APPLICATION OF DESIGN OF EXPERIMENTS (DOE) IN QUALITY BY DESIGN (QBD) IN HPLC METHOD DEVELOPMENT AND VALIDATION

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ABSTRACT:

The pharmaceutical industry uses Quality by Design (QbD) principles to create quality in the early stages of development/pharmaceutical product production, not just at the end of a product's evaluation. This document illustrates the application of QbD principles to High-Performance Liquid Chromatography (HPLC) method development/validation using "design of experiments" (DOE) methodology. The QbD approach to HPLC method development and validation includes key elements such as: setting the Quality Target Product Profile (QTPP), determining the Analytical Target Profile (ATP)'s appropriate level of acceptance / rejection for CQAs, conducting risk analyses, and/or establishing control strategies to assure all analytical methods are repeatedly developed with reliable results. Multiple types of designs (e.g., full factorial design, central composite) may be used to develop/validate the analytical methods/results.The regulatory agencies, e.g., ICH and FDA, expect the use of QbD and DoE when developing and validating high-quality pharmaceutical products; therefore, the use of QbD and DoE produces robust, efficient, and reproducible analytical methods.

Keywords: QbD; DoE; DoE in HPLC method validation and development.

INTRODUCTION:[1-4]

Pharmaceutical development's objective is to produce quality products utilizing processes that ensure that the finished product delivers what it was designed to do. Insights gained from developmental studies may support the quality risk management process by helping to inform design space, specifications, and controls for manufacture. The integration of quality into the design of a product is more important than testing for quality after the design has been made. Any changes occurring in development should be viewed as opportunities to increase one's knowledge. The act of operating within the original intended design space of the product is not considered a change; however, when operating outside of the original intended design space, the regulatory post-approval change process is triggered. The regulatory authority will assess the proposed design space.

The statistical method known as "Design of Experiments" (DoE), used by engineers and process chemists to optimize reactions by modifying several variables simultaneously, can fully examine the "reaction space." However, the use of this method in the chemistry community is limited by an overall lack of understanding of this method; thus, presenting an additional barrier for researchers who seek to develop new synthetic methodologies, which usually require extensive amounts of time to determine an optimal reaction. There are many academic projects that work with industry partners to enable the use of DoE for optimization of reactions.

DoE research examines the influences (and their interactions) of various factors on response results using statistically sound methods. The statistical method also has the additional benefit of including a "cross-check" of the various screened responses, thereby allowing for the prompt identification of any unusual results. As with the classic OVAT method, for repeatability to be accomplished, each investigation must be replicated; if this does not occur, then the findings from a single aberrant investigation may cause the entire optimization process to be in error.

Chemometrics is a recently developed branch of chemistry that uses formal logic, statistics, and mathematics to create the optimal experimental protocol. It uses few selected experiments to gain an overall appreciation of the system in question. Among the chemometric disciplines available, experimental design, also known as Design of Experiments (DoE), is the most commonly used for validation and optimization of chromatographic techniques. Furthermore, experimental design is used to develop design spaces, which are integral components of the AQbD paradigm as recommended by the FDA. By giving a thorough review of DoE applications in HPLC pharmaceutical research, this study will advance our grasp of this broad topic and promote the growing use of chemometric techniques in drug analysis.

QbD:[3-5]

Quality by Design or QbD, is a methodology for continuous improvement through the use of knowledge obtained over time throughout product life-cycle becoming more effective by developing protocols and testing based on the limits of science and increasing the scientific knowledge of critical features of products and processes.

The manufacture of a safe and effective product depends implementing QbD principles in the development process leading to proper formulation designs, development of manufacturing processes, and the development of systems designed to meet specifications. Therefore, it is essential to assure the development of practices and protocols will be utilized as intended within the same parameters used for developing and transitioning from development to an independent use.

Additionally, a guideline or model defined through mathematics is required to develop and assured to have obtained a reasonable level of cohesiveness when transferring the knowledge obtained through the QbD process(es).

DEFINITION FROM FDA PAT GUIDELINE:[5-9]

"Production Systems must include an acceptable level of assurance that final products are safe by establishing their safety through the effective, timely measurement (during processing) of key quality and performance attributes of new and in-production materials/processes.

