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QUALITY BY DESIGN APPLICATIONS IN BIOSIMILAR PHARMACEUTICAL PRODUCTS

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Abstract: A process is well understood when all critical sources of variability are identified and explained, variability is managed by the process design and monitoring, and product quality attributes is accurately and reliably predicted over the design space. Quality by Design (QbD) is a systematic approach to product development can process control that begins with predefined objectives, emphasizes product and process understanding and sets up process control based on sound science and quality risk management. The Food and Drug Administration (FDA) and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) have recently started promoting QbD in an attempt to curb rising development costs and regulatory barriers to innovation and creativity. QbD is partially based on the application of multivariate statistical methods and a statistical Design of Experiments strategy to the development of both analytical methods and pharmaceutical formulations. In this paper, we review the basics of QbD and their impact on the innovative, generic and biosimilar pharmaceutical industry. In particular, we consider the challenge of mapping the control space in biotechnological processes and how advances in statistical methods can contribute to QbD.

Keywords: Quality by Design, Design of Experiments, Simulation Experiments, Multivariate Methods, Analytical Methods, Specification Limits, Biosimilars.

1. Introduction

A process is well understood when all critical sources of variability are identified and explained, variability is proactively managed by the process, and product quality attributes can be accurately and reliably predicted over the design space. Processes must meet current good manufacturing practices to ensure that drug products meet safety and efficacy requirements. Traditionally, this requirement has been met in the pharmaceutical industry by performing process validation studies on three batches. It has been recognized that this approach is unlikely to fully represent routine manufacturing and therefore unlikely to cover all potential sources of variability (e.g., raw materials, operators, shifts, reactor vessels). The Office of New Drug Quality Assessment at the FDA, has identified this issue as a challenge to the regulatory process and stated that there is currently a "focus on process validation and not process understanding" [15]. Quality by Design is about changing this approach.

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Quality by Design (QbD) is a systematic approach to development that begins with predefined objectives, emphasizes product and process understanding and sets up process control based on sound science and quality risk management. In the traditional approach, product quality and performance are achieved predominantly by restricting flexibility in the manufacturing process and by end product testing. Under the QbD paradigm, pharmaceutical quality is assured by understanding and controlling manufacturing and formulation variables. End product testing is used to confirm the quality of the product and is not part of the on going consistency assurance and/or process control ([24]). The Food and Drug Administration (FDA) and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) have recently started promoting QbD in an attempt to curb rising development costs and regulatory barriers to innovation and creativity [4, 8, 10, 16, 23, and 24]. Historically, the QbD paradigm was first integrated in the pharmaceutical industry, before being considered by the biopharmaceutical industry. Although ICH guidelines that define QbD (e.g. [9]) apply to both sectors, the biopharmaceutical industry is striding to catch up.

An operational definition of QbD in analytical methods has been proposed by Borman et al from GlaxoSmithKline (see [2]). We build on their approach, emphasizing the specific needs of biosimilar products. QbD can be described as a four stage process addressing both design and control. The stages are:

I. Design Intent: The Active Pharmaceutical Ingredient (API) chemical and physical characteristics and Drug Product (DP) performance targets are identified for the commercial product.

II. Design Selection: The API manufacturing process and the DP formulation and manufacturing process are selected to achieve the Design Intent for the commercial product.

III. Control Definition: The largest contributors to Critical Quality Attributes (CQA) variability are established and controls defined to ensure process performance expectations are met.

IV. Control Verification: The performance of the API and DP processes in manufacturing are measured to verify that the controls are effective and the product performance acceptable.

QbD is partially based on the application of multivariate statistical methods [5, 6, 7, 11, 25] and a Statistical Design of Experiments strategy [10, 11, 12, 25] to the development of both analytical methods and pharmaceutical formulations. In defining the controls (Stage 3), a QbD process is applied again to the design of the analytical methods in order to provide the necessary controls. The next section is a general review of the application of QbD to the development of analytical methods in the chemical and biotechnological pharmaceutical industry.

