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

Lori Sandford[1] and Graham Shelver[2]

[1]Varian Inc. Walnut Creek, CA, 94598, [2]S-Matrix Corp. Eureka, CA 95501

Introduction

Chromatographic method development can be a time consuming and subjective process. As companies accelerate drug development programs and candidate compounds move through this process, fast and robust HPLC method development becomes increasingly important. Most method development is done using a manual, one-factor-at-a-time (OFAT) process where the approach is to vary one system parameter at a time and examine the resulting 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 robustness. This can be particularly 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.

The method development process can be dramatically improved by applying 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. This can be done using a software-based Design of Experiments (DOE) applications that relies on multivariate modeling to automatically predict and generate optimized analytical HPLC methods that can be transferred to preparative HPLC systems and rapidly scaled up, significantly increasing productivity. This work describes the use of Fusion QbD® – an integrated LC method development software application to a) develop and optimize the separation of a multi-component pharmaceutical mixture comprising six structurally similar sulfa drug compounds that include at least one unresolved critical peak pair, (Sulfadiazine & Sulfacetamide) in the shortest time possible, and b) 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:

- Chromatography Data Software (CDS): Varian Galaxie™, (Varian Inc., Palo Alto, CA.)
- QbD Method Development Software (DOE, modeling, Simulation, Robust Method Optimization: Fusion QbD Software Platform (S-Matrix Corp., Eureka, CA.)
- System parameters included as experiment variables: Flow Rate, Gradient Slope, Gradient Time, Column Temperature, Organic Modifier

Experimental

Analytical Method Development

Five study factors were selected for experimentation: Flow Rate, Gradient Slope, Gradient Time, Column Temp, Organic Modifier. These factors were varied according to a model-robust DOE 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. Figure 1 shows the general structure of a factorial-type DOE design. Figure 2 shows the first 11 runs of the Fusion QbD designed experiment, which The Fusion QbD design automatically includes Wash (Column Conditioning) runs for mobile phase chemistry changes between experiment runs [4]. 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 the peak results data were automatically modeled. 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:

Response Goals: USP Resolution: > 2.0

USP Tailing: 0.95 — 1.05

Retention Time Max < 17 minutes

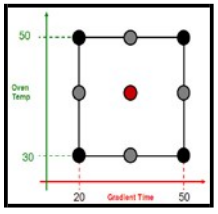

Figure 1. DOE-type design showing overall centroid (●), vertices (●), and edge midpoints (●).

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. First 11 runs of a software-generated statistical experimental design.

Results

The chromatogram shown below, from the initial method using a standard acetonitrile gradient, shows 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, with model prediction error ≈ experimental error). 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.

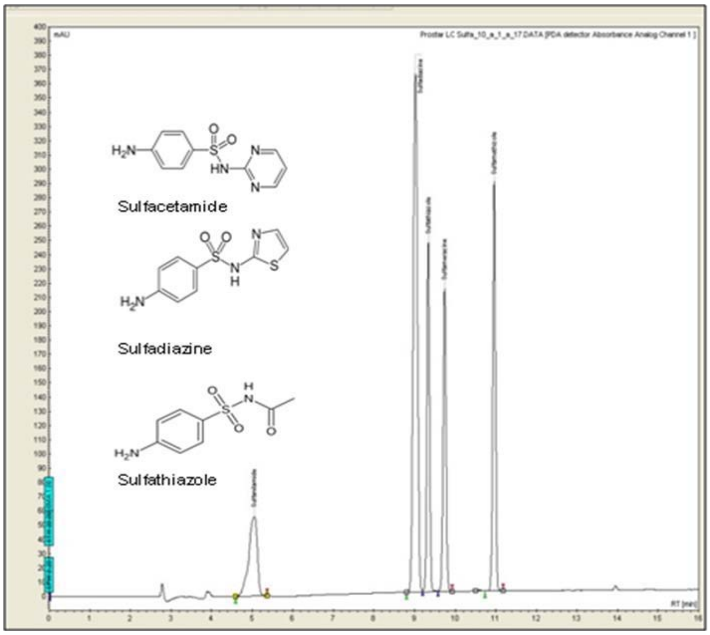

Figure 3. Initial Acetonitrile gradient separation showing co-elution of Sulfadiazine and Sulfacetamide and poor resolution of Sulfathiazole.

Response surface plots generated using the results data models indicated significant resolution responses and non-linear interaction effects. One such set of responses is shown in Figure 4, where the effect of the interaction between gradient time, flow rate and increasing column temperature on the resolution of Sulfacetamide is plotted. Figure 4 shows a clearly poorer Sulfacetamide resolution response when the organic modifier is acetonitrile across the entire study range of oven temperature (left vertical graph series) versus methanol (right vertical graph series). The interaction effect of oven temperature with organic modifier type (methanol versus acetonitrile) is also evident in these graphs, with resolution increasing as column temperature increases (top-to-bottom graph progression).

