Using a Design of Experiments Approach to Develop Fast LC Methods for Automated Scale-up to Preparative Chromatography of Sulfa Drugs

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Introduction

Chromatographic method development can be an extremely time consuming and subjective process. As companies accelerate their drug development programs and candidate compounds move through this process, fast and robust HPLC method development becomes increasingly important. Most method development is being done by a manual, one-factor-at-a-time (OFAT) process where the approach is to vary one system parameter at a time and examine the resultant performance. This procedure is continued until no further improvement is obtained, at which time another parameter is selected for study. These separations are often sub-optimal in terms of resolution, tailing, retention time and lack robustness. This can be problematic when preparative chromatography is required to purify milligram to gram amounts of product, as compounds that appear to be well resolved at the analytical scale, may no longer separate efficiently when scaled up, necessitating either further method development or additional product purification steps. One way to improve this process is to apply a Quality by Design (QbD) strategy that develops analytical LC methods to meet performance requirements using sound statistical experimentation principles that accurately quantify system behavior and then scale these up for preparative separations. (1, 2, 3) This can be done using a software based Design Of Experiments (DOE) applications that relies on multivariate modeling to automatically generate optimized analytical HPLC methods that can then be transferred to preparative HPLC systems and rapidly scaled up, significantly increasing productivity. This work describes the use of Fusion QbD - an integrated DOE method development software application to - a) to develop and optimize a the separation of a multi-component pharmaceutical mixture comprising 6 structurally similar sulfa drug compounds that included at least one unresolved resolved critical peak pair, (Sulfadiazine & Sulfacetamide) in the shortest time possible and - b) to transfer the resultant method to a preparative LC system and test the ability of this method to efficiently separate the actives when on-column sample loadings were scaled up.

Materials and Methods

Analytical HPLC – Varian Model 920 LC 335 Diode Array detector 270nm
Preparative HPLC – Varian Prepstar LC410P/218/510/325 UV Detector 270nm
Analytical Column – Varian Pursuit XRs C_{18} 5um 4.6 x 250mm
Preparative Column – Varian Pursuit XRs C_{18} 5um 10 x 250mm
Aqueous Phase – Water/0.1% (v/v) TFA,
Organic Phase 1 - Acetonitrile/0.1% (v/v) TFA
Organic Phase 2 - Methanol/0.1 % (v/v) TFA

Rapid Method Development Software Platform – Instrument control, chromatogram generation, peak processing: Varian Galaxie chromatography data system (CDS), (Varian Inc., Palo Alto, CA.) Statistical experimental design, data analysis, modeling, optimization: Fusion QbD (S-Matrix Corp., Eureka, CA.) System parameters included as experiment variables – Flow Rate, Gradient Slope, Gradient Time, Column Temperature, and Organic Modifier.

Analytical Method Development – Study factors were varied according to a model-robust screening design generated by Fusion QbD, which constructed the 28-run design as a set of ready-to-run methods and the corresponding sequence in the CDS. The experiment was run overnight on the 920LC under Galaxie CDS control. Peak result data were automatically imported from the CDS into Fusion QbD, using a file-less data exchange module, and analyzed. The data were subjected to experimental error, transformation, regression, outlier, residuals and pareto ranking analyses. Optimization solution searches were conducted with Fusion QbD numerical and graphical optimizers using the following goals: USP Resolution: > 2.0, USP Tailing: 0.95 — 1.05, Retention Time Max < 17 minutes.

Analytical to Preparative Method Transfer - The optimized analytical method was transferred to the Prepstar LC system and analytical scale injections were made to confirm that the method was transferable. Preparative scale injections of 8 -10 times greater column loading were made and run at equivalent linear flow velocities.