2. Quality by Design Challenges in Biopharmaceutical Products

The biopharmaceutical industry has emerged from the development of recombinant DNA techniques in the 1980s. Today more than 120 therapeutic proteins are widely used for treating cancer, anemia, rheumatoid arthritis etc and are saving millions of lives. The most common cellular expression systems used to manufacture therapeutic proteins include bacteria, yeast, mammalian cells and insect cells. The choice of expression system depends on factors such as type of target protein, posttranslational modifications, expression level, intellectual property rights and economy of manufacture. Bacterial systems offer rapid and cheap expression, but cannot express complex proteins which are glycosylated and require an in vitro folding and tag removal step during downstream processing. Yeast generally expresses the target protein in its native form to the medium but expression levels are very low. Insect cells provide many advantages of the mammalian cell characteristics but currently there are no such products approved. A large stake of the 40 billion dollar biopharmaceutical market is composed of recombinant proteins produced in large bioreactors of 10-25,000 liters with engineered mammalian cells. The production cost of such processes is very high and can reach 1 million dollars per batch.

In recent years, some of the early patents set by the biotechnology innovator companies are beginning to expire opening the grounds for generic versions of biopharmaceuticals called biosimilar or follow-on biologics. Although no regulatory infrastructure presently exists in the U.S. for biosimilars, the regulatory systems are fast responding to this need, in response to intense political pressure to provide cheaper life saving drugs. Fore more background see [17]. In fact, by definition, the development and the production of biosimilar products fits very well the QbD paradigm as the target quality attributes sought of a biosimilar product are those of the innovator product on the market. Therefore from early development stages, comparability assessments need to be performed with innovator product by an array of analytical methods which probe the structure, the modifications, the activity, the specificity, the purity, the size and the stability of the product. As stated by health authorities (e.g. EMEA, the European Medicines Agency, http://www.emea.europa.eu), the success of the biosimilar development approach depends on the ability to characterize the product and therefore to demonstrate the similar nature of the concerned products. Biological medicinal products are usually more difficult to characterize than chemically derived medicinal products. In addition, there is a spectrum of molecular complexity among the various products (recombinant DNA, blood or plasma-derived, immunological, gene and cell-therapy, etc.). Moreover, parameters such as the three-dimensional structure, the amount of acido-basic variants or post-translational modifications such as the glycosylation profile can be significantly altered by changes, which may initially be considered to be ‘minor’ in the manufacturing process. Thus, the safety/efficacy profile of these products is highly dependent on the robustness and the monitoring of quality aspects.

As a consequence of these conditions, the standard generic approach (demonstration of bioequivalence with a reference medicinal product by appropriate bioavailability studies) is not appropriate, while the approach based on a comparability exercise needs to be followed. Innovators of well characterized biotech products also perform comparability exercises after manufacturing changes. The challenges and approaches used to demonstrate comparability of a well characterized biopharmaceutical can be as varied and complex as the products themselves. The quality attributes of a biotech product are traditionally associated with specifications. The International Conference on Harmonization (ICH) defines specifications as "a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described." Ideally, the acceptance criteria should be set to meet well-established requirements such as that representing product performance. However, this information is often missing and specifications are set by establishing the likely range of acceptable values to be seen in production data. When reviewing acceptance criteria set using preproduction data, regulators tend to favor the 3-sigma limits that are used for control charts. In practice, limits set using data from a small number of batches are almost always too tight [18] with the risk of rejection of "good batches". Some outstanding issues relate to comparability (e.g. process scale up and scale down versus current process scale of biosimilar versus innovator product) and to the setting of product specifications, when only a limited number of batches are available. This situation is particularly relevant for products produced in large bioreactors. Under the traditional Quality by Testing approach (QbT) ,a product specification is often set by observing data from a small number of batches believed to be acceptable and then setting upon them acceptance criteria for future batches. Under QbD consistency comes from the design and control of the manufacturing process. Moreover specification of the drug product should be clinically relevant and generally determined by product performance. Although specifications are set for a drug product under both the QbT and QbD paradigms, the roles that the specifications play are completely different. Under QbT, each batch has to be tested against the specification to ensure its quality manufacturing consistency while under QbD, the specification is solely for the confirmation of product quality and not for manufacturing consistency or process control. ([24]).

In sum, pharmaceutical Drug Products (DP) are typically small molecules, often very stable, mostly without a drug delivery device and produced by chemical synthesis. In contrast, biopharmaceutical Drug Products are large, complex molecules where stability requires special handling and the delivery device is often a key differentiator. Biopharmaceutical DP are produced in living organisms at comparatively high costs and are highly sensitive to manufacturing changes. In the next section we focus on the QbD development of analytical methods emphasizing statistical methods that help address these challenges.

