

The Challenges of Developing a Purity Method for the Analysis of a Single Dose DPI Inhaler

Victoria Sillis¹, Frank Chambers¹ & Bob Lott²

¹ Inhalytic Ltd, Discovery Park, Sandwich, Kent, CT13 9ND, United Kingdom

² CI Informatics, PO Box 300, Didcot, OX11 1EU, United Kingdom

Summary

The USP HPLC method for albuterol was redeveloped using Fusion QbD to reduce solvent use, improve sensitivity, and enable impurity analysis of albuterol within a lactose formulation matrix. The original USP method employed a high mobile phase flow rate, increasing consumables cost and environmental impact, and lacked sufficient sensitivity. The redevelopment was particularly important because of a requirement for a single-dose test. Fusion QbD was used to optimize the method by adjusting flow rate, column dimensions, UV wavelength, gradient conditions, and particle size.

Firstly, the flow rate was reduced to conserve solvent and enable Fusion QbD optimization. Column dimensions were shortened using an online Merck™ HPLC method transfer calculator, reducing solvent consumption thus providing a more environmentally sustainable method.

Secondly, sensitivity was enhanced to allow lower sample concentrations. The UV wavelength was shifted from 230 nm to 225 nm, aligning with albuterol's peak maxima and improving UV response. Fusion QbD then evaluated the impact of gradient, organic content, oven temperature, and flow rate on method performance.

Thirdly, Fusion QbD was used to assess the impact of gradient, organic percentage, oven temperature and column flow rate on sensitivity and robustness of the critical pair resolution, both key method performance requirements set out in the Analytical Target Profile.

With these modifications, it was possible to take the USP method and improve it to allow the analysis of impurities from a single dose DPI inhaler.

Key Message

The USP HPLC method for albuterol was redeveloped using Fusion QbD to improve sensitivity, reduce solvent use, and enable impurity analysis from single-dose DPI inhalers by optimizing flow rate, column, UV wavelength, and gradient conditions.

Introduction

The USP albuterol purity monograph [1] to determine and quantify albuterol impurities from a pressurised metered dose inhaler (pMDI) is provided as a draft method, summarised in Figure 1. The aqueous portion of the mobile phase is based on sodium dodecyl sulphate (SDS) at approximately 5mM. The solution concentration required for this method is 0.3mg/mL of albuterol, which is considered acceptable when analysing a full canister of a pMDI, but not acceptable when analysing a single dose of a DPI product containing a 100 µg albuterol and lactose monohydrate as a carrier in the formulation.

Time / mins	Mobile Phase A / %	Mobile Phase B / %	Mobile Phase A: 5mM SDS in Water/Acetonitrile (80/20%v/v) Mobile Phase B: 5mM SDS in Water/Acetonitrile (60/40%v/v) Flow Rate: 2 mL/min Injection Volume: 50 µL UV Wavelength: 230 nm Column Temperature: 30°C Column: Pursuit 5 C18, 150 x 4.6mm 5 µm
0	90	10	
35	45	55	
45	45	55	
45.1	90	10	
55	90	10	

Figure 1 - USP chromatography conditions

Problem Statement

The current USP HPLC method for albuterol is unsuitable for low-level impurity analysis when applied to a single-dose DPI, as it uses high flow rates, consumes excess solvent, and lacks sensitivity. Therefore, the method required redevelopment to improve sensitivity, reduce solvent use, to enable impurity analysis from a single-dose DPI.

Materials and Methods

A proprietary blend containing 100 µg albuterol and lactose monohydrate was purposefully degraded over two weeks at 70 °C in an open beaker without humidity control, to generate a sample enriched with degradation products of interest. Method development was primarily conducted using an Agilent 1100 HPLC (High Performance Liquid Chromatograph) equipped with a UV detector. Test sequences were designed using Fusion QbD® method development software (S-Matrix Inc.) [2], a data-driven HPLC method development algorithm. The Fusion QbD® LC Method Development software was further employed to establish the Method Operable Design Region (MODR) in alignment with Analytical Quality by Design (AQbD) principles.