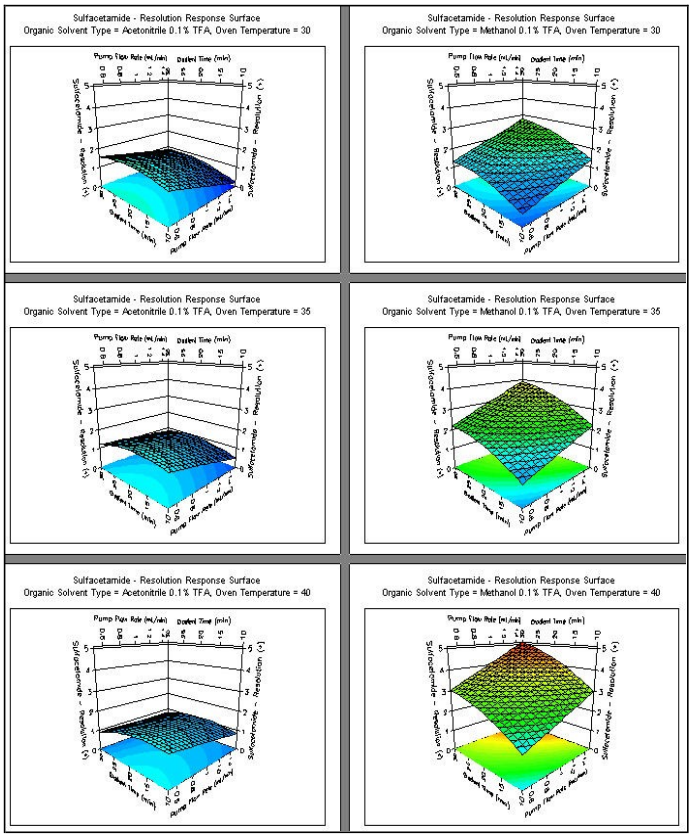

Figure 4. Single response series graphs showing effect of column temperature on Sulfacetamide resolution eluted with a) Acetonitrile and b) Methanol.

The differing ability of methanol versus acetonitrile to resolve all peaks is also clearly seen in the response overlay graphs shown in Figure 5. The completely shaded acetonitrile overlay graph (left graph) confirms that this solvent is unable to meet all the separation goals. Using the generated models to modify the four study variables failed to generate any unshaded region where all goals are simultaneously met. However, the large unshaded area associated with the methanol graph (right graph) indicates that this solvent can resolve all sample peaks while also meeting the tailing factor and maximum retention time goals.

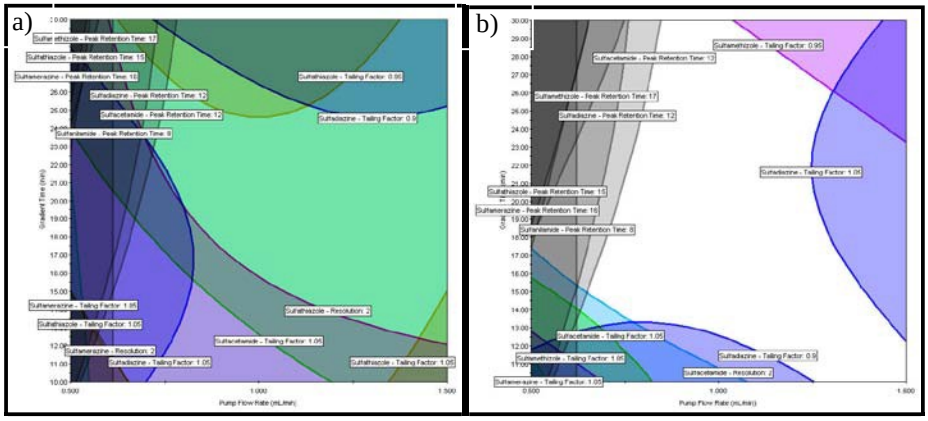

Figure 5. Overlay graphs of all responses showing areas where all separation goals are simultaneously met (unshaded region) and where the goals are not all met (shaded regions) for a) Acetonitrile, and b) Methanol as the organic modifier.

The chromatogram presented in Figure 6.a was obtained from the predicted optimum method when the optimization search included all separation, shape, and assay time goals using Methanol as the organic solvent. In this case all peaks eluted within 9 minutes. The chromatogram presented in Figure 6.b was obtained from the predicted optimum method when the optimization search goals were restricted to only resolution (shape and assay time not included. In this case all peaks eluted within 12 minutes with significantly improved resolution.

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. The chromatogram presented in Figure 7 demonstrates that the linear velocity and peak resolution goals were maintained when the separation was transferred to the Prepstar LC system and scaled up using the resolution optimized method with methanol.