3. Development of Analytical Methods

The application of QbD to the development of analytical methods applies the same four steps outlined above to stage III of *Control Definition*. Specifically, we have the following steps first presented in [2]:

i. Method Design Intent: The analytical method performance criteria are identified for the methods intended to be used for commercial product release.

ii. Method Design Selection: The method conditions are selected to achieve the Design Intent for the commercial release methods.

iii. Method Control Definition: The largest contributors to variability in the method performance characteristics are established and controls defined to ensure method performance expectations are met.

iv. Method Control Validation: The performance of the analytical method in use in a manufacturing environment is measured to verify that the method controls are effective and the method performance acceptable.

Analytical methods performance criteria are driven by an understanding of the process monitoring and control requirements, including the process critical quality attributes (CQAs) and specification limits. CQAs are identified by uncovering the characteristics of a drug substance or a drug product that needs to be controlled, to ensure the safety or efficacy of a product. The criteria for evaluating methods measuring these CQAs include:

Precision: the requirement for the method variability to be a small proportion of the specification.

Selectivity: the determination of which impurities actually need to be monitored at each step and ensuring adequate discrimination between them

Sensitivity: ensuring the method is sufficiently sensitive relative to the specification limit in order to achieve effective process control.

These criteria address the aspects of the method that are required to facilitate ease of use in routine operation (e.g., analysis time, acceptable solvents, and available equipment). Opportunities for the implementation of improved or new technology also need to be identified. These criteria can be generated by performing an analysis of the voice of the customer (VoC) (i.e., the aspects of a method that are considered important for the quality control laboratories within manufacturing, where the commercial methods will be operated). Fundamental to design selection, is the method-development phase. To develop a QbD method, the method performance criteria must be understood as well as the desired operational intent that the eventual end user would wish to see in the method. To deliver the latter, methods must take into account the VoC. Traditional approaches to analytical method validation and analytical method transfer, rely on a one time evaluation of the method and do not provide a high level of assurance of method reliability. The limited understanding of a method obtained through these traditional approaches has often led to poor technology transfer exercise from development into use in commercial manufacturing facilities. The fact that significant analytical method variables are not fully explored, leads to post transfer method failures. When transfers fail, significant

resources are often required to attempt to remedy the causes of the transfer failure, usually at a time when there is considerable pressure to support the introduction and launch of a new product.

The desired state is to be able to prove that the method will be reliable (robust and rugged) throughout the life cycle of its use. This can be achieved by a risk assessment for identifying potential variables and determine which robustness and ruggedness experiments are to be performed. The latter can be tested as part of a measurement systems analysis study in which the most likely sources of variability are identified and studied. All the knowledge (not just the technology) should then be transferred; any future changes to the method or the environment in which it is operated should be risk assessed; and, if appropriate, equivalency should be demonstrated as part of an overriding change-control procedure.

Analytical method robustness testing typically involves evaluating the influence of small changes in the operating conditions ([20]). Ruggedness testing identifies the degree of reproducibility of test results obtained by the analysis of the same sample under various normal test conditions such as different laboratories, analysts, and instruments. The term "robustness" has been used differently when describing chemical processes where processes are defined as robust if they have the ability to tolerate the expected variability of raw materials, operating conditions, process equipment, environmental conditions, and human factors. In the analytical world, it includes factors affecting robustness and ruggedness.

4. Statistical Methods in the Application of QbD

The application of QbD to the development of analytical methods is supported by an extensive statistical methodology. In particular we briefly review some of the following topics:

- Statistical Design of Experiments
- Simulation experiments
- Stochastic emulators
- Variable fidelity experimentation
- Combining expert opinions with physical end simulation experiments
- Response surface methodologies
- Multi-objective optimization
- Multivariate methods

4.1. *Statistical design of experiments*

Statistically designed experiments are now recognized as essential for rapid learning and thus for reducing time-to-market while preserving high quality and peak performance (see [11, 25]). The application of statistically designed experiments, in the development of analytical methods, is reported in [2, 16, 23]. One such example is the design of an HPLC method used to determine eight

biogenetic amines as dabsyl derivatives [20]. The system consists of an Agilent 1050 High Performance Liquid Chromatography, with a variable-wavelength UV-vis detector and a model 3396-A integrator. The list of factors and their levels, used in the statistically designed experiment is presented in Table 1.