PRINCIPLES OF QBD:[1-4]

QbD is built on the understanding of, and controlling, all the elements that will impact the quality of the end product. The major elements are:

• Quality Target Product Profile (QTPP) - defines the qualities expected of the product.

• Critical Quality Attributes (CQAs) - identifies the attributes or characteristics of a product that must be met to ensure high quality.

• Risk Assessment - provides an evaluation of all of the potential risks that could have an effect on CQAs.

• Design of Experiments (DoE) - provides the means to identify Key Process Parameters (CPPs) and optimize the various processes of producing the product.

• Control Strategy - provides a means of putting in place controls to ensure continued high-quality products.

• Lifecycle Approach - emphasizes the need for the ongoing monitoring of processes and continuous improvement.

ANALYTICAL TARGET PROFILE (ATP) AND QUALITY TARGET PRODUCT PROFILE ASSIGNMENT:[1-7]

A Quality Target Product Profile (QTPP) is a list of the product quality attributes that must be attained to assure the drug's safety and efficacy.

QTPP defines the expectations for the final product based on the drug and how it will be used in a clinical setting, as well as the method of administration, the type of dosage form, the type of delivery system, the strength of the dosage form, the type of container closure system used, the significant factors that affect the pharmacokinetics of the drug, and the criteria for product quality, such as sterility, quality, and stability, and so forth.In short, the QTPP ensures that all aspects of the final product have been considered and will be met.

Analytical Quality by Design (AQbD) first begins with defining the Analytical Target Profile ("ATP"). The objective of the analytical method as defined by ATP will determine how to choose, design and develop the analytical method. Essentially, the ATP provides direction on what was/should be measured (e.g., the active pharmaceutical ingredient, or API), how to measure it (e.g., pharmaceutical dosage forms), and why/when to do so (e.g., during stability studies). The definition of ATP must include the required level of confidence (target measurement uncertainty) to support the quality of analytical results, typically derived from accuracy and precision.

ANALYTICAL METHOD PERFORMANCE CHARACTERISTICS (AMPC) AND CRITICAL QUALITY ATTRIBUTES (CQA) IDENTIFICATION:[15-20]

The next step of the pharmaceutical quality by design (QbD) approach is to determine critical quality attributes (CQA) which is essentially the chemical, physical, biological and/or microbiological characteristics that pharmaceutical products, whether during production or following completion, must be within defined specifications in order to ultimately meet quality requirements. CQA may include, but are not limited to, identity, assay, uniformity, degradation, end product, residual solvent, drug release and dissolution, moisture level, microbiological limits, and physical properties (e.g., colour, shape, size, and friability). The potential CQA derived through QTPP serves as a basis for developing products and processes. It is possible to also determine critical material attributes (CMA) and critical process parameters (CPP) in achieving the identified CQA and QTPP. CMA are the physical, chemical, biological, and/or microbiological characteristics that are necessary for input materials to achieve the intended CQA.

RISK ASSESSMENT:[1-4]

The orderly way that we take and put together knowledge data into something that is helpful for determining potential risks is referred to as risk assessment. There are three elements related to conducting a risk assessment:

a) Risk identification: Methodically determining possible sources of hazard through the use of historical data, theoretical analysis, and stakeholder concerns.

b) Risk analysis: Determining the level of risk associated with those hazards that were previously identified.

c) Risk evaluation: Assess the significance of the estimated hazards by comparing them on either a quantitative or qualitative scale.

DESIGN OF EXPERIMENTS (DoE):[9]

Design of Experiments (DoE) is a systematic and organized way to discover the relationship between independent variables (input) and dependent variables (output). The output of the experiment will be analyzed in relation to the established mathematical equation representing the relationship. In the manner of a scientific approach, the independent variables of the experiment will be varied in a way that will provide you with the data necessary to determine the magnitude of effect each of the independent variables has on the dependent variable. By varying the independent variables you will be able to determine the independent variables that have the greatest, and least, effect on the dependent variable; to determine the settings of the independent variable that will provide the optimum setting of the dependent variable; and to determine how the independent variable settings interact.