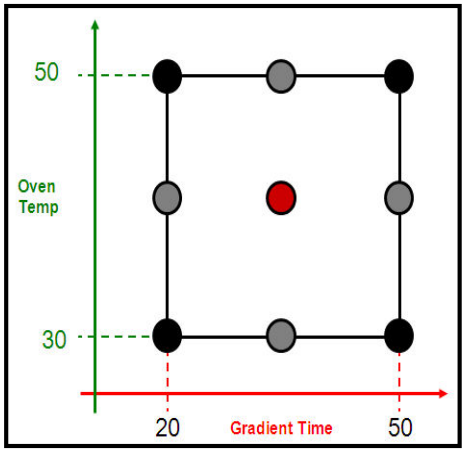

Figure 1. A model-robust screening type mixture-process design showing the centroid (●), vertices (●) and the edge mid-points (●) was used to screen ranges of the study variables and quantify their effects on method performance.

Run No.	Sequence No.	Pump Flow Rate (mL/min)	Gradient Time (min)	Organic Solvent Type (*)	Oven Temperature (°C)
Wash - 1	1	1.0	0.1	Acetonitrile 0.1% TFA	30.0
1.a.1.a	1	1.5	30.0	Acetonitrile 0.1% TFA	30.0
2.a.1.a	1	0.5	30.0	Acetonitrile 0.1% TFA	30.0
3.a.1.a	1	1.5	10.0	Acetonitrile 0.1% TFA	30.0
4.a.1.a	1	0.5	10.0	Acetonitrile 0.1% TFA	30.0
Wash - 2	1	1.0	0.1	Methanol 0.1% TFA	30.0
5.a.1.a	1	1.5	30.0	Methanol 0.1% TFA	30.0
6.a.1.a	1	1.5	10.0	Methanol 0.1% TFA	30.0
7.a.1.a	1	0.5	10.0	Methanol 0.1% TFA	30.0
Wash - 3	1	1.0	0.1	Methanol 0.1% TFA	30.0
Wash - 4	2	1.0	0.1	Acetonitrile 0.1% TFA	32.5
8.a.1.a	2	1.3	25.0	Acetonitrile 0.1% TFA	32.5
Wash - 5	2	1.0	0.1	Methanol 0.1% TFA	32.5
9.a.1.a	2	0.8	25.0	Methanol 0.1% TFA	32.5
Wash - 6	2	1.0	0.1	Methanol 0.1% TFA	32.5
Wash - 7	3	1.0	0.1	Acetonitrile 0.1% TFA	35.0
10.a.1.a	3	1.0	20.0	Acetonitrile 0.1% TFA	35.0
11.a.1.a	3	1.5	20.0	Acetonitrile 0.1% TFA	35.0

Figure 2. Software generated a statistical experimental design. The variables included process and mixture types. Fusion QbD therefore selected mixture process algorithm design (4)

Results

The initial chromatogram, run using a standard acetonitrile gradient prior to the application of DOE, showed co-elution of Sulfadiazene and Sulfacetamide as well as a poorly resolved Sulfathiazole peak. (Fig. 3). Peak results responses were modeled for the five critical peaks in the 28 run DOE experiment. All models fitted the data (all coefficients were significant, model prediction error ≈ experimental error) (6) Response surface plots indicated significant resolution responses and non-linear interaction effects. One such set of responses is shown in Fig 4 where the effect of the interaction between gradient time, flow rate and increasing column temperature on the resolution of Sulfacetamide is plotted.