Table 1. Factors and levels in HPLC experiment described in [20].

Factor	Nominal Value	Lower level (-1)	Upper level (1)
Gradient Profile	1	0	2
Column Temp (^{o}C)	40	38	42
Buffer Conc. (mM)	40	36	44
Mobile-phase Buffer pH	5	4.8	5.2
Detection Wavelength (nm)	446	441	451
Triethylamine (%)	0.23	0.21	0.25
Dimethylformamide (%)	10	9.5	10.5

The specific experimental array used in the experiment is a 2^{7-4} Fractional Factorial experiment with 3 center points, is described in Table 2. The level "-1" and "1" correspond to the lower and upper level listed in Table 1, "0" corresponds to the nominal level.

Table 2. Experimental array of experiment described in [20].

Gradient	Col Temp	Buf Conc	Buf pH	Det Wave	Trie perc	Dim Perc
1	1	1	1	1	1	1
1	1	-1	-1	1	-1	-1
1	-1	1	1	-1	-1	-1
1	-1	-1	-1	-1	1	1
-1	1	1	-1	-1	1	-1
-1	1	-1	1	-1	-1	1
-1	-1	1	-1	1	-1	1
-1	-1	-1	1	1	1	-1
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

The statistically designed experimental array consists of 11 experimental runs that involve combining the design factors levels in a balanced set of combinations. The statistically designed experiment approach is maximizing the learned information for a given experimental budget. From the experimental plan in Table 2, one can deduce the main effects of the 7 factors on various responses such the method resolution (see Figure 1).

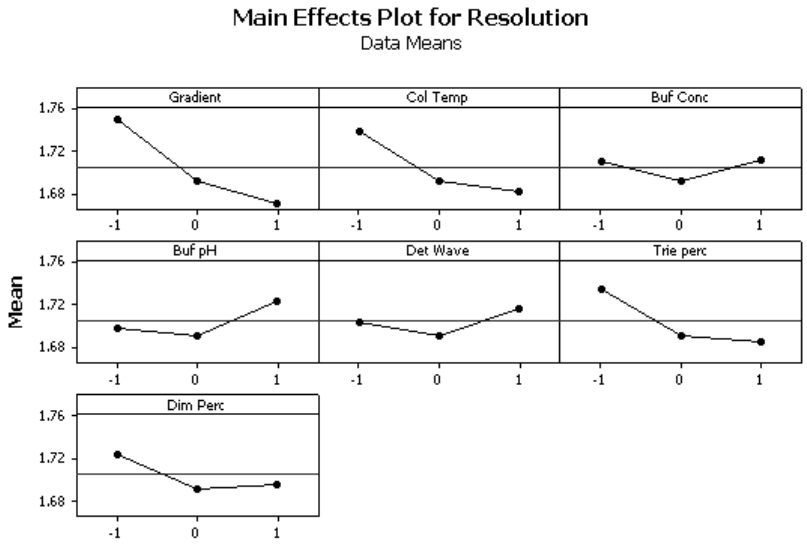

Figure 1. Main effects of factors on method resolution from data in [20].

From this analysis, one can determine that Gradient Profile and Column Temperature are active factors affecting resolution. A contour plot display of the method's design space, accounting for non linear effects of Gradient Profile and Column Temperature is presented in Figure 2.

