

Design of Experiments Demonstrates Robustness of Biopharmaceutical Process

The manufacture of chemical reagents used for clinical diagnostics is one of the most critical manufacturing challenges because in some situations the health of the patient can depend on an accurate test. DiaSorin, a leader in producing immunoreagent kits, continually evaluates the efficacy of its manufacturing processes to ensure their robustness. Recently the company has begun using design of experiments (DOE) to provide an added level of confidence in its evaluation efforts. These efforts have taken advantage of a new generation of DOE software that greatly simplifies the design and analysis of experiments. These experiments can provide high levels of statistical confidence with far fewer runs than would be required by traditional one-factor-at-time experimental methods. For example, the company recently used DOE to evaluate the robustness of its process for manufacturing an alpha-1-antitrypsin (AAT) assay.

AAT is a protein that protects the lungs. The liver makes this protein and releases it into the bloodstream. Because of a genetic disorder, some people have little or no AAT so they are at risk of developing emphysema or liver problems. With an incidence of 1 in 1600, AAT deficiency is one of the most common hereditary diseases. Three in four adults with a severe deficiency will get emphysema, some before they reach 40. Children with AAT deficiency can develop liver problems that last their whole lives. One treatment involves adding to or replacing the missing protein. A lung transplant may be an option for some seriously ill patients. Smoking cigarettes substantially increases the risk. AAT deficiency can be treated but not cured. A blood test can determine whether or not someone has the deficiency. If someone tests positive, their family members should also take the blood test.

Manufacturing AAT test kits

DiaSorin manufactures AAT reagent sets by injecting purified AAT protein into a goat, which then produces antibodies to the protein. DiaSorin takes serum from the goat and purifies the antibodies and then inserts them into an acceptable buffer system. Each batch is subjected to a series of demanding in-process tests in order to assure its compliance with key quality control criteria. These tests include measuring the pH of the batch with a target of 7.5 and a range of 6.0 to 8.0. A spectrophotometric absorbance measurement is also performed on each batch to ensure it falls between the limits of 271 nm to 442 nm. When the processing is concluded, end point tests are performed. These include measuring the background signal generated on the Roche FARA automated analyzer with the reagent but with no sample present. The reactivity of the assay to AAT is determined by measuring standards.

Scott Bergmann, quality control engineer for DiaSorin, decided to perform a study to determine whether meeting the in-process specifications ensured achieving the company's demanding quality standards for the finished product. No batch that met the company's in-process specifications had ever failed the final quality tests. However, the in-process specifications for every batch are very close to the target values so day-to-day production data provides little insight into their robustness. The easiest approach would have been to intentionally produce batches at the limits of the manufacturing

specifications and then test them against the finished product quality standards. But this approach would not have determined whether or not every possible combination of acceptable in-process parameters would have met the final requirements. Another weakness of this approach is that it would not have provided statistical measurements indicating the level of confidence which could be placed in its results.

Developing a controlled experiment

Bergmann made use of DOE tools to determine whether DiaSorin’s in-process measurement criteria were capable of controlling its manufacturing processes under any possible conditions. “While I could have designed the experiment and analyzed the results manually, I felt that the right commercial software package would save a lot of time and increase the rigor of the statistical analysis. I selected Design-Expert® software from Stat-Ease, Inc., Minneapolis, MN because it provides a very easy-to-learn-and-use interface which is perfectly suited for scientists and engineers who only use DOE occasionally. Design-Expert software also provides the power that is needed to design efficient experiments and generate powerful statistical analysis of the results.”

Bergman selected a full-factorial experiment which tests every combination of the factors under evaluation. The factors include two different reagents (A and B), three different titres of antibodies, three different pH levels (6.0, 7.5 and 8.0) and the use or non-use of water dialysis, a process designed to lower the background. The experiment incorporated 32 runs. Bergmann measured eight different responses including the spectrophotometric absorbance of several samples, the bias of measurement error of several standard samples of AAT, and the y intercept and slope of a line used to correct for the bias of the test kit.