An online HPLC method transfer calculator [3] (ex. Merck™), was used to provide a set of baseline chromatographic condition suitable for a HPLC column based on a stationary phase silica support of 2.7 µm (cf the monograph column using 5 µm silica) and reduced length of 10cm (cf 15 cm for the monograph column). This change allowed the flow rate to be reduced from 2.0 mL/min to 1.0 mL/min, thus conserving solvent in subsequent Fusion QbD runs.

Results and Discussion

A comparison of typical chromatograms obtained using the USP method versus the shorter column with small particle size is shown in Figure 2.

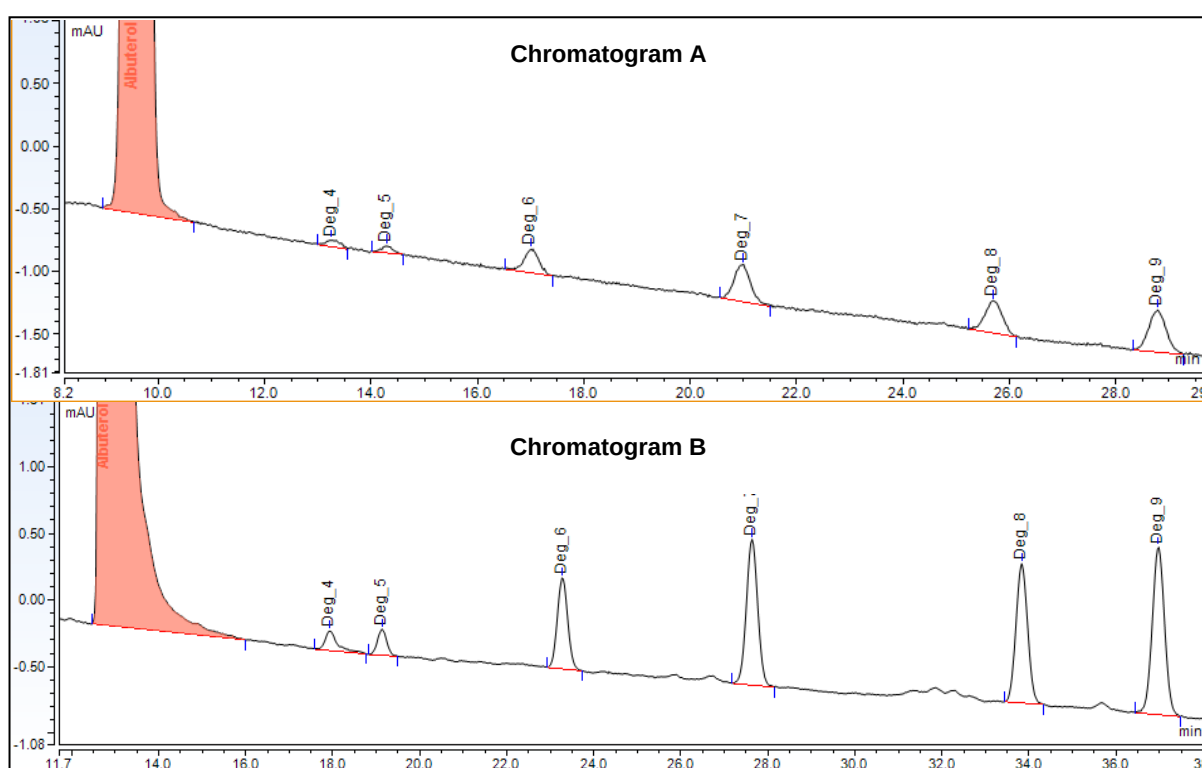


Figure 2 - Comparison of chromatograms from the USP method conditions (Chromatogram A) and reduced flow rate, short column and smaller particle size (Chromatogram B). Albuterol main band is shaded.

Fusion QbD was then used to determine the effect of gradient time, final organic percent, oven temperature and flow rate on sensitivity and robustness of separation. The software proposed a 30 run DOE to properly evaluate all main and interaction effects of the four critical process parameters under study. These experiments were automatically built in the Thermo Scientific Chromeleon Chromatography Data System (CDS) ready for analysis. The resultant chromatograms were first integrated in Chromeleon then imported into Fusion QbD for peak tracking and data analysis.

Peak height of Deg-4 and Deg-5 were used as surrogate responses for sensitivity. These peaks were chosen being the smallest peaks in the degraded albuterol sample and therefore the hardest to detect, while also representing the methods critical pair.