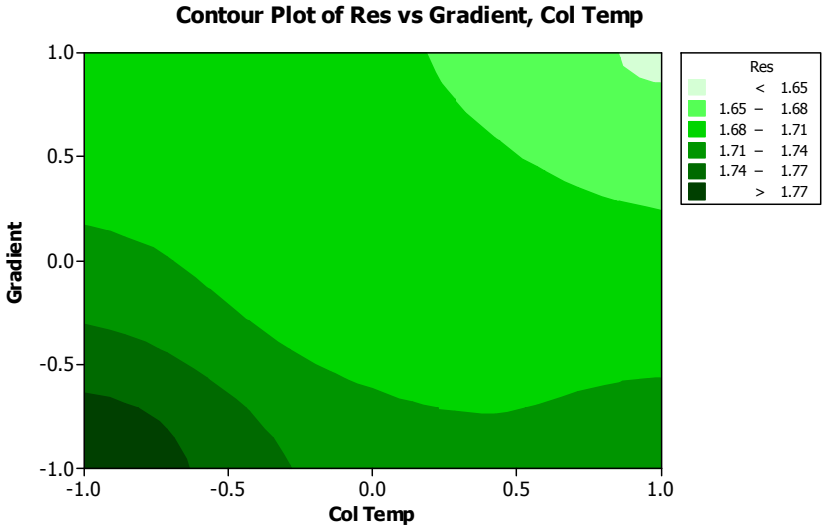

Figure 2. Contour plot of effect of Gradient Profile and Column temperature on method resolution from data in [20].

The Fractional Factorial design used in the development of the HPLC method provided a full exploration of the design space in terms of main effects and robustness properties. These experiments are physical experiments and require optimization in experimental set up complexities and budget limitations.

In contrast, simulation experiments provide new opportunities for quick and extensive learning (see [12]). Such experiments are described next.

4.2. *Simulation experiments and stochastic emulators*

Modern simulation platforms provide efficient and effective opportunities for conducing experiments and learn about the impact of design factors on CQAs. For example *VisiMix* enables mathematical modeling of mixing phenomena. It conducts calculation of average and local characteristics of mixing flow and distribution of concentration, including simulations of "non perfect" mixing. Another software product focused on the development of analytical methods from the Molnar-Institute is *DryLab*. This simulator simplifies and speeds the process of developing good chromatographic separations or methods by allowing to model changes in separation conditions using a personal computer. One more example is *DynoChem* which is used for fitting chemical reaction models, prediction of scale-up conditions, optimization of laboratory and production results, equipment characterization and shows the effect of scale dependent physical phenomena (mixing, heat transfer, mass transfer). *Dynochem* can be used for simulation of reactions performed in homogenous environment. When mixing is not ideal and the solution is not homogenous, *VisiMix* can be used for finding the required mixing conditions. In recent years, a comprehensive statistical methodology has been developed to exploit such simulation platforms, see [1, 12, 21, 22, 25].

Simulation experiments also provide an effective approach to address issues of robustness. Products are robust when they have low variation. So it might appear that simulator experiments, in which there is no outcome variation, might not be useful at all here. In fact, they can be effectively used by exploiting one of the central ideas in Taguchi's strategy for robust design experimentation, the use of both design factors and noise factors in the experiment (see for example chapter 13 in [11]). Any factor that can be set to a nominal value as part of the engineering specification of the product is a design factor. Factors that are subject to random variation in the production or usage environment are noise factors. This paradigm can also be applied to many computer simulations by specifically including noise factors as inputs to the simulator and studying how variation in these inputs affects product performance. These noise factors might describe variations of components or raw materials about their specified nominal values or uncontrollable variation in the usage environment, such as the loads to which a plane frame will be subjected. The goal of these robust design experiments will typically be to optimize one or more features of the output distribution, for example to achieve low variation about a target outcome.

The new experimental framework of computer simulators has stimulated the development of new types of experimental designs and methods of analysis that are tailored to these studies. The guiding idea in experimental design has been to achieve nearly uniform coverage of the experimental region. The most commonly used design has been the so-called Latin hypercube. In Latin

hypercube designs, each factor is given a large number of levels, an option that is virtually impossible in the lab but very easy when experimenting on a simulator. Schemes to mate the levels from the different factors, forming design points, include random mating and methods for limiting correlation or achieving first-order orthogonally. Some experiments are designed using deterministic "space filling" sequences, such as those offered by so-called "uniform designs" and special point sets for numerical integration in high dimensions (for more on these and related topics see [25]).

In using computer experiments for robust design problems, outcome variation is induced via uncertainty in the inputs. The most direct way to assess such variation is to generate simulator output for a moderate to large sample of input settings. However, if the simulator is slow and/or expensive, such a scheme may not be practical. The stochastic emulator paradigm ([1]) provides a simple solution, by replacing the simulator with an emulator for the bulk of the computations. The key steps of the stochastic emulator approach are as follows:

1. Begin with a Latin hypercube (or other space-filling) design of moderate size.
2. Use the simulator to generate data at points in the design.
3. Model the simulator data to create an emulator, called the *stochastic emulator*.
4. Use cross-validation to verify that the emulator accurately represents the simulator.
5. Generate a new space-filling design. Each configuration in this design is a potential nominal setting at which we will assess properties of the output distribution.
6. At each configuration in the new design, sample a large number of points from the noise factors and compute output data from the stochastic emulator.
7. Construct statistical models that relate features of the output distribution to the design factor settings. These models might themselves be emulators.