TO ASSURE POSITIVE RESULTS FROM YOUR DOE APPLICATION IN HPLC, YOU WILL FOLLOW THESE STEPS: [9-15]

1. Determine the objective of your experiment. Traditionally, the way you think about your goals will help you draw any significant conclusions based on the results of your experiments after the data has been collected. This way of thinking is based on the premise that you should perform the maximum number of trials before reaching any final conclusions that will yield accurate data about what you are studying. Therefore, when conducting any experiments that will utilize the DoE methodology, you should specifically identify the purpose of your research prior to conducting any experiments (e.g., separating very close elution of analytes, determining how your analyte behaves chromatographically). In this way, DoE will improve the quality of your HPLC research and provide you with justification for performing the research.

2) Do some basic research Selecting the initial settings for HPLC, the type of column to use and the composition of the mobile phase, is the first step to completing your work using the HPLC. As a result, several studies have been conducted using a design of experiments (DOE) to choose the mobile phase.

3) Variable selection as well as its concentration needs to be determined. To create high-quality DoE-supported HPLC experiments, accurate identification of all relevant variables is a critical aspect. If an important variable has not been correctly identified as an input (factors), an analytical system may display unstable behaviour. Conversely, when a variable that is not importatn is identified as a factor, the remaining phases of the research were likely to be overly complicated. Within the DoE framework, HPLC experiments are conducted for various levels of the variables being studied. For numerical variable inputs, magnitude is represented by the factor level. The levels of variables have been assigned a coded number of either -1 (lower) or +1 (higher). Coded levels of nominal level are represented as 0. Using one scale, the impact of different factor types could be compared. In addition, codes provide a qualitative factor with coding also within the DoE.

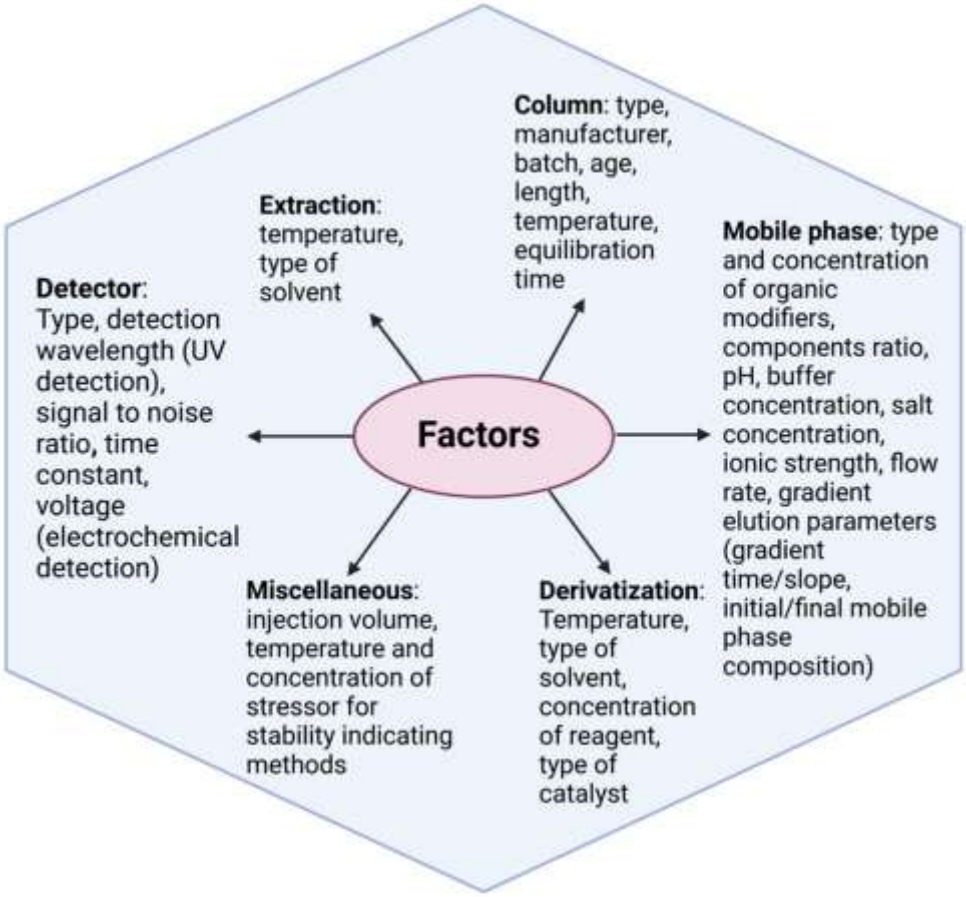

Fig:1.Factors affecting HPLC separation[7]

The design's purpose will dictate the number of components and the selection of their matchings. In general, when applying the method of Experiments (DOE) in Method Development or Robustness Tests, there are several aspects to which two views should be applied for analysis. However, the analysis will be limited to examining the most critical aspects at three or more levels during the optimisation phase (results). The overall methodology will depend on the analyst's past abilities, available resources, and time restrictions. The arrangement of factor levels around the nominal (average) level is typically symmetric. For measurement based on previous results or on precision (where a factor value is uncertain) may be determined by amount or time.