The model term pareto ranking charts, Figure 3, indicated the primary factors influencing peak height are those relating to the steepness of the gradient, namely final organic % and Tg. This can also be observed in the surface plots, Figure 4.

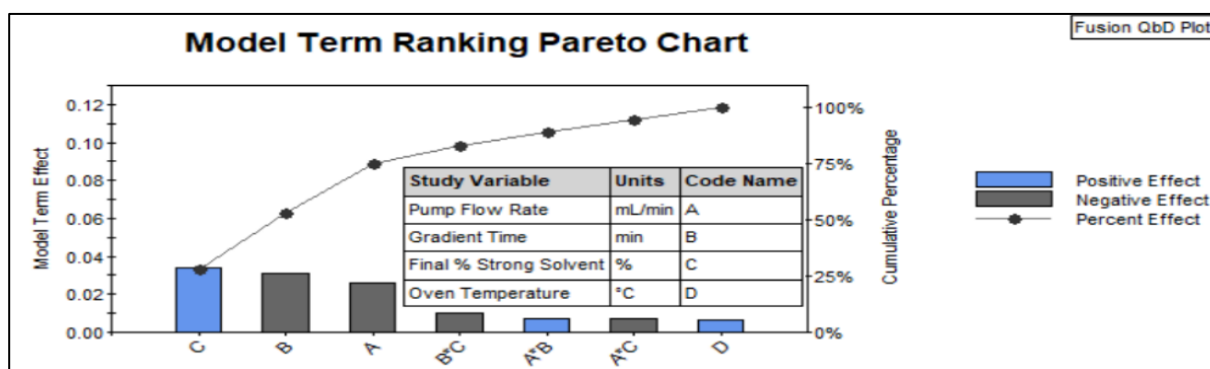


Figure 3 – Deg-5 (Height) Model Term Ranking Pareto Chart

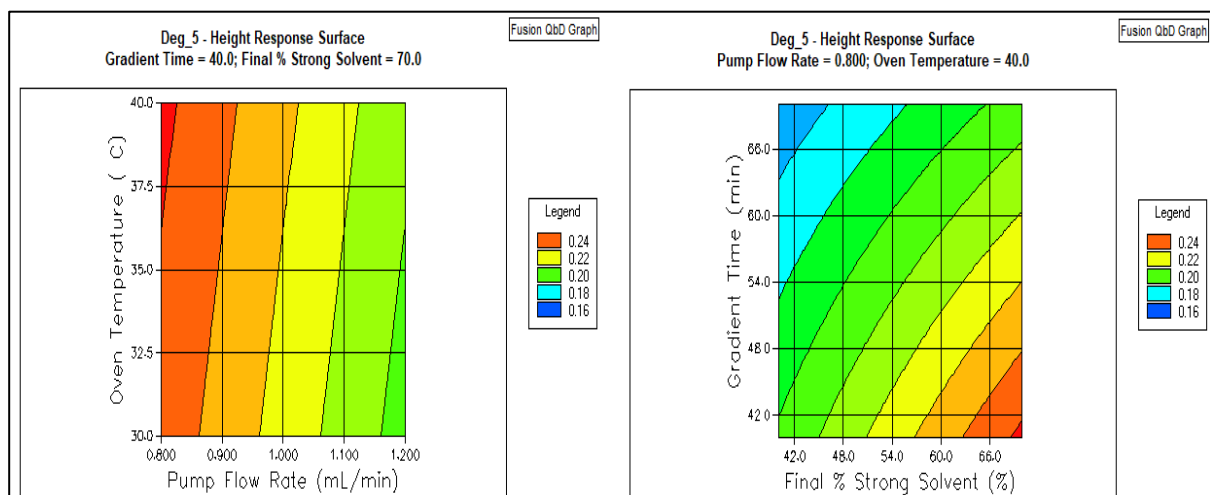


Figure 4 – Deg-5 (Height) Response Surface Plots

The aim therefore was to find the gradient of maximum steepness without compromising the robustness requirements set out in the analytical target profile (ATP). To this end a Monte Carlo simulation was performed to generate the distribution of Deg-5 resolution results from virtual robustness experiments performed throughout the experimental region. Process capability metrics (Cpk) were then calculated using a value of 2 as the minimum resolution specification limit as stated in the ATP. An optimisation was then performed to simultaneously maximise Deg-4 and Deg-5 height and Deg-5 resolution robustness (Cpk).