This approach can dramatically reduce the overall computational burden by using the stochastic emulator, rather than the simulator, to compute the results in step 6. Stochastic emulators are a primary Quality by Design tools in organizations that have successfully incorporated simulation experiments in the design of drug products, analytical methods and scale up processes.

4.3. *Bayesian models*

In [19], information from expert opinion, computer experiments and physical experiments are combined in a simple regression model of the form:

$$\mathbf{Y} = f(\mathbf{X}, \boldsymbol{\beta}) + \boldsymbol{\varepsilon} \tag{1}$$

In this model $\mathbf{X}$ represents the design space corresponding, for example, to the values of the factors in Table 1. In this example $\mathbf{X}$ is an 8xk matrix, k representing the number of observations in the experiments, the first column

consisting of the value '1" used to accommodate the average response. The vector $\boldsymbol{\beta}$ represents the values of the model coefficients, in this case of dimension 7, and $\mathbf{Y}$ represents the k observations, for example of method resolution. This is achieved by modeling physical experimental data as:

$$\mathbf{Y}_p \sim N(\mathbf{X}_p\boldsymbol{\beta}, \sigma^2\mathbf{I}) \tag{2}$$

where σ^2 is the experimental variance representing the uncertainty of responses due to experimental conditions and measurement system.

Instead of relying solely on the physical experiments to establish the distribution of the response in the design space, we start by first eliciting estimates from expert opinion and, later, add results from computer experiments. Results from physical experiments are then superimposed on these two sources of information. Suppose there are *e* expert opinions. Expert opinions on the values of $\boldsymbol{\beta}$ can be described as quantiles of:

$$\mathbf{Y}_o \sim N(\mathbf{X}_o\boldsymbol{\beta} + \boldsymbol{\delta}_o, \sigma^2\boldsymbol{\Sigma}_o) \tag{3}$$

where $\boldsymbol{\delta}_o$ is the expert specific location bias.

Assuming the following prior distributions for the unknown parameters $\boldsymbol{\beta}$ and σ^2:

$$\boldsymbol{\beta} \mid \sigma^2 \sim N(\boldsymbol{\mu}_o, \sigma^2\mathbf{C}_o) \tag{4}$$

$$\sigma^2 \sim IG(\alpha_o, \gamma_o) \tag{5}$$

Where $N(\boldsymbol{\mu}, \sigma^2)$ stands for a normal distribution and $IG(\alpha, \gamma)$ is the inverse gamma distribution.

Using Bayes's theorem, the resulting posterior distribution of $\boldsymbol{\beta}$ becomes:

$$\pi(\boldsymbol{\beta} \mid \sigma^2, \boldsymbol{\eta}, \mathbf{y}_o) \sim N((\mathbf{X_o}'\boldsymbol{\Sigma_o}^{-1}\mathbf{X_o} + \mathbf{C_o}^{-1})^{-1}\mathbf{z}, \sigma^2(\mathbf{X_o}'\boldsymbol{\Sigma_o}^{-1}\mathbf{X_o} + \mathbf{C_o}^{-1})^{-1}) \tag{6}$$

with $$\mathbf{z} = \mathbf{X}_o'\boldsymbol{\Sigma}_o'(\mathbf{y}_o - \boldsymbol{\delta}_o) + \mathbf{C}_o^{-1}\boldsymbol{\mu} \tag{7}$$

The computer experimental data can be described as:

$$\mathbf{Y}_c \sim N(\mathbf{X}_c\boldsymbol{\beta} + \boldsymbol{\delta}_c, \sigma^2\boldsymbol{\Sigma}_c) \tag{8}$$

Combining these results with the expert opinion posteriors we derive a second posterior distribution and then adding estimates from physical experiments trough Markov Chain Monte Carlo we calculate the final distribution for $\boldsymbol{\beta}$.

$$\text{Stage 1 } (\mathbf{Y}_o) \rightarrow \text{Stage 2 } (\mathbf{Y}_o + \mathbf{Y}_c) \rightarrow \text{Stage 3 } (\mathbf{Y}_o + \mathbf{Y}_c + \mathbf{Y}_p) \quad (9)$$

A related approach called "variable fidelity experiments" has been proposed in [8] to combine results from experiments conducted at various levels of sophistication.