4) Select the response:

We measure or observe responses as they relate to relevance of some factor and how we can improve them. During the experiment, the definition of several responses to be recorded is often made. Selectivity and retention factors are two of the most common response types that are measured during the screening phase of method development. During the optimization phase, chromatographic response function, desirability function, retention factors and resolution factors can be tracked. Separating quality responses (resolution factor, relative retention time, retention factor, number of theoretical plates, plate height, tailing factor and peak-to-valley ratio) and quantity responses (API's and/by-products content, peak height and area) can be considered for robustness testing.

5) Plan the experiments:

In the development of a new laboratory study, an appropriate experimental design will be selected in this stage. The preferred design is one that possesses good statistical properties, such as orthogonality or rotatability, with independent variable changes (orthogonality) and lack of correlation between the distance from the center of a design and the variance of prediction (rotatability). Experimental designs can also be separated into two major categories based on the final outcome of HPLC experiments: response surface designs and screening designs. Screening designs will identify the independent variables and their combinations that have the largest degree of effect on the desired response. Once those variables that do not have a significant effect on the response have been eliminated, only the remaining variables will be investigated in detail using a response surface design. Since response surface designs are frequently used to establish an experimental condition that results in the best possible response and/or criterion (maximum, minimum, or range), they are sometimes called optimization designs.

6) Perform the experiments:

Even though it's necessary for completing experiment and arriving at valid harmonized results, experiments themselves are not as highly regarded in Design of Experiments (DoE). Trials need to be done randomly to minimize the influence of any extraneous variables. One should also perform experiments in duplicates (two separate runs using identical procedures) to assess for experimental errors.

7) Examine the information collected from the analytical process:

The next step of the process will turn the experimental data into the knowledge necessary to make valid inferences about the HPLC system. Two forms of conducting statistical analysis of data collected are analysis of variance (ANOVA) and regression analysis. Regression analysis evaluates the relationship between the independent and dependent variables. Because the mathematical model requires many independent variables to be fit into the model, multiple-linear regression (MLR) will be the most commonly used form of regression analysis. ANOVA is a useful method of confirming that the derived model is valid due to the F-test used to assess if an independent variable or group of independent variables is significantly different from one another or from zero.

The graphical analyses of data provide another means of investigating important characteristics and discovering how variables correlate. This applies to using 2D graphs, (for example) half-normal probability plots, Pareto diagrams, contour plots, response surface plots, perturbation plots and residuals plots etc., to mechanistically quantify HPLC data through analysis of specific plots. Of the three earlier referenced charts, the chart types majorly (from minor) will be evident through the first three types of charts referenced. The best-fitting mathematical model exists graphically in contour and response surface plots. These diagrams can also demonstrate how the response can change depending on the values of each parameter being measured. Many more than 2 variables will currently account for statistical significance. Therefore, a perturbation graph can be used to plot or Super Number Graph (or another statistical method not mentioned here). In addition, residual plots can demonstrate that outliers exist and/or indicate that a systematic error was made. An experienced HPLC analyst would be able to perform the numerous procedures quickly due to their attempting to perform tasks related to the DoE methods of analysis; however, utilizing the available standard statistical programs and/or specialized software programs will aid in simplifying the analysis procedure.