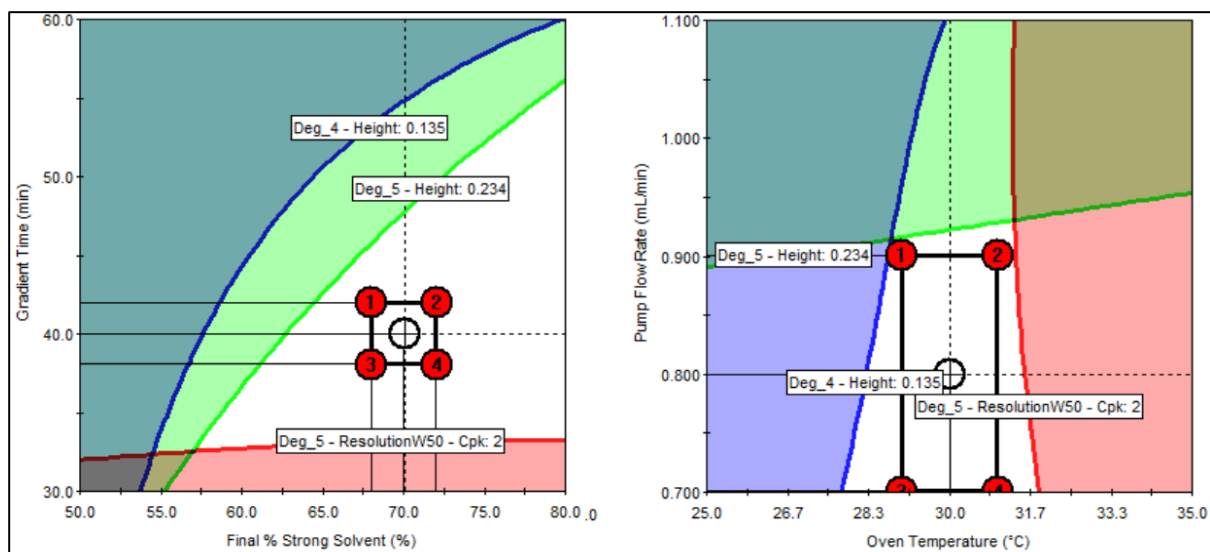


Figure 5 – Extrapolated Design Spaces with defined MODR depicted.

The optimum method was found to be in the corner of the studied experimental region. Optimum conditions were the minimum column temperature studied (30°C), minimum flow rate studied (0.8 mL/min), minimum gradient time studied (40 min), and maximum final organic % (70%). Extrapolation of the derived empirical models allowed for the visual identification of threats beyond the studied region and a Method Operable Design Region (MODR), Figure 5. A summary of the optimised chromatographic conditions is included in Figure 6.

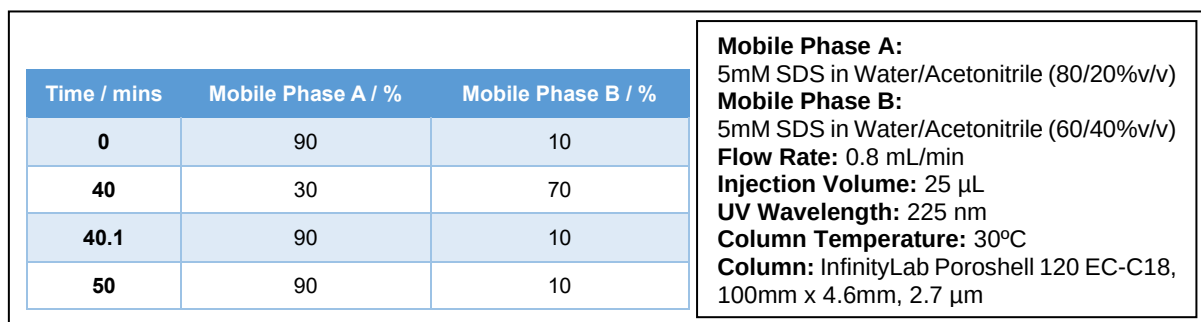


Figure 6 - Optimised chromatography conditions

The optimized method identified by Fusion QbD, is shown in Figure 7. The optimised method demonstrated a sevenfold increase in sensitivity compared to the USP monograph conditions, along with a reduction in solvent consumption of 70 mL per injection.

