Wyeth Increases Yield 11.6% in Chiral Separation with Response Surface Methods

The separation of chiral compounds, molecules that are the mirror image of each other, is a major challenge in biopharmaceutical manufacturing because most bioorganic molecules exhibit this chemical property. In a typical chiral separation process, Wyeth Pharmaceuticals originally obtained a relatively unstable yield of 37.7% of the eutomer, or desired molecule. This compares to a theoretical maximum of a 50% eutomer yield.

Robert Tinder, Senior Research Scientist for Wyeth, performed a response surface method (RSM) experiment that graphically depicted the effect of key process factors on the yield and purity of the separation process. He identified a combination of factor values that increased eutomer yield to 42.1%, an 11.6% improvement over the original process. The new process conditions are also much more robust.

The challenge of chiral separation

Chiral compounds often have different levels of safety and effectiveness. It is necessary to separate them to ensure that the pharmaceutical product consists entirely of the approved compound. In this application, Wyeth used one of the most popular methods of chiral separation. The drug molecule was produced as racemic base, one that contains both chiral compounds (called enantiomers).

The chiral separation process began with adding a chiral acid to the racemic base to produce a chiral salt which is composed of a 50/50 mixture of each enantiomer. As is usually the case, one of the enantiomers was more soluble than the other. When the solution was crystallized, the desired enantiomer crashed out much more readily.

The original process conditions were 2 equivalents of water, 1 equivalent of chiral acid, and a cooling temperature of 23^{o}C. These conditions resulted in a racemic yield of 40% and a chiral purity of 94.2% for a eutomer yield of 37.7%. It’s important to note that yield does not necessarily correlate with purity. Often a process with a high yield will provide a low level of purity and vice versa. In this case, 90% purity was required by the next step in the chiral separation cycle.

There are a large number of process parameters with the potential to affect purity and yield. One is the choice of which chiral acid to use. Which solvent – such as water, ethanol or methanol – to use as the resolving agent may also be at issue. Other parameters that can impact the eutomer yield include how much chiral acid is used for a given quantity of chiral base, whether or not seeds are added for crystallization, at what temperature the resolution is performed, at what temperature the mixture is cooled, and how long the mixture is cooled.

Designing an RSM experiment

Based on an earlier screening experiment, Tinder isolated three process parameters as having the most critical impact on yield and purity in this application:

A. Temperature (^{o}C)
B. Chiral acid (Equivalents)
C. Water (Equivalents)

He designed an experiment in which 300 mg of the chiral base and an appropriate amount of chiral acid were weighed into vials. 3 mL of solvent was added followed by a prescribed amount of water. The mixture was heated to 50^{o}C for 30 minutes while being stirred at 900 rpm. Then the mixture was cooled to a specified temperature over 30 minutes. The mixture was held at that temperature for 96 hours before filtration.

Tinder said that Wyeth used the one-factor-at-a-time (OFAT) method to develop the original process. “The problem with this approach is that varying just one factor ignores interactions between factors so it usually identifies local peaks that are far from the global optimum,” he said. “On the other hand statistical design of experiments (DOE) varies the values of chosen factors in parallel so it uncovers not just the main effects of each variable but also the interactions between factors. This makes it possible to identify ideal combinations of factors in far fewer experimental runs than with the OFAT approach.”

“I selected Design-Expert® software from Stat-Ease, Inc., Minneapolis, Minnesota, because unlike general purpose statistical software it is designed specifically for DOE,” Tinder said. “It provides a wide range of experimental designs, each of which offers advantages for certain applications. I selected an RSM design here because it makes it easy to visualize the impact of factors in the form of a surface map. Design-Expert also greatly simplifies RSM by making it easy for a user without any statistical background to design an experiment and analyze the results."