Mathematical modeling in DoE (Design of Experiments). Multivariate data is fitted to a linear or quadratic model based upon predetermined objectives after response measurements have been made under various conditions for each of the different circumstances. Mathematical models are used to relate how experimental conditions produced the observable response. Linear models (also first-order models) and quadratic models (also second-order models) are identified by polynomial order, as in equations (1) and (2). (e.g. the example uses three components, x1, x2, x3).

$$y=b_0+b_1x_1+b_2x_2+b_3x_3+b_{12}x_1x_2+b_{23}x_2x_3+b_{13}x_1x_3+b_{123}x_1x_2x_3+\varepsilon \quad (1)$$

$$y= b_0+b_1x_1+b_2x_2+b_3x_3+ b_{11}x_1^2+ b_{22}x_2^2+b_{33}x_3^2+b_{12}x_1x_2+ b_{23}x_2x_3+b_{13}x_1x_3+b_{123}x_1x_2x_3+\varepsilon \quad (2)$$

CATEGORIZATION OF EXPERIMENTAL DESIGNS USED IN THE DEVELOPMENT OF HPLC METHODS:[9-20]

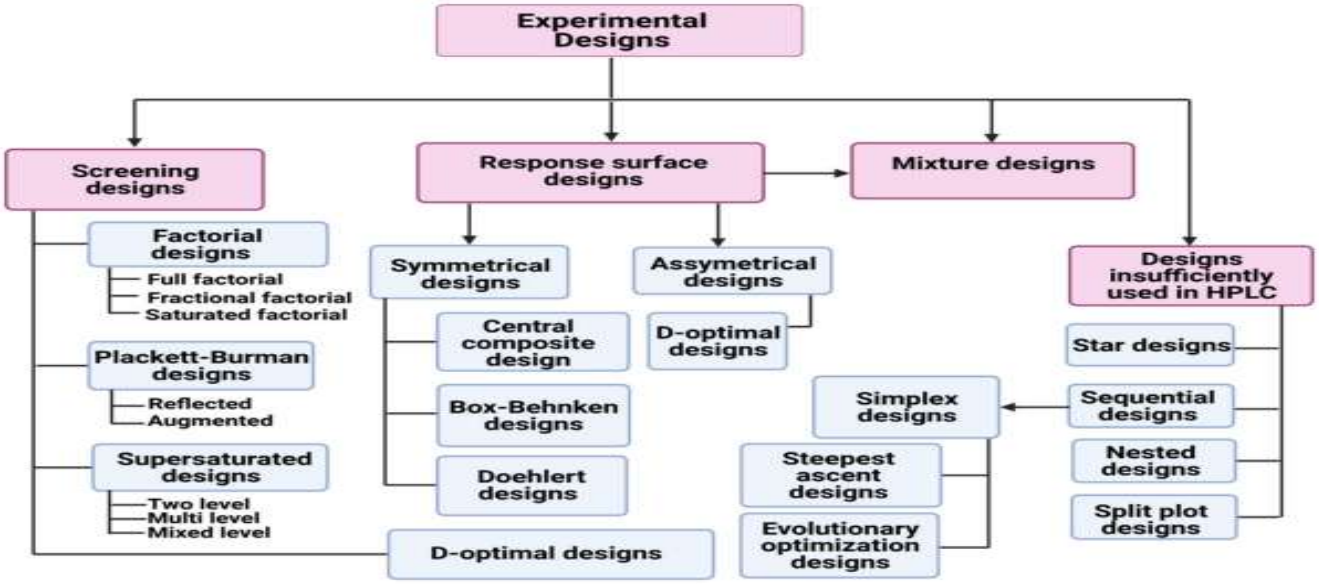

Fig:2.Classification of Experimental designs[7]

SCREENING DESIGNS:

HPLC pharmaceutical investigations benefit greatly from the use of two-level factorial designs as screening tools. Additional categories for factorial-based design include:

- Full Factorial Design (FFD)
- Fractional Factorial Design (FrFD)
- Plackett-Burman Design (PBD)

1.Full Factorial Design:

To perform *n*k tests in Fractional Factorial Design (FFD) requires n levels for each of k different factors. A trial for all combinations of factor values is made to enable the determination of all linear and interaction components in a mathematical model.

2. Fractional factorial design :

In FrFD, n^{k-p} (where n represents the number of levels - often two), k the number of components, and p the fraction size) will indicate how many trials there are. For an example, in FrFD, there must be more experiments than factors. There will not have enough degrees of freedom in FrFD to compute each of their interaction terms separately, so that the major effects will be confused (aliased) with the interaction effects instead. As the primary purpose of screening is to identify primary effects, this is expressed as 288.