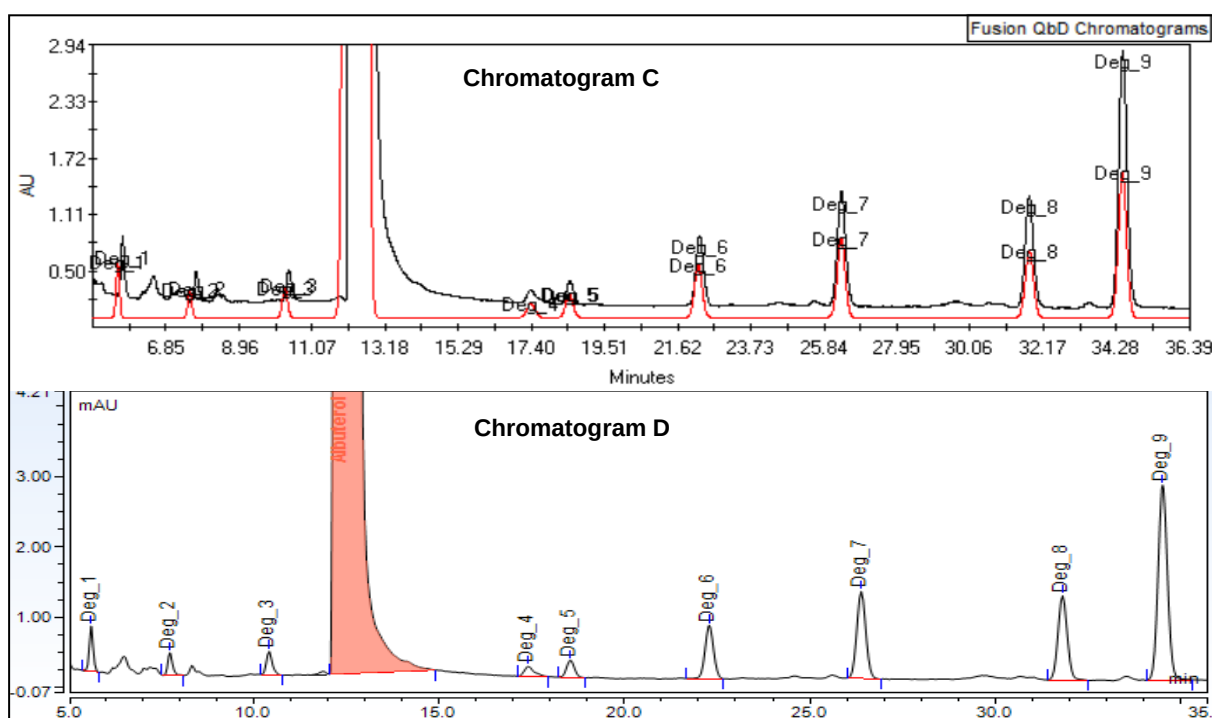


Figure 7 – Predicted “Best Method Centre Point” chromatogram (Chromatogram C in red) compared to the obtained verification chromatogram (Chromatogram C in black) along with the representative chromatogram from Chromeleon (Chromatogram D). Albuterol main band is shaded.

Conclusions

The USP albuterol purity monograph was successfully redeveloped to improve sensitivity and reduce solvent usage and the impact on the environment.

The sensitivity improvements were significant, with contributions made from alternative column technology, use of albuterol's $\lambda_{\max}$, and optimisation of HPLC parameters proven to effect sensitivity.

The Fusion QbD software allowed for the optimisation of sensitivity while proving excellent robustness ($C_{pk} > 2$) of the separation under the modified conditions.

The data obtained suggests further improvement may be possible with an extension of the experimental region studied. Further work to translate to a UPLC column and instrument would also likely prove advantageous.

References

- [1] Pharmacopeial Forum; PF Vol.27, No. 3, page 2505.
- [2] <https://www.sigmaaldrich.com/GB/en/support/calculators-and-apps/hplc-method-transfer-calculator>.
- [3] S-Matrix Inc; Fusion QbD Method Development Software.