Pharmaceutical Manufacturer Increases Yield with Response Surface Methods

Design of experiments software handles complex manufacturing variables

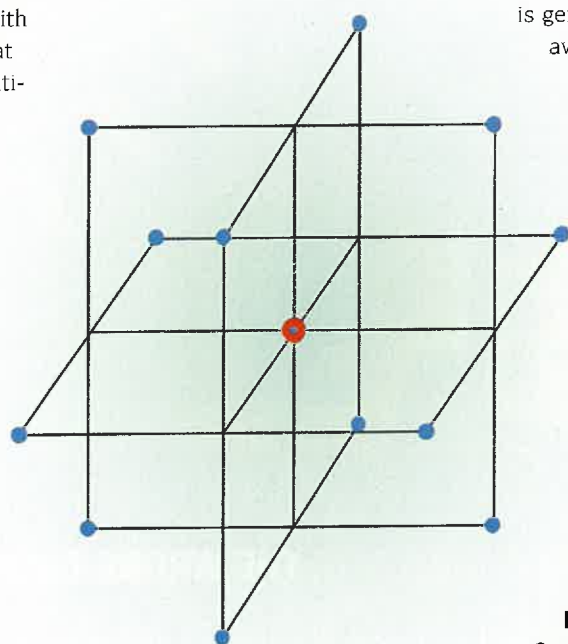
Design of Experiments (DOE) varies the values of chosen factors in parallel so it uncovers not just the main effects of each variable but also the interactions between variables.

Pharmaceutical manufacturing typically involves a series of process steps, each with many variables that can have a major impact on critical responses such as product yield. It's often difficult to gain an understanding of the impact of these variables during the critical period when production must be quickly scaled to meet the demand generated by clinical trials. MannKind Corporation overcame this challenge by using designed experiments to identify and optimize critical process variables involved in producing a small molecule substrate for use in pulmonary drug delivery. Optimizing these variables increased the overall production yield in a multistep synthetic process from 7% to 35% at the pilot scale.

MannKind Corporation is a biopharmaceutical company focused on the discovery, development and commercialization of therapeutic products for diseases such as diabetes and cancer. The company's lead investigational product candidate, the Technosphere® Insulin Inhalation System, is currently in Phase 3 clinical trials in the United States, Europe and Latin America to study its safety and efficacy in the treatment of diabetes. This therapy consists of a proprietary dry powder formulation of insulin that is inhaled into the deep lung using a proprietary device. Clinical trials to date have shown that the TIS produces a profile of insulin levels in the bloodstream that approximates the profile normally seen in healthy individuals immediately following the beginning of a meal, but which is absent in patients with diabetes.

Insulin delivered via TIS is rapidly absorbed into the bloodstream following inhalation, reaching peak levels within 12 to 14 minutes. As a result of this rapid onset of action, most of the glucose-lowering activity occurs within the first three hours of administration — which is generally when glucose becomes available from a meal — instead of the much longer duration of action observed when insulin is injected. MannKind scientists believe the relatively short duration of action of TIS reduces the need for patients to snack between meals in order to manage ongoing blood glucose excursions. Clinical trials have shown that patients have achieved significant improvements in overall glucose control without the weight gain typically associated with insulin therapy.

Figure 1: Box-Behnken design on three factors.



Pharmaceutical Manufacturing Scale-up Challenge

MannKind was recently faced with the challenge of scaling the manufacture of the small molecule raw material (a diketopiperazine or DKP) used to prepare Technosphere® particles. "We started producing the product at a laboratory scale and the volumes were so low that it wasn't necessary to fully optimize the process," said Dr. Karla Somerville of MannKind. "But as we scaled up for later stage clinical development, yield became much more important. As is common in pharmaceutical manufacturing, our process was very complex with many variables, each of which has the potential to interact with others. Trying to improve the process using traditional one-factor-at-a-time (OFAT) experiments would have been very expensive and time-consuming. We turned to design of experiments (DOE) because it is specifically intended to identify interactions between process variables that play a critical role in pharmaceutical manufacturing."

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 variables. This powerful approach makes it possible to identify ideal combinations of factors in far fewer experimental runs than the OFAT approach. The DOE protocol demands statistical validity, so costly development can be restricted to significant results with great confidence.

Designing the Experiment

There are several steps involved in MannKind's process for DKP manufacture. Reaction temperatures, reaction times, solvent ratios, catalyst quantities, reagent quantities, and wash volumes are critical parameters in the various process steps.

"The effects of some of these variables are fairly obvious," Somerville said. For example, in some steps it is necessary to wash solvent from a solid using water. Increasing the amount of water in the wash may reduce impurity levels but may also increase the amount of product that is lost to the wash water. Optimizing the washing step requires balancing purity against yield and is complicated by the fact that this step interacts with all the others in determining the yield and purity of the finished product."