Results demonstrate validity of in-process specifications

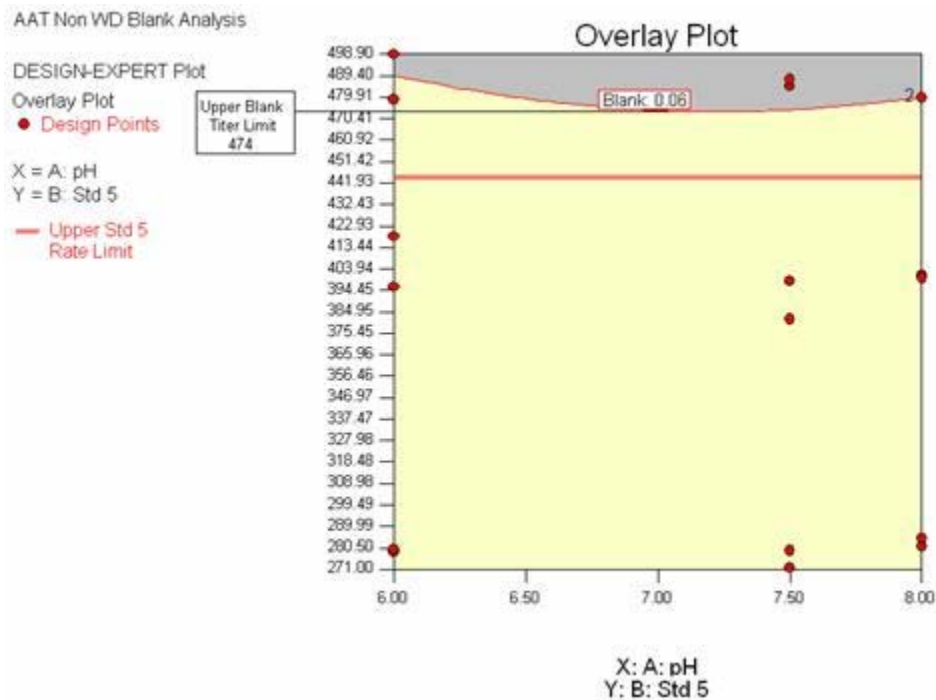


Figure 1: Blank analysis without water dialysis.

Figure 1 shows one of the major outputs provided by Design-Expert to analyze the experimental results. The x and y axes of the graph plot two key in-process measurements, pH and spectrophotometric absorbance in nm. The red dots show the runs of the designed experiment. The red horizontal line on the chart indicates the upper limit of the acceptable range for the spectrophotometric absorbance measurement. The DOE software calculated the range of conditions associated with the blank or background exceeding the final specification of 0.06 and this is indicated by the gray area on the chart. “A simple visual examination of the chart shows that the gray area where final product does not meet specifications is safely distant from the area below the red line in which the specifications are met,” Bergmann said. “This examination alone gave us a considerable degree of confidence that our existing in-process quality control criteria are sufficient and that by meeting these specifications we can be sure of meeting our finished product requirements.”

“Our in-process measurement specifications were developed by experiential and experimental methods back in the 1980s,” Bergman concluded. “While these specifications have served us well over the years, our technology is continually being improved so we can’t automatically assume that they are still valid under the current conditions. The use of a designed experiment made it possible to provide statistical evidence of the robustness of our in-process specifications. The statistical output provided by Design-Expert software showed that our criteria were valid at much better than a 95% level of confidence. This gave us assurance that our in-process measurement criteria were still valid and provided documentation of our manufacturing procedures to ensure compliance with Food and Drug Administration Good Manufacturing Practices. This application provides an excellent example of how DOE can reduce the time required to perform a latitude study while delivering statistical analysis that increases the degree of confidence in the study. It also shows how a PC-based DOE tool can greatly simplify the process of designing an experiment and analyzing the results.”

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