Consider for example combining simple calculations in Excel, to results from VisiMix and actual physical mixing experiments.

The combined model is:

$$Y(\mathbf{x}, l) = \mathbf{f_1(x)}'\boldsymbol{\beta}_1 + \mathbf{f_2(x)}'\boldsymbol{\beta}_2 + Z_{sys}(\mathbf{x}, l) + \varepsilon_{means}(l) \quad (10)$$

where $l = 1, ..., m$ is fidelity level of the experimental system, $Z_{sys}(\mathbf{x}, l)$, is the systematic error and $\varepsilon_{means}(l)$ is the random error ($l = 1$ corresponds to the real system). There are also primary terms and potential terms, only the primary terms, $\mathbf{f}_1(\mathbf{x})$, are included in the regression model.

Assuming that the covariance matrix **V** is is known and **Y** is a vector that contains data from n experiments, the GLS estimator of β_1 is:

$$\hat{\beta}_1 = (\mathbf{X}_1'\mathbf{V}^{-1}\mathbf{X}_1)^{-1}\mathbf{X}_1'\mathbf{V}^{-1}\mathbf{Y} \quad (11)$$

The next section deals with response surface methodology, a comprehensive approach for running experimental designs.

4.4. *Response surface methodology*

Response surface methodology (RSM) is a strategy for exploring the relationship between controllable factors and important response variables. RSM makes extensive use of statistically designed experiments and regression models. A key element to RSM is the notion of sequential learning where initial experiments help focus attention on the most important factors, subsequent steps help to bring these factors to settings that achieve good results, and finally efficient statistical modeling techniques are used to estimate how these factors affect the response variables of greatest importance ((see [10, 11, 14, 25]).

The following steps are typical in RSM studies:

- screening – identifying which of the factors are really critical for performance;
- effect estimation – estimating the effect of each of the important factors;
- steepest ascent – following an estimated direction of steepest ascent to achieve better results;
- response modeling – fitting a more complex regression model that includes nonlinear effects of the factors;
- optimization – using the fitted surface to estimate optimal operating conditions.

The screening step is quite intuitive. Suppose the screening step identifies two key factors and we proceed with these factors to the next steps. An effective analogy to understand the remaining journey is to think of climbing to the top of a mountain. Imagine a contour map in which our two factors form the north–south and east–west axes and the contour lines map regions of high and low response such as in Figure 3. The map is derived from information in experiments that expose the likely height at particular factor settings. Early in the RSM program we are often near the base of the mountain. Our experiments for estimating effects will pick up the direction of the slope that leads us uphill. Experiments along the line of steepest ascent follow this route up until we reach a crest. Using this approach one can optimize the design of the HPLC method described in Table 1, to achieve maximum resolution on the bottom left corner of Figure 2.

4.5. *Multivariate methods*

Data collected by analytical methods is typically multivariate. DP specifications are almost always presented in a univariate format, one variable at a time. Multivariate statistics offer effective approaches to set specifications and describe DP performance. For example, in [5], Multivariate tolerance regions are used to specify the performance of a product by considering the combined distribution of its CQA characteristics. Such an approach accounts for the correlation structure between CQAs thus enhancing sensitivity and specificity of the analytical methods. For more on multivariate approaches to process control and specification setting see [5, 6, 7, 11, 25].

5. A case study from the process development of a biopharmaceutical

To conclude this paper we describe a typical example from a downstream purification step of a monoclonal antibody adapted from the case study presented in [3]. Initially, the hydrophobic interaction chromatography (HIC) process step is mapped by the process verification methodology which allows identifying key process variables. Subsequently, designed experiments are used to identify the critical operational parameters and their targets.

An experiment was designed with six factors: column bed height, protein loading density, pH, temperature, flow rate, and salt concentration of the load. Typical response or dependent variables are yield, purity, elution gate, and resolution between product and impurity peaks.

