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Research paper

Use of response surface methods and path of steepest ascent to optimize ligand-binding assay sensitivity

Alison P. Joyce *, Sheldon S. Leung

Pfizer, Inc. 1 Burtt Road, Andover, MA 01810, United States

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ABSTRACT

Response surface methods (RSM) combined with a steepest ascent approach is a powerful technique to optimize assay performance. In this case, a ligand-binding assay (LBA) to quantitate a peptide biotherapeutic was optimized for improved sensitivity using this technique. Conditions were elucidated to enable pg/mL quantitation of the peptide in human plasma using steepest ascent to efficiently optimize assay factors. Instead of relying solely on assay development experience and intuition to improve assay sensitivity, this systematic approach takes advantage of a predictive mathematical model generated through response surface methods that defines a specific path towards greater predicted assay sensitivity. The actual response observed along the steepest ascent path was in good agreement with the model for several steps, until reagent concentrations moved beyond the physical limits of the system, and model breakdown occurred. RSM combined with steepest ascent method proved a useful tool for sensitivity optimization in three ways: (1) The required LBA sensitivity performance (approximately 200 pg/mL), measured as a signal-to-noise ratio (SNR) at the targeted lower limit of quantitation (LLOQ), was efficiently achieved in only two optimization experiments; (2) Steepest ascent confirmed that the desired sensitivity was found within the initial RSM design space, and no further gain in sensitivity was found venturing beyond this design space along the steepest ascent path; (3) The desired assay sensitivity was maintained over a reasonable range of reagent concentrations along the steepest ascent path, indicating assay robustness for this parameter.

1. Introduction

Assay performance parameters such as sensitivity and specificity may be difficult to obtain in complex matrices such as serum or plasma (Selby, 1999; DeForge et al., 2007; Doucet et al., 2009; Johan Schiettecatte et al., 2012). Assays may be multi-step processes, and contain many factors that can affect assay sensitivity. These factors may interact with each other to affect desired responses. For ligand-binding assays, these responses are often assay performance parameters, such as sensitivity, dynamic range, precision and accuracy. Instead of using one-factor at a time method development to optimize assay factors separately, or using inefficient large "checkerboard" experiments, a multi-factorial Design of Experiments (DOE) approach may be used.

Applying DOE in assay development can involve (1) running 2-level factorial screening experiments to identify key factors and eliminate unimportant ones; (2) optimizing the method using response surface methods (RSM) and steepest ascent or descent, which provides a more detailed look at how responses behave as relevant factors at multiple levels (greater than two) are simultaneously varied; and (3) testing robustness to see if perturbations in the optimized settings affect the system (Fig. 1). This paper will focus on the RSM optimization phase of this DOE process.

RSM experiments result in a mathematical model or equation that describes a response as a function of the varied factors and levels. This mathematical equation contains several key pieces of information about the system being studied; for example, identified significant factors, factor interactions, and

* Corresponding author at: 1 Burtt Road, Andover, MA 01810, United States. Tel.: +1 978 247 1331; fax: +1 978 247 2842.
E-mail address: alison.joyce@pfizer.com (A.P. Joyce).

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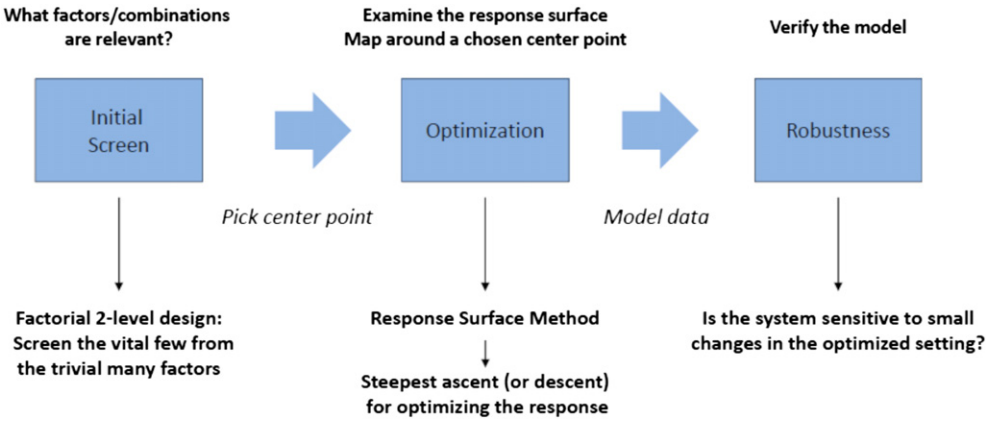