3. Plackett-Burman design:

One type of experimental design is called Plackett-Burman Design, which typically has two levels and many advantages to it, such as being orthogonal and balanced. With regard to the number of experiments (N) in a PBD, this number will always be a multiple of four (i.e., 4, 8, 12, 16, 20 or 24). When using N experiments, N - 1 variables will be evaluated. Thus, PBD's main benefit is being able to evaluate many parameters while using only a small number of runs. A PBD can begin with the first row of a table which is used for this type of design, also known as "generator".

Response surface designs include:

a) symmetrical designs and

b) asymmetrical designs.

The previous category of design has a focal point and looks at a symmetrical experimental space. Examples of symmetrical designs include: the Central Composite Design (CCD); the Box-Behnken Design (BBD); the Taguchi Design (TD); the Doehlert Design; and the three level FFD. The second category of design looks at different levels of variables and consequently produces an asymmetrical shape for the variable's associated space. D-Optimal Design is an example of an asymmetrical design. Mixture designs are used only to evaluate mixture factor(s); in effect, to optimize the composition of the HPLC mobile phase.

Central composite design :

Central composite design (CCD) is by far the most complex design type used in optimizing the HPLC process. It consists of trials equal to $2^{k-p}+ 2k +Cp$, where k indicates the number of variables or factors, p indicates the size of the components for each experiment, and Cp indicates the central point of the experimental domain. Central composite design is made up of the basic central point, as well as star design (2k axial points) and complete or partial factorial design (2k-p). Central points are a key component of response surface designs, whereas they are not present with screening designs. It is also possible to perform ANOVA and determine experimental error by replicating in the central location of the trials. Based upon the distance from the axial points to the central point (α), CCD can be fashioned in either a 3-level or 5-level setup.

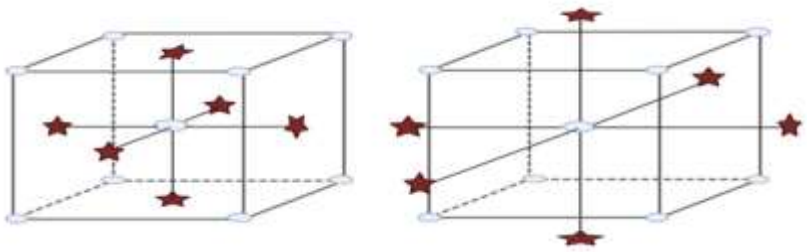

Fig:3. Central composite design with 3 factors (a) face-centered (b) circumscribed[7]

Box-Behnken design:

A Box-Behnken design (BBD) is a type of three level response surface design. The BBD is described mathematically by the equation, 2k(k-1) + cp, where k = number of factors, cp = number of center points. Therefore, BBD requires fewer total experiment runs than CCD (e.g., 13 for three factors and 15 for CCD). BBD is based on an incomplete factorial design and is considered to be almost rotatably designed. Figures Four and Five indicate those studies where all study factors are conducted at their highest and lowest levels are not included; therefore, BBD would most commonly be applied when the highest and lowest levels are known to be in the middle of the designated study area, or will not benefit from having all study factors conducted at the highest and lowest levels.

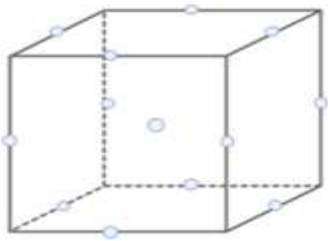

Fig:4. Box-Behnken design (BBD)[7]

Doehlert design :

An analyst can assign different levels to different factors in a Doehlert design (a uniform shell design that cannot be rotated), so you can assign more levels to more important factors. Also, when there are real-life constraints (e.g., only having limited resources), this is useful because you can then "move" to a different part of your experimental domain. However, if you do not include vital information relating to the experimental location when you do this, then that would not be an appropriate research strategy.