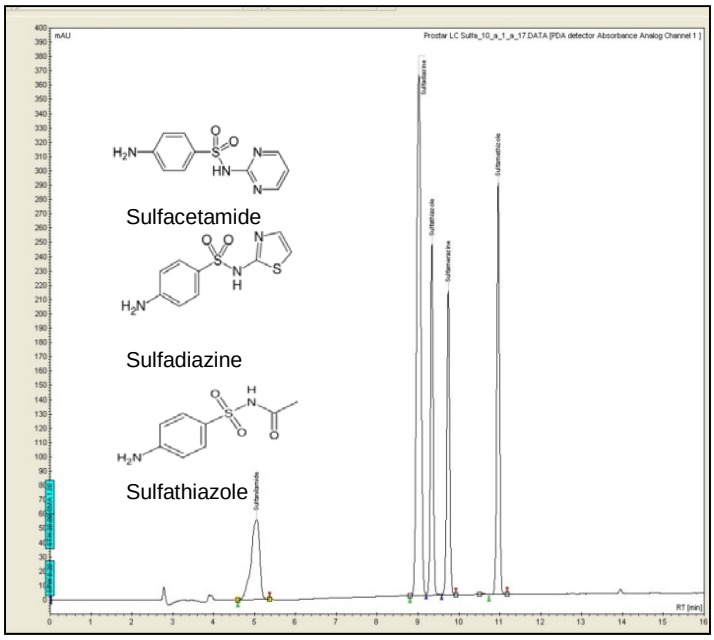

Figure 3. Initial Acetonitrile gradient separation showing co-elution of Sulfadiazine and Sulfacetamide and poor resolution of Sulfathiazole

There is a clearly observable difference in the resolution response when the organic modifier is methanol whereas, when acetonitrile is used, there is no observable response indicating that for this particular peak, methanol is better able to resolve Sulfacetamide than acetonitrile. The temperature interaction effect associated with methanol is also clearly visible, (Fig. 4) increasing as column temperature increased. The differing abilities of methanol and acetonitrile to resolve all the peaks of interest is also clearly shown in the overlay graphic response plots (Fig 5) below. The completely shaded acetonitrile overlay graphic confirmed that this solvent was unable to meet all the separation goals while the large un-shaded area associated with the methanol plot indicated that this solvent was able to resolve all the peaks within the set retention times and still meet the tailing factor goals. Using the generated model to modify any of the 4 study variables failed to improve peak separation when acetonitrile was the organic modifier suggesting that this solvent was simply unable to achieve any of the separation goals within the designated design space on the Pursuit XRs C18 column.

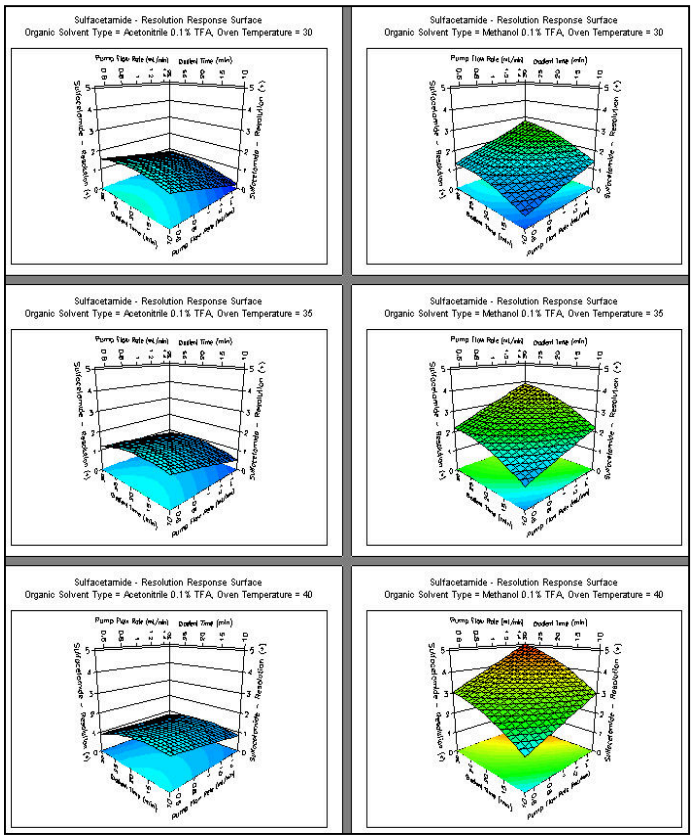

Figure 4. Single response series graphs showing effect of column temperature on Sulfacetamide resolution eluted with a) Acetonitrile and b) Methanol.