Fig. 1. DOE in assay development: 3 main objectives (initial screen, optimization and robustness testing). RSM and steepest ascent are utilized in the optimization phase.

curvature, which identifies if the response is linear in nature. Another vital clue gained from the RSM driven mathematical model is how the direction and magnitude of the factor levels may be changed to explore a new area of improved assay performance. This process improvement using the model can be carried out using path of steepest ascent. Steepest ascent provides experimental moves in a specific direction to explore a new area of potentially improved method performance. It behooves the experimenter to take advantage of all the information captured within the mathematical model that describes a particular response, instead of relying on intuition or guessing what experimental conditions to perform next.

During the optimization phase of assay development, the initial starting conditions may be far away from a more appealing region exhibiting improved assay performance. A basic RSM design, essentially a two-level factorial experiment with repeated center points, may be applied to fit a first order model, which is linear in nature. If there are only two significant factors impacting the desired response, then a simple 5 point design is applied (two factor, two level factorial with center points) as shown on Fig. 2. If the fitted model for the exploratory RSM experiment is linear, as it tends to be if the area of improved response is far from the initial starting conditions, then the magnitude and sign of the linear terms in the following equation may be used to determine the steepest ascent up the directional path toward an area of improved response Y (Eq. (1)):

$$\text{Response}\,\hat{Y} = b_0 + b_1 \times_1 + b_2 \times_2 + \ldots + b_k x_k \qquad (1)$$

where x_1 and x_2 are significant factor effects in coded form and b values represent parameter estimates independent of the scaling convention for the factors, characterizing the magnitude and direction of the effects (Myers et al., 2009; Natrella, 2010). Alternatively, if the desired response requires minimizing instead of maximizing, traveling down the path via steepest descent would be required. Using coded variables removes units of measure and facilitates model interpretation. To code the variables, low and high levels of each factor are set as -1 and $+1$, respectively, and midpoint level coded as 0 (Anderson and Whitcomb, 2005).

The goal of this work was to initially use RSM in an exploration experiment to obtain a mathematical equation describing the sensitivity response as a function of the assay factors tested, which were coating and detector antibody concentrations. The method of steepest ascent was then utilized to quickly and efficiently follow the path described by the mathematical equation to optimize assay sensitivity. RSM and steepest ascent have been utilized to improve other biological methods, such as medium composition for optimum production of elastase by bacteria (Chen et al., 2002), improve growth conditions for CHO cells (Liu et al., 2001), optimization for antifungal active substance production from bacteria (Wang and Liu, 2008), and reagent modification conditions for a dye labeled protein (Wang et al., 2007). However, to our knowledge, there have been no reports of utilizing steepest ascent approach to improve ligand-binding assay performance parameters, such as sensitivity. In this paper, we demonstrate how a simple RSM experiment was used to predict a linear model describing the ELISA sensitivity response as a function of factor concentrations. Using steepest ascent, a series of experimental runs were conducted where the reagent concentrations were incrementally increased or

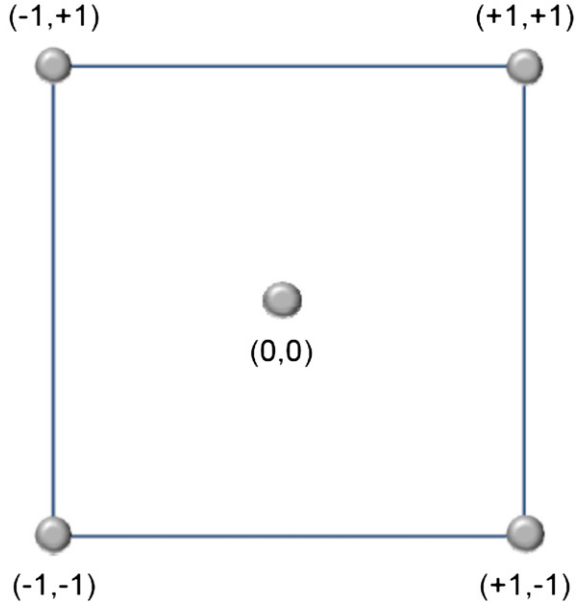

Fig. 2. Simple RSM design using replicated center and corner points. $+1$, -1 and 0 represent high, low and mid levels of the two factors, which were coating and detector antibody concentrations, respectively.