Results show how factors affect purity and yield

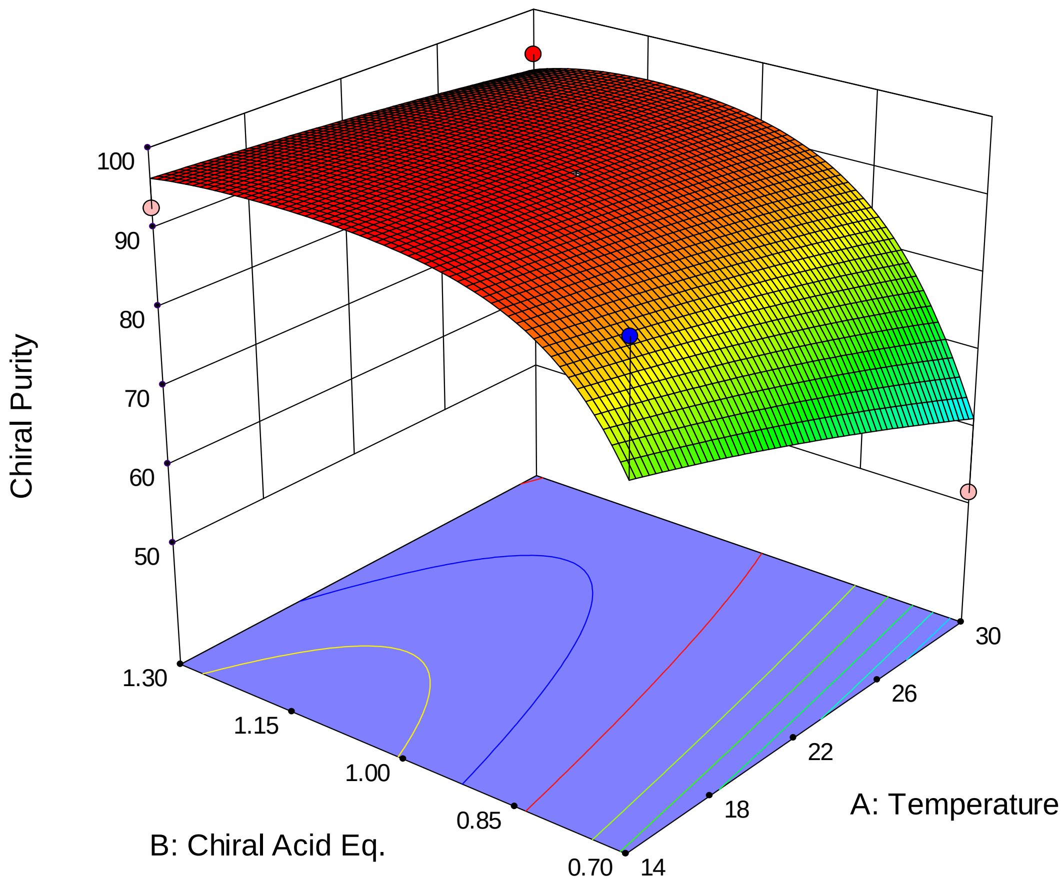

Figure 1: Effect of chiral acid equivalents and temperature on purity with 3 equivalents of water

The analysis of variance (ANOVA) for the yield response shows a predicted R-squared value of 0.888, which is excellent for modeling purposes. Figure 1 depicts the effect of water, amount of chiral acid and temperature. A redder color indicates a higher yield. "The data suggests that at high water concentrations, good yield can be obtained at a wider range of chiral acid stoichiometry and temperatures," Tinder said. "On the other hand, lower temperature and stoichiometry close to 1.1 equivalents is needed to obtain optimal yield with a low water concentration."

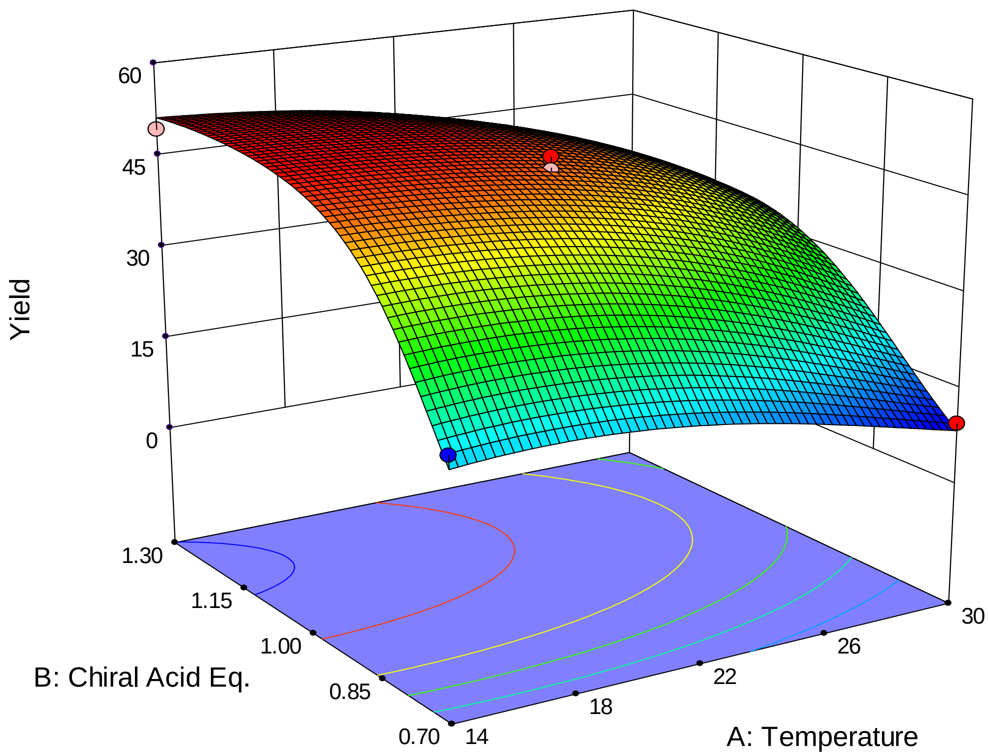

Figure 2: Effect of chiral acid equivalents and temperature on yield with 3 equivalents of water

Figure 2 shows the effect of the three factors on yield of the eutomer. Again, higher levels of red indicate higher levels of chiral purity. "The data shows again that at 3 equivalents of water there is a larger operating space to obtain larger amounts of eutomer," Tinder said. "It further suggests that the optimal operating space with respect to temperature is likely within the operating regions of the design space even at 3 equivalents of water. The data suggests that even more water may be beneficial for this reaction."