Speed optimized and resolution optimized prediction chromatograms were automatically created and run on the analytical LC system using the Fusion QbD point prediction tool.

a) b)

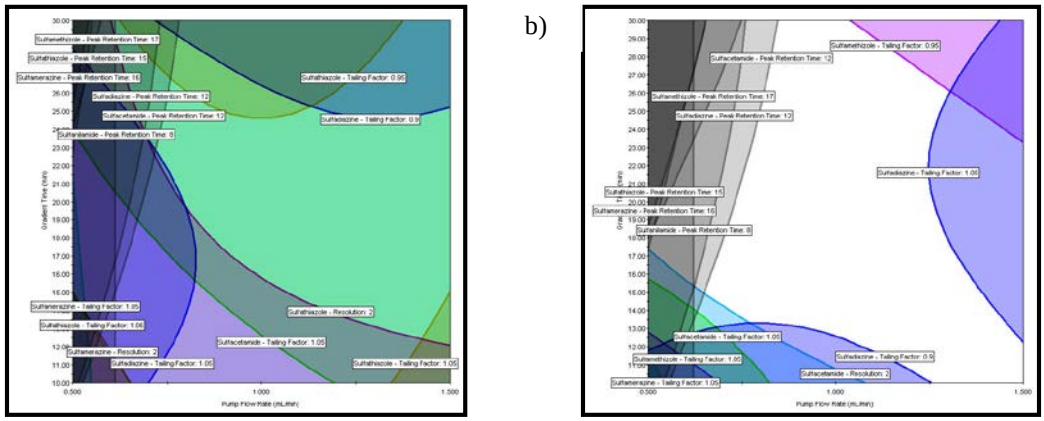

Figure 5. Overlay graphic plots of all responses showing areas where all separation goals are met (un-shaded) and where the goals are not met (shaded) for a) Acetonitrile, and b) Methanol as organic modifier.

The resultant chromatograms showed that when methanol was used as the organic modifier, and separation goals were set to maximize resolution (minimum R = 2.0) and minimize retention time, all the peaks were eluted within 9 minutes (Fig. 6a). When retention time was dropped and only the maximize resolution goal retained, all the peaks were eluted in 12 minutes with significantly improved resolution (Fig. 6b). When the separation was transferred to the Prepstar LC system and scaled up using the resolution optimized method with methanol, linear velocity and peak resolution goals were preserved (Fig. 7)

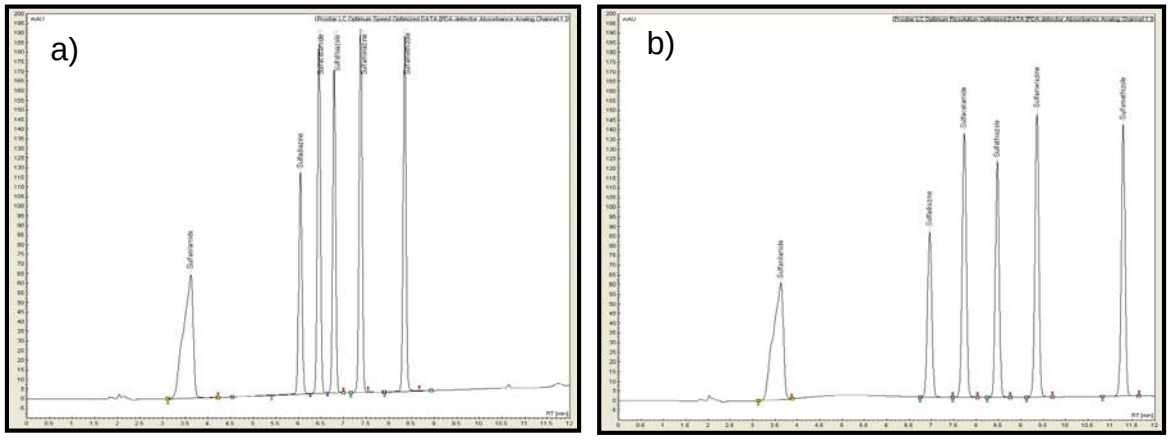

Figure 6. a) Speed optimized, and b) Resolution optimized sulfa drug separation on n XRs Pursuit C_{18} 250x4.6mm column generated from Fusion QbD automated optimizer.