A large number of runs would have been required to capture interactions between all of the factors. To reduce the time and cost of the research, Somerville decided to run a screening experiment first to determine which of these variables had a significant impact on the yield and other objectives. She used Design-Expert® software from Stat-Ease, Inc., Minneapolis, Minnesota, to configure a highly-fractionated two-level factorial screening experiment that evaluated 8 variables at a time for their main effects – called a "resolution IV" design in the jargon of DOE.

The template provided by the program is unique – a design invented by statisticians at Stat-Ease that requires the minimum runs needed for screening at this level of resolution. Depending on the number of factors, the 'min-run res IV' option provides substantial savings in experimental resources. For example, 10 factors can be screened in only 20 runs by a min-run res IV versus 32 for a standard fraction at this resolution.

"We selected Design-Expert software because it is considerably easier to use than other DOE programs," Somerville said. "I can simply enter in factors, and then select the experiment design that fits my needs. The software immediately provides feedback on the design, giving me the number of runs required and the effects resolution. This makes it easy to quickly evaluate the pros and cons of different designs." Dr. Somerville was trained in DOE in Stat-Ease's Experiment Design Made Easy workshop.

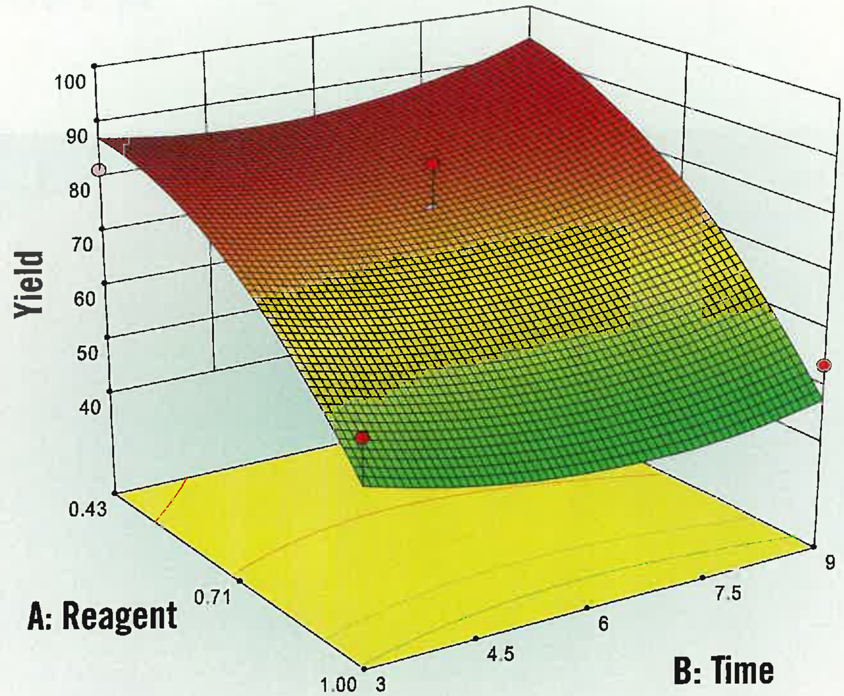


Figure 2: Response surface for the effect of Reagent A amount and reaction time on % yield. Yield decreased dramatically with increasing amounts of Reagent A.

Optimizing the Process

MannKind scientists completed the screening experiment by running two combinations of the 8 factors per day according to a 'recipe' sheet laid out in random order by the software. The scientists measured yield, purity, isomer ratio, and impurity levels for the output of each batch and entered the results of each run into Design-Expert software. The software then showed the statistical significance of each factor. The experiment identified three variables as being responsible for the vast majority of the variation in the results.

For the second experiment - to optimize the process, Somerville then used Design-Expert software's response surface methods (RSM) to configure a Box-Behnken design that models the curvature of a non-linear response. The Box-Behnken design requires only three levels of each factor and only a fraction of all combinations. It puts points at the center and midpoint of each side. Somerville selected the Box-Behnken design because it requires relatively small numbers of runs to generate optimum parameters for problems with large numbers of factors.

A total of 17 runs were needed to generate the response surface for the three critical factors. The RSM model provided optimal values for each of the variables. When Somerville used the numerical optimization tool in Design-Expert software to dial in these optimal values, the program predicted that yield would increase by 10%. One last experiment was conducted to confirm that, using the optimal values generated by the model, the yield was increased nearly exactly as predicted. The company is now running production of the scaled-up process and is achieving the same results. "Design of experiments enabled us to optimize our process in a fraction of the time that would have been required using conventional methods," Somerville concluded.

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