Optimizing assays for automated platforms

Experimental design and automation accelerate the development of drug assays.

BY PAUL B. TAYLOR

Pharmaceutical researchers today face the challenge of developing assays that are robust in a robotic screening environment (i.e., that maintain target performance over extended periods of operation). Automation and miniaturization have enabled high-throughput screening (HTS) labs to screen hundreds of thousands of compounds against a biological target in a short amount of time, and, accordingly, the focus has shifted to reducing the costs and time frames associated with assay development. Many researchers believe that the optimization of assays for HTS is one of the most significant bottlenecks in drug discovery.

At GlaxoSmithKline, significant increases in the number of targets and the throughputs required to screen them in modern HTS environments have exacerbated this bottleneck. Improvements in robustness, stability, signal separation, and assay variability are often required before assays can be transferred onto automated platforms. When reagent supplies are limited, miniaturized formats must frequently be developed.

Traditional methods of optimizing assays (varying one factor while the others remain constant) typically require significant amounts of time and produce information on main effects only. Interaction information is either difficult to interpret or not available. Supplementing classical scientific inquiry with a combination of statistical design principles and robotics is one approach to reducing the assay optimization bottleneck.

Because we already had substantial experience with these principles in a screening environment, we believed that they could be applied to the acceleration of assay development. With robotics capable of performing rapid randomized tasks, we realized that it would now be possible to design statistically complex optimization experiments that would be either difficult or impossible to carry out manually.

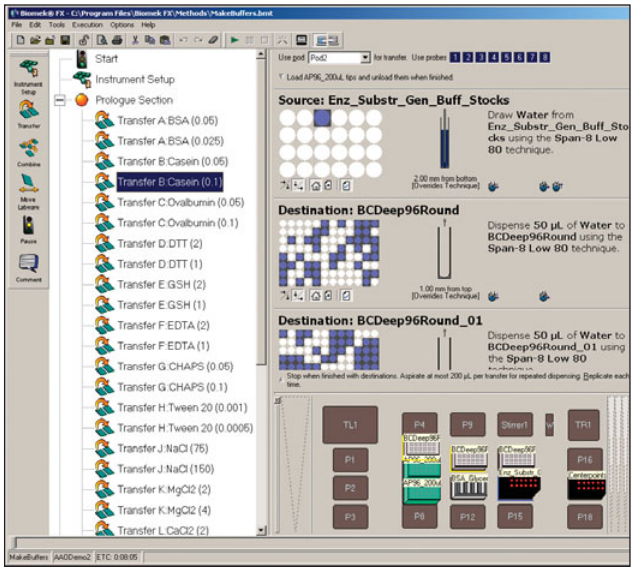

Figure 1. Example of a liquid-handling method and deck layout from AAO on a Biomek FX. Each highlighted source has its associated destination plate map displayed for verification while the liquid handler is active.

Statistical experimental design uses techniques for designing sets of experiments that are based on mathematical probabilities. These designs produce tables that contain complex combinations of assay variables such as buffers, reagents, plate types, temperatures, and assay run times. The tables are used as the common currency for generating plate maps on liquid-handling workstations.

Assays are affected by many factors, each of which can be included in the design of assay optimization experiments. The resulting complexity needs to be translated rapidly into robotic methods to be useful in the laboratory. In the initial stages of proof of concept, it commonly took 1–2 weeks to program randomized plate maps manually into a Biomek 2000 automated liquid-handling workstation (Beckman Coulter, Fullerton, CA). As we used this semiautomated process to achieve benchmark data, it became clear that an improved assay optimization process would involve software that could import tables generated by statistical design packages, create plate maps from them, and then use these as templates for methods creation in the liquid-handling workstation. We realized that a fully automated process had the potential to facilitate not only empirical discovery but also speed.

We approached Beckman Coulter about collaborating with us in the development of what is now called SAGIAN Automated Assay Optimization (AAO) software. The fully developed AAO process encompasses the ability to download information to a robot and have liquid-handling methods automatically created. The first two versions of AAO were developed for use on a Biomek 2000, whereas the latest version uses a Biomek FX. The Biomek FX has a significantly expanded deck and the ability to configure two liquid-handling mechanisms per instrument, for example, a span-8 head for randomized deliveries in combination with a 384-tip head for assay initiation.

The import capabilities of AAO have allowed researchers to experiment with a range of statistical designs, including fractional factorial, full factorial, and response-surface designs such as Box-Behnken, D-optimal, and central composite. Designs use recommendations from earlier experiments and are applied iteratively (usually 2–3 cycles) until optimizations are achieved. In the final stages of development, multilevel designs provide the opportunity to identify optimum factor levels with high resolution.

The design development process requires expert scientific knowledge with respect to the choice of factors and their corresponding levels. Some conditions, for example, may be known to be unsuitable in a biochemical context and therefore detrimental to the assay. It is important to incorporate this knowledge into the design phase if benefits are to be gained.

AAO: A five-step process

Step 1: Design the experiment. In this stage, the user selects factors of importance and applies the most appropriate statistical design according to available reagents and the size of the desired sample set. In the AAO FX version, import and export capabilities have been significantly improved so that with minimal intervention, the user can choose any statistical software package for design and analysis.

Step 2: Generate plate maps for the experiment set. After the design phase, the software takes the imported statistical table and automatically generates randomized plate maps. This mapping also takes into account plate section restrictions and well-level protocols (totals, backgrounds, and standards). The SAGIAN AAO package supports 96- and 384-well plates. Should an experiment require a 1536-plate environment, any liquid handling completed in a 384-well plate can be reformatted, the assay initiated by an appropriate liquid handler, and the resulting data then decoupled for analysis by the AAO software. Graphic displays list the contents of each well in the experiment, the labware assigned to every source, and the deck layouts for each liquid-handling method.