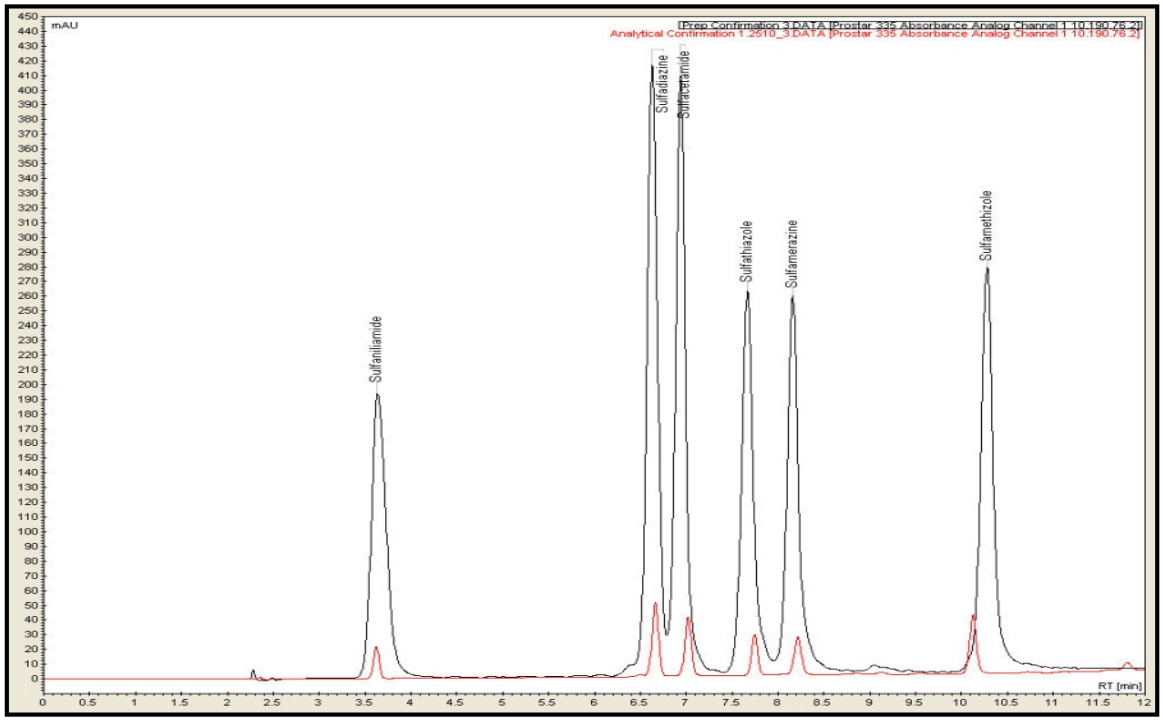

Figure 7. Analytical and preparative separation of sulfa drug mixture on Pursuit C_{18} 250 x 10mm semi-prep column.

Conclusions

- Fusion QbD was able to automatically predict and test speed and resolution optimized analytical methods that separated all the sulfa drug peaks of interest.

- Results showed that when methanol was used as the organic modifier it was significantly better at resolving the peaks of interest than acetonitrile.

- Analytical to prep scale-up of the sulfa drug peaks was successful with sufficient resolution of the critical peak pairs to ensure that maximum recovery of pure fractions would be possible.

Literature Cited

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