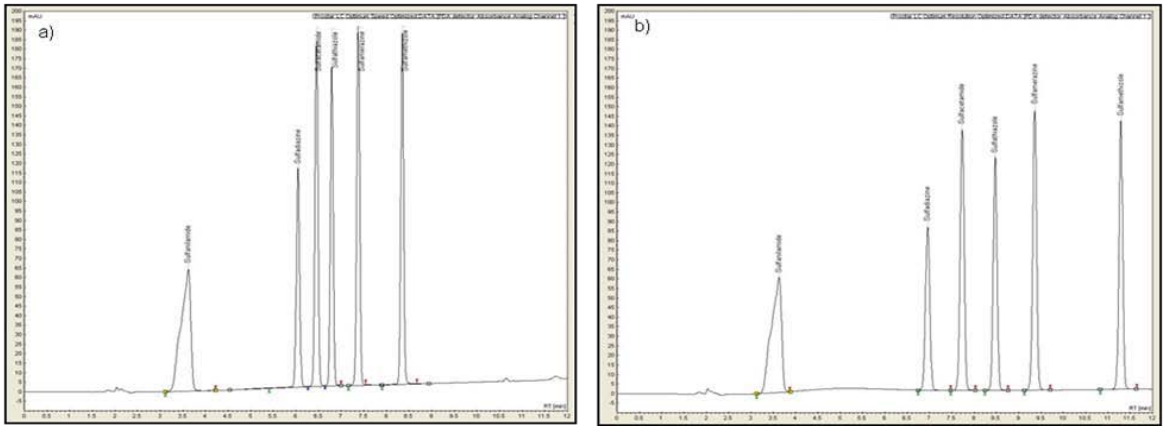

Figure 6. a) Speed optimized and b) Resolution optimized sulfa drug separation on n XRs Pursuit C18 250x4.6mm column generated from Fusion QbD automated optimizer.

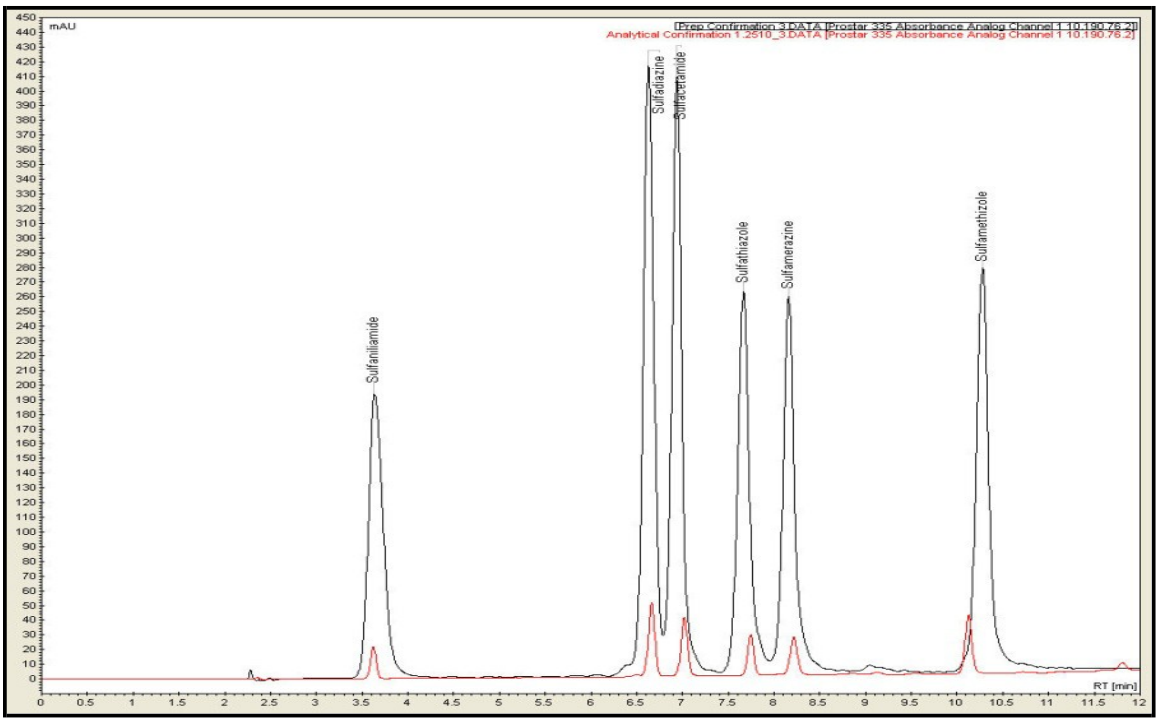

Figure 7. Analytical (red trace) and preparative (black trace) separations of sulfa drug mixture on Pursuit C18 250 x 10mm semi-prep column.

Conclusions

- Fusion QbD was able to automatically predict speed- and resolution-optimized analytical methods that separated all the sulfa drug peaks.
- Results showed that methanol performed significantly better than acetonitrile as an organic modifier in terms of resolving all sample peaks.
- 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 was possible.

Literature Cited

1. Cornell, John A., Experiments With Mixtures, 2nd Edition, John Wiley and Sons, New York, NY, 1990.
2. ICH-Q8 (R2) – Guideline for Industry. Pharmaceutical Development. August, 2009.
3. Montgomery, D., Design and Analysis of Experiments, Fourth Edition, John Wiley & Sons, New York, New York, 1996.
4. Myers, Raymond H. and Montgomery, Douglas C., Response Surface Methodology, John Wiley and Sons, New York, NY, 1995.

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