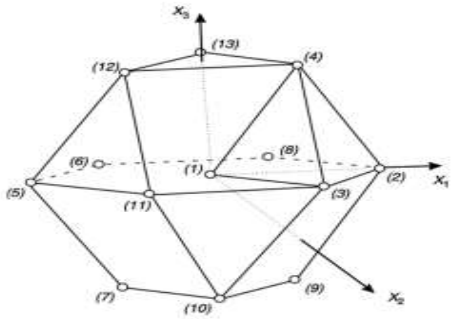

Fig:5. Doehlert design [7]

D-optimal design:

Symmetrical optimization strategies may be limited in their application\, due to experimental restraints. In this scenario, D-optimal can offer an alternative as it performs equally well in asymmetric and symmetric experimental areas. The advantages of D-optimal design lay in defining the mathematical model ahead of time and estimating both quantitative & qualitative variables. The number of coefficients in the proposed model (most commonly quadratic without interaction terms) is the same as the number of experiments required in a D-optimal design. A set of trials is selected from among all possible combinations of factor values to conduct. By combining trials with the largest determinant for XT X (where *X* is the model matrix and *X*T is its transpose), this group of trials will possess maximal information. The maximum amount of determination has been met prior to the experiments or performing statistical analysis\, thereby assuring that all of the coefficient values for the mathematical model will be accurate. This is one of the primary characteristics of D-optimal design.

DOE-RELATED REGULATORY FACTORS IN HPLC ANALYSIS:[16-20]

The concept known as Quality-by-Design (QbD) is focused on ensuring that overall quality has been built into the final product during the development of new medicines. Several regulatory guidelines (ICH Q8, Q9, Q10 and Q11) have been published to encourage the adoption of the QbD approach to the development of pharmaceutical products due in large part to the significant contribution made by analytical techniques in evaluating quality. The US FDA published a guidance document in 2015 that describes how analytical methods for drug and biologic products should be evaluated through a systematic approach involving both early risk assessment and multivariate experimental design methods such as DOEs to establish the robustness of the analytical method.

AQbD (Analytical Quality By Design) is the formulation of analytical methods through the use of QbD (Quality By Design) principles. The goal of AQbD is to produce a reliable and well-defined analytical method which consistently produces the intended result. Critical Method Attributes (CMA), Risk Assessment, Analytical Target Profile (ATP), Critical Process Parameters (CPP), Method Optimization, Design Space (DS), Control Strategy and continual Monitoring of the Method (CMM) constitute basic tenets of AQbD. Numerous statistical methods can support the implementation of AQbD; however, the Design of Experiments (DoE) methodology has been identified as the most effective. Literature has demonstrated that DoE based AQbD methods have been developed for many different HPLC applications in recent years.

APPLICATIONS OF DOE IN HPLC:[15-18]

Developing stability-indicating LC techniques is a challenging endeavour because of the complex and multi-component nature of the samples to be analysed. During evaluation, samples of pharmaceutical products and/or APIs will be submitted to a wide variety of stressful conditions; either the API(s) or product may be highly unstable when exposed to different types of conditions. These "stress samples" contain many unknown degradation products in addition to the API(s). Because of this multi-faceted nature, a large number of chromatographic parameters must be appropriately adjusted in order to achieve the best possible separation of all

analytes, which adds to the complexity of method development. Consequently, today, many stability-indicating LC techniques are developed using DoE-based AQbD methodology. Examples include previously reported use of PBD (a study to screen and identify the most statistically relevant CMPs) to optimise three of the most important CMPs during development of a stability-indicating LC technique for cloxacillin.

The hyphenation of HPLC and MS to improve pharmaceutical and related research has resulted in a tremendous boost in quality and quantity of data. However, as the number of variables increased, the difficulty of separating and detecting compounds also increased. In order to address these problems, a DoE (design of experiments) approach was used to investigate the genotoxic impurity of interest in the LC-MS study. All important variables in this study were explored using CCD (central composite design) at three levels: mobile phase flow rates, gradients, and injection volumes (LC variables), as well as collision energies and cone voltages (MS variables). The most recent literature describes the use of Design Space along with PBD and CCD to develop the LC-MS process for simultaneous analysis of five different statins.

The modified LC system is used for the separation of complicated pharmacological combinations and/or API(s) with similar retention behaviour. The retention behaviour of analytes in a more complicated HPLC system cannot usually be modelled using a linear or quadratic polynomial model. The application of artificial neural networks (ANN) as a model-predictive tool could therefore be useful in those situations where it is not possible to predict the retention behaviour of analytes using standard models. DoE (CCD) is used in conjunction with ANN to achieve optimal ion-pair HPLC separations of neuroprotective peptides. ANN is a good method for modelling the nonlinear relationships between variables. More recently, ANN-QSRR was used to develop a green LC method for the isolation of specific antipsychotics. In the green LC concept, the hazardous solvent acetonitrile was partially replaced by cyclodextrin in the mobile phase. QSRR was also used to optimise the separation conditions and to predict the retention. The molecular interactions and molecular mechanisms involved in the separations in a certain separation system can also be studied using QSRR, as the QSRR studies depend on the physical-chemical properties of analysed from molecular descriptors.

