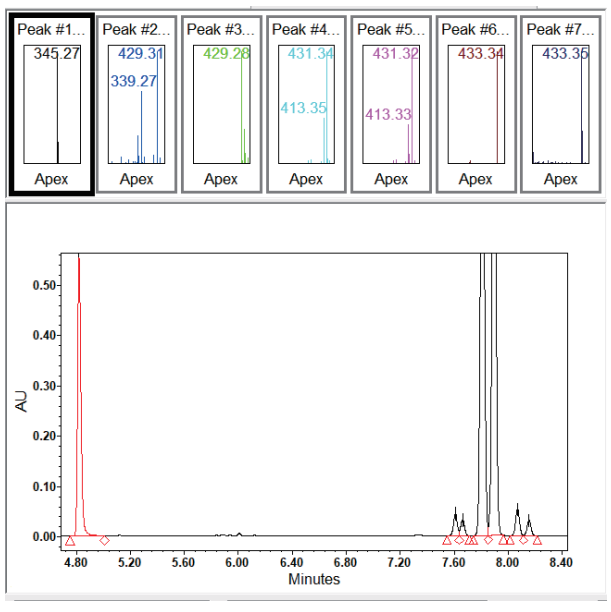
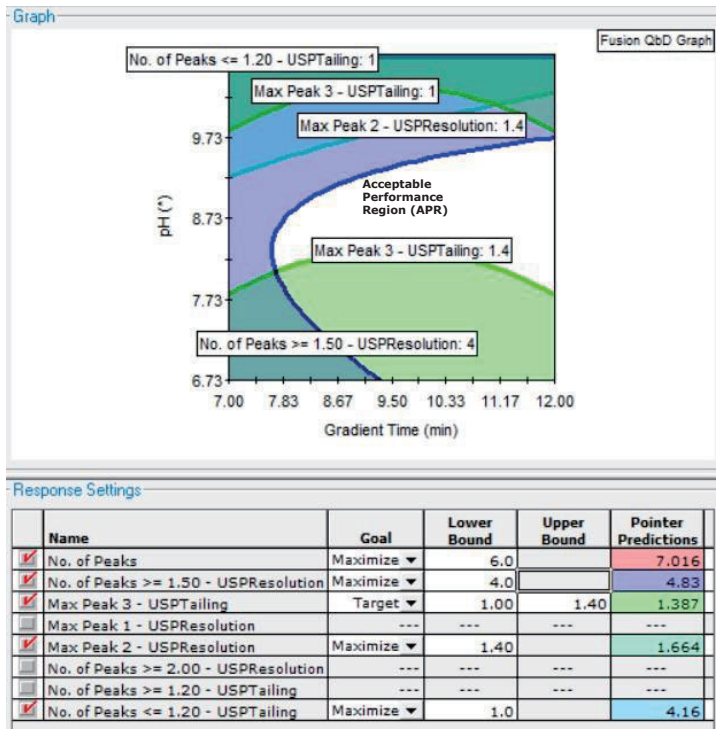


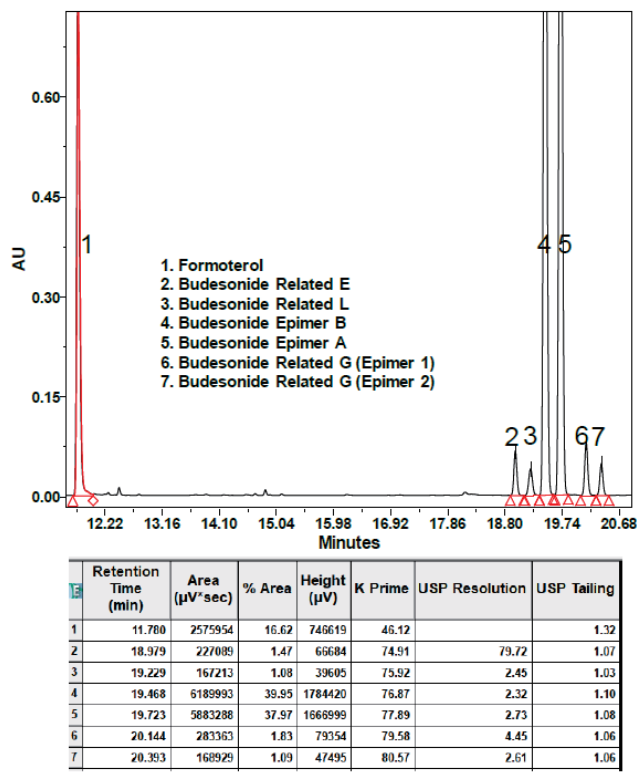
Figure 4. The "best looking" separation of Formoterol Budesonide, and its related compounds in the High pH screening experiment.



Response Settings

	Name	Goal	Lower Bound	Upper Bound	Pointer Predictions
✓	No. of Peaks	Maximize	6.0		7.016
✓	No. of Peaks >= 1.50 - USPResolution	Maximize	4.0		4.83
✓	Max Peak 3 - USPTailing	Target	1.00	1.40	1.387
	Max Peak 1 - USPResolution	---	---	---	---
✓	Max Peak 2 - USPResolution	Maximize	1.40		1.664
	No. of Peaks >= 2.00 - USPResolution	---	---	---	---
	No. of Peaks >= 1.20 - USPTailing	---	---	---	---
✓	No. of Peaks <= 1.20 - USPTailing	Maximize	1.0		4.16

Optimization
Optimization in LC method development is normally done by fine tuning some of the less important parameters such as the temperature, gradient slopes, gradient times, and flow rates. In this experiment the flow rate and the temperature were varied. Data were processed in Fusion to create the design space for a robust method. Results have shown that the desired performance goals can be achieved under wide ranges of experimental conditions. For example, all analytes of interest (7 peaks) can be separated with a minimum resolution of 2 and with a tailing factor of less than 1.3 over a wide range of temperatures (32.2-38.6 °C) and a wide range of gradient times (13-25 min). Figure 6 shows the separation of the compounds of interest under the "BOA" conditions.



	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)	K Prime	USP Resolution	USP Tailing
1	11.780	2575954	16.62	746619	46.12		1.32
2	18.979	227089	1.47	66684	74.91	79.72	1.07
3	19.229	167213	1.08	39605	75.92	2.45	1.03
4	19.468	6189993	39.95	1784420	76.87	2.32	1.10
5	19.723	5883288	37.97	1666999	77.89	2.73	1.08
6	20.144	283363	1.83	79354	79.58	4.45	1.06
7	20.393	168929	1.09	47495	80.57	2.61	1.06

Figure 6. A representative separation of Formoterol Budesonide, and its related compounds under the "BOA" conditions that were obtained from the optimization experiment. These conditions are: pH=8, Temperature=33 ° C, and Flow Rate=0.350 ml min-1. The gradient profile was: initial hold of 2 minutes at 5% Acetonitrile and 95% Ammonium followed by a linear gradient of Acetonitrile from 5-60% over 25 minutes.

CONCLUSION

Using Fusion QbD as a tool for developing LC methods in conjunction with an ACQUITY UPLC H-Class Plus system can be advantageous as it:
·Automates the entire process of method development
·Saves time by determining the minimum number of experiments needed for valid results
·Provides tools to visualize the impact of chromatographic parameters

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