Optimizing the process

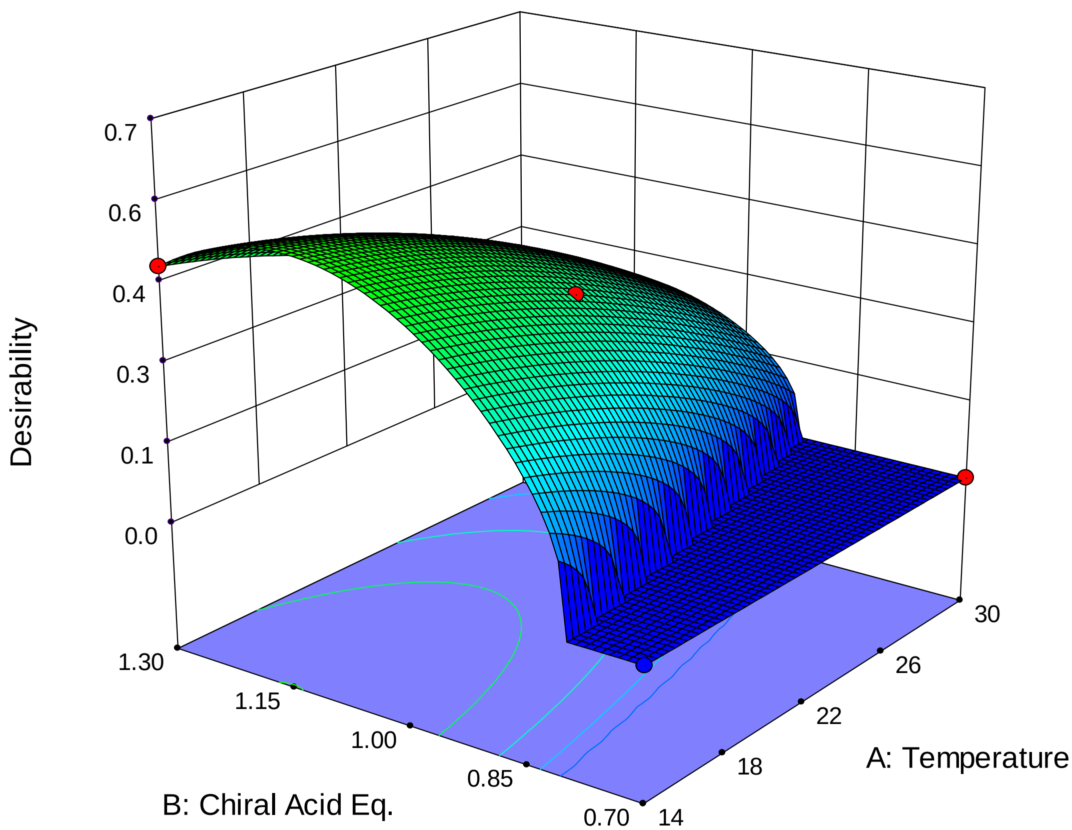

Figure 3: Optimizing the factors with water at 3 equivalents

The optimization feature in Design-Expert was used to calculate the optimal operating parameters. Tinder selected an optimization function that maximizes eutomer yield while maintaining a lower limit of 90% for chiral purity. This resulted in an optimization surface where solutions having a low chiral purity were omitted from the response surface. The graphic solution suggested that an optimum exists at 3 equivalents of water, 1.13 equivalents chiral acid and 15.8^{o}C. It predicted that these conditions will provide a yield of 46% and a chiral purity of 91.6% for a eutomer yield of 42.1%.

A one-gram scale run was performed to validate the optimal solution. One gram of racemic base and an appropriate amount of chiral acid were loaded into a reactor followed by the proper amount of solvent and water. The reaction was heated to 50^{o}C for 30 minutes, stirred and cooled to the prescribed temperature over 30 minutes. The mixture was held at the standard temperature for 48 hours before the crystalline solid was isolated by filtration, washed 2 times and dried overnight under a high vacuum to recover . This run provided a eutomer yield very close to the 42.1% predicted by the DOE.

Using response surface methods (RSM) Robert Tinder discovered new process conditions which provided an 11.6% improvement in yield and were much more robust. This application illustrates perfectly the ability of DOE to deliver substantial improvements in pharmaceutical processing throughput.

For more information, contact:

--Robert Tinder, Principal Scientist at Roche Pharmaceuticals. He was previously at Wyeth Pharmaceuticals.

--Stat-Ease, Inc.; 2021 E. Hennepin Avenue, Ste. 480, Minneapolis, MN 55413-2726. Ph: 612-378-9449, Fax: 612-746-2069, E-mail: info@statease.com, Web site: http://www.statease.com