APPLICATIONS OF EXPERIMENT DESIGN IN QbD AND AQbD:[20-24]

The FDA has approved a new strategy called Quality by Design, which meant they would change their rules about how to make safe and effective drugs in 2004 (Quality by Design); this new strategy (Quality by Design) provided criteria under which drugs should be made according to international standards. The ICH Q8, Q9, and Q10 provide guidance on how to design and manufacture drug products using the ICH Q8, Q9 and Q10 guidance documents and QbD, with DoE being used to enhance understanding of the manufactured product and process. Since December 2004, when the FDA approved the use of QbD/DoE to support drug development/manufacture, companies from all over have been using DoE as a key aspect of their drug development to understand their product and process more effectively.

The literature contains several examples of how the design of experiment (DoE) technique has been used to support screening and optimizing both the product and process of pharmaceutical manufacturing. The purpose of the DoE is to identify the best combination for each independent variable that provides the optimum dependent responses (e.g. particle size, entrapment efficiency, importantly within that there is the agglomeration of drugs) by optimally screening a set of independent variables (e.g. temperature, pressure, concentration of excipients, time, and speed of stirring) with dependent variable values (e.g. dissolution rate).

Screening designs can additionally be used in developing quality target product profiles (QTPP) to identify Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs), which are classified as independent variables, that affect the Critical Quality Attributes (CQAs), which are classified as dependent variables in a Pharmaceutical Quality by Design (QbD) environment. Additionally, utilizing improved statistical techniques such as Multiple Response Optimization, Surface Response Methods and experimental design will allow for the establishment of design space areas where both CQAs and QTPPs are considered. The establishment of a design space area provides regulatory flexibility for a designated area in which a manufacturer may make changes without prior regulatory approval based upon product and process knowledge.
The Department of Energy (DOE) has recently been used in a logical manner to improve and advance procedures in the laboratory. Examples of independent variable inputs that DOE can be used for screening and optimisation include media composition, mobile phase, flow rate, and incubation period. The dependent variables listed in the literature as possible outputs include, but are not limited to, microbial growth, retention time, and resolution between peaks.

CONCLUSION:

In conclusion, the application of Design of Experiments (DoE) and Quality by Design (QbD) for developing HPLC methods represents a significant advance in both analytical science and pharmaceutical research. QbD provides an organized and scientific foundation to ensure quality is built-into products and analytical methods from the start, rather than waiting and depending on traditional trial and error techniques. Careful definition of the Quality Target Product Profile (QTPP), Analytical Target Profile (ATP) and Critical Quality Attribute (CQA) will provide a sound basis for developing methods with confidence, together with a structured evaluation of risk.In this approach, the use of Design of Experiments (DoE), as a powerful statistical method, will enable the identification and optimization of Critical Material Attributes (CMA's) and Critical Process Parameters (CPP's) by the researcher through the use of structured experimental designs which allow for the simultaneous evaluation of multiple variables, understanding of interactions, development of predictive mathematical models and development of a reliable design space. Types of structured experimental designs include factorial designs, Plackett–Burman designs, Central Composite Design (CCD), and Box–Behnken Design (BBD). This technique also results in improved reproducibility, reduced resource requirements, reduced time to develop methods and increased robustness of methods. Incorporating QbD & DoE into HPLC & LC-MS analyses not only makes feasible the development of stable and chromatographically satisfactory techniques but also adheres to FDA guidance and ICH mandates. The use of AQbD further supports this approach, as it assures that analytical techniques continue to meet pre-established performance specifications throughout their lifetime.Therefore, QbD with DoE enhances the HPLC method development process by being knowledge-based, risk-analytical, and data-driven. Ultimately, by enhancing product quality, regulatory flexibility, and

ongoing improvement, this novel methodology ensures the development of safe, effective, and high-quality pharmaceutical products.

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