Load material was produced by processing clarified harvest material from two 10-L cultures over a Protein A capture column. The Protein A eluate was adjusted to pH 6.0 and frozen in aliquots at –70 °C. For each chromatography run, thawed Protein An eluate was filtered and diluted to the appropriate ammonium sulfate concentration with 2 M (NH4)2SO4 at pH 6.0, 6.5, or 7.0.

The protein solution was filtered again and UV absorbance at 280 nm was measured to calculate the total protein concentration of the load volume required for each run. The load was equilibrated in the water bath to attain the specified temperature before starting the chromatography run.

The SEC-HPLC assay was used to determine the percent of product and impurity species A, B, and C present in a test sample. A Tosoh G3000SWXL column (catalog number 08541) with a guard column was used to analyze Octyl fractions. The mobile phase used was 100 mM sodium phosphate, 200 mM sodium chloride, pH 6.5. The target load was 30 μg of protein per injection, and total run time was 35 minutes. A gel filtration standard was used (Bio-Rad, 151–1901) to verify system suitability.

None of the variables tested had any impact on resolution between product and species B. Octyl Sepharose Fast Flow may be incapable of better separation, or the controlling parameter has yet to be discovered. Resolution of species C only seemed correlated with the percent A in the load. These observations reaffirmed that the primary role of the Octyl column is to remove species A; other species may be reduced, but not removed, except at a high cost to yield. However, as long as the key variables are controlled, the prediction profiler generated by JMP or MINITAB calculates that the resolution of species A should vary only between 0.53 and 0.59 for 6.9–12.0% A in the load for the Octyl column.

The non-critical parameters that were identified were pH and flow rate or residence time. Key parameters were temperature, loading density, and the concentration of ammonium sulfate in the load. The critical parameter for this case study was the percent of species A present in the load. It affected yield and separation of product from impurities and was not well controlled at that point in development. The Octyl step was designed to consistently remove amounts of species A between 7 and 12% by controlling the key parameters. Meanwhile, upstream conditions were refined so that the percent of species A in the load during the manufacturing runs was 9.5% ± 0.6%. Thus percent A was down-graded from a critical to a key parameter.

Following development of the purification process, four manufacturing runs were conducted at the 2,000-L culture scale. The bed volume of the Octyl column was 40.5 L The yield and purity were 66% ± 6% and 92% ± 2%, respectively. Those are narrow ranges, particularly for a product in its first manufacturing campaign.

The key and critical parameters for the manufacturing runs were analyzed. However the number of data points and the breadth of the ranges of the data was insufficient to yield any statistically significant correlations. Loading density and conductivity, used here in place of ammonium sulfate concentration, appeared to result in earlier elution as predicted. Increasing conductivity resulted in less

resolution of species A from the main product, and more species A in the load had a negative impact on yield.

The results of the manufacturing runs showed that the process was robust when the key parameters identified in process development were controlled. The small variation among the runs coincided with the results of the bench-scale experiments, demonstrating the predictive power of the model.

6. Summary and Conclusions

The application of QbD to the development of drug products and analytical methods is providing an opportunity to revise traditional concepts and procedures for designing and validating analytical methods and setting specifications in drug products. In this paper, we outline the challenges faced by modern medicinal products, and their generic and biosimilar counterparts. These challenges can be addressed by statistical methodologies such as statistically designed experiments, multivariate methods, Bayesian models, simulation experiments and stochastic emulators. The paper is a high level introduction to these topics referring the reader to references for more details.

Acronyms

QbD - Quality by Design
API - Active Pharmaceutical Ingredient
DP - Drug Product
CQA - Critical Quality Attribute
VoC - Voice of the Customer
FDA - Food and Drug Administration
ICH - The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Software Products for simulations and QbD statistical analysis

Simulation experiments:

VisiMix - http://www.visimix.com
DryLab - http://www.molnar-institut.com/cd/indexe.htm
DynoChem - http://www.scale-up.com/index.html

Statistical design of experiments:

JMP - http://www.jmp.com
MINITAB - http://www.minitab.com/
Design-Expert - http://www.statease.com/dx71descr.html
Empower 2 Method Validation Manager - www.waters.com

Multivariate methods:

Clementine - http://www.spss.com/clementine/capabilities.htm
GeNie - http://genie.sis.pitt.edu
R - http://www.r-project.org
TITOSIM - http://www.titosim.com

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