Step 3: Create liquid-handling methods. After plate maps have been generated, the AAO software guides the user in the creation of liquid-handling methods. During this stage, the user has control over the order of pipetting and associated parameters such as volumes, aspiration and dispensing speeds, heights, mixing, and tip touches. Pipetting settings may be saved as liquid classes with user-assigned names for use in future experiments, producing significant time savings. Components that are not factors in the experimental design, such as reagents or quality controls (e.g., the original buffer), can be added and configured independently from the experimental design. Per-well scheduling (an individual time-scheduling protocol for each well) has been added as a feature in the latest version of software for situations in which time is to be included as a factor. Figure 1 shows the resulting liquid-handling methods and deck layouts.

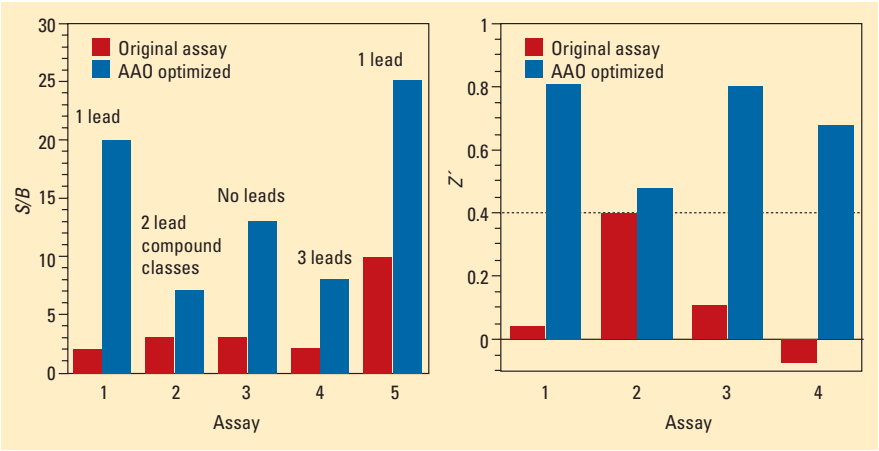

Figure 2. Improved performance demonstrates an increased probability of identifying viable leads in optimized assays. Improved assays show increases in the signal-to-background ratio (*S*/*B*) and *Z*′, where for the latter, a value of 0.5–1 indicates that the variability in the signals is sufficiently small compared with the range of the data.

Step 4: Perform the assay. Once the liquid-handling methods are prepared and validated, the user runs the assay. During this stage, the user is responsible for manipulating the labware, including loading the plates onto the liquid-handling workstation and other devices such as shakers, incubators, or readers. Once plates are on the deck (as graphically guided by the software), the user selects the appropriate methods to run.

Step 5: Collect the data and analyze results. After data has been pasted into an AAO-generated MS Excel spreadsheet template, the results are deconvoluted and parsed for easy inspection across replicates and exported to a statistical package for further analysis. Graphical plots and other visual data presentation tools assist in identifying significant effects for inclusion in a statistical model.

Benefits of assay optimization

The combination of experimental design techniques and AAO software vastly increases the speed at which assays can be optimized, reducing costs in several areas. The most obvious is the reduction in the amount of time an employee must spend on each target optimization. Costs are brought down further by minimizing reagent usage, because statistical design reduces the number of experiments that are needed. Improving the robustness of assay performance has allowed us to miniaturize assays, saving tens of thousands of dollars. In some cases, the inability to miniaturize the assay would have precluded a full screen from being run.

Improved assay performance also facilitates miniaturization. It is difficult to miniaturize a marginally performing assay. Frequently, as miniaturization takes place, losses in performance manifest as lower signal separation, increased variability, and loss of robustness. Reasons for this can include increased exposure to oxygen, higher surface-to-volume interactions, and the increased error associated with miniaturized liquid handling and detection. As we improved assay performances via wider dynamic ranges with lower variabilities and increased robustness, miniaturization was facilitated and resulted in significant cost savings.

Optimization also simplifies the assay process. Typically, by the second or third iteration of AAO, those factors having either negligible or detrimental effects have been removed. Attention can then be focused on identifying optimum level ranges of the remaining factors, which typically are few and known to be beneficial. This frequently allows simplification of the format, thereby reducing error and preparation times.

The ultimate measure of success for any screening group is the quality of sustainable leads delivered to drug development areas. To demonstrate the effectiveness of AAO, we ran full HTS on a sampling of assays that had been optimized with and without AAO. We found that the optimized assays were generally more sensitive to hit detection, enabling us to identify leads that were missed in the original screens. Figure 2 shows the results of five antagonist–inhibitor assays optimized for screening with and without AAO. Seven lead compounds were delivered subsequent to assay optimization with AAO.

The ultimate measure of success is the quality of leads delivered.

Unstable assays need to be watched at all times to ensure that performance does not require intervention. Statistically, the ability to identify true hits becomes more difficult as performance is lost and usually results in inflated false positive rates. In contrast, robust assays can be automated to run unattended. As we have improved the stability of our assays, we have been better equipped to run them overnight, increasing throughput and allocating resources more efficiently.

A bottleneck removed

With this new process, we have reduced assay development cycle time significantly. Optimization using traditional methods usually takes 4–12 months. By using statistical design with the SAGIAN AAO software, we have been able to optimize performance in 4–6 weeks. This has produced workforce and reagent cost savings of 10–40 cents per well. By combining the power of statistical design and analysis with the speed of automated pipetting for running experiments, AAO has provided a framework for increased pharmaceutical productivity at the assay development stage.

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