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REVIEW ARTICLE **OPEN ACCESS**

Review on Analytical Method Development and Validation of Phytoconstituents based on Design of Experiments

Keshavnam M. Chitte[1], Disha Prajapati[2*], Tanvi Dodiya[2], Janvi Patel[1]
[1]Department of Quality Assurance, Parul Institute of Pharmacy & Research, Parul University Limda, Vadodara 391760, Gujarat, India.
[2]Department of Pharmacognosy, Parul Institute of Pharmacy & Research, Parul University Limda, Vadodara 391760, Gujarat, India.
***Corresponding Author:** Dr. Disha Prajapati
disha.prajapati@paruluniversity.ac.in

ABSTRACT
Design of Experiments is a systematic and efficient approach for optimizing processes, products, or experiments by varying multiple factors simultaneously and observing their effects. The majorly employed designs include Box-Behnken, Fractional Factorial, Full Factorial, Central Composite, Two-Level Factorial, Taguchi, Plackett-Burman, Latin Square, etc. The choice of design depends on the number of factors and interactions required for the study. The key steps involved in the design of experiment for analytical method development include planning, screening, modeling, optimizing, and verification. Retention factor values, resolution, peak area, and retention time are the most commonly employed dependent variables. Independent variables include the mobile phase composition, solvent front, chamber saturation time, solvent composition, temperature, time, particle size, and concentration. The present review compiles the application of the design of experiments in analytical method development for various phytoconstituents in medicinal plants, herbal products, and ayurvedic formulations. The application of the design of experiment for analytical method development can prove to be a beneficial and powerful tool that can help systematically optimize the method and enhance its reliability in compliance with regulatory requirements.
Keywords: *Design of Experiments, Box-Behnken Design, Central Composite Design, Full Factorial Design, Method Development, Validation*

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INTRODUCTION

The design of experiments is a mathematical framework that is applied to the organization, analysis, and interpretation of experiment data [1]. This area of statistical application is utilized in scientific examinations of an entire system, procedure, and product where the impacts of modified input factors (Xi) on the observed response factor (Y) are examined [1,2]. Experimental design was first explored in 1920 by the renowned researcher Ronald Fisher, who is regarded as "a brilliant person who almost independently created the framework for current statistical the field of science" and whose contributions to statistics are generally acknowledged [3]. In the design of experiments, the intention is to get as much data as possible using the fewest possible experiments. Additional motivations for statistical experiment design include the requirement to evaluate errors in experimentation, understand variable interactions, estimate effects, and model connections [4]. Design of Experiments is a methodical and structured approach that establishes mathematical models y = f(xi) to ascertain the correlations between input parameters (xi) influencing one or more outcomes (y) [2].

Advantages of design of experiments

- It eradicates the effect of confounding, which is the mixing of the effects of design factors.
- Assists in managing experimental mistakes.
- Aids in identifying the significant factors that require control and unnecessary factors that may be left uncontrolled.
- Aids in measuring connections, a crucial task [5].

DOE Software

The appropriate statistical software can assist in the quick design and analysis of DOE. Several freeware statistical packages and commercials are available for this specific purpose. Several popular commercial software programs are available, including Statgraphics, SPSS, Prisma Statistica, Minitab, Design-Expert, SAS, etc. The most popular freeware programs are Action for Excel from Microsoft and R. with Microsoft Excel, DOE design and analysis can be completed quickly and simply by following the steps and formulas [1,6].

Statistical Methods

Analysis of Variance (ANOVA)

It evaluates possible variations in a categorical-level (ordinal or nominal) independent variable with two or more categories compared to a continuous-level (ratio or interval) dependent variables. An ANOVA, for instance, can look at possible variations in beels' fish yield according to stocking density or the degree of macrophyte infestation (Low, Middle, High Levels) [7].

Linear Regression

The linear relationship between an scalar response and a number of explanatory variables commonly referred to as input and output factors in statistics is computed using a statistical model called linear regression. When there is just one explanatory factor, a straight forward linear model approach is employed; when there are several explanatory factors, a multivariate linear regression model is utilized [8].

DOE TECHNIQUES

Box-Behnken Design

Building quadratic response surfaces and employing Box-Behnken design variables numbers 3–6. 12 trials and additional five center-point replications are needed for the Box-Behnken design for three variables. It applies the identical mathematical approach as CCD and looks at the variables on three different levels. BBD does not look at borderline zones, although CCD does contain the extreme factor combos. BBD requires fewer experiments, which is another clear distinction. Nevertheless, fewer experiments also imply fewer degrees of freedom [9].

Central Composite Design

The most popular fractional factorial design for the response surface model is the most commonly used central composite design. A key element of response surface methodology is the CCD model. This kind of optimization model's greatest benefit is that it can generate second-order quadratic models without the necessity for a three-level factorial experiment, making it more accurate.

Crucial steps in the responses surface methodology

- **a)** Following the required screening, the experiment's multiple factors and their ensuing interactions were determined.
- **b)** The established different levels of features were prioritized.
- **c)** After optimization, the most appropriate model has been chosen.
- **d)** It is also possible to select the suitable model, which is excellent for experimental design.
- **e)** In order to do experimental research, one must first perceive the in tangible factors and qualities that require methodical analysis.
- **f)** The chosen model is validated.
- **g)** A surface graphical representation is generated if needed [10].

There are three different types of CCD.

The CCD model, also known as the Box and Wilson design, consists of three factorial designs: factorial 1, factorial 2, and factorial 3[11]. The response surface plot is determined in part by the center point, which represents the experimental domine, and the star point outside the domine [12].

Face cantered (CCF)

Star points serve as a central point for every face of the factorial space for the design. Consequently. α is equal to ±1. Each factor is needed at three levels for this variable. The designs that are face-cantered (CCF) cannot be rotated. A single thing is made very evident by this design: CCC utilizes the biggest processing space, whereas CCI discovers the smallest [13].

Circumscribed design (CCC)

Each of the corner points of a CCD model is always magnified and shown with red spots. The extraction points are limited to the green spots on the sides, starting from the center point (blue). Every factor in the CCD model would have five levels. Novel extremes for the high and low sets for all factors are being established by the star points. These layouts demanded five levels for every variable and were either spherical, hyper-spherical, circular, or symmetrical. It was discovered that the circumscribed (CCC) was a rotational design [14].

Inscribed design (CCI)

The CCI design used the parameter settings as star points and produced factorial designs within the boundaries when the variable settings had a stated limit. Stated differently, CCI layout is an altered form of

CCC design, with CCC design split by α to produce the CCI model. In the end, it was discovered that CCC and CCI were rotating models [15,16].

Taguchi Design

In the late 1940s, Dr. Taguchi used design of experiment techniques to conduct important research. His goal was to develop an effective and user-friendly experimental technique that could be used to raise the standard of manufactured goods (Ross, 1988; Roy, 1990).an alternative method of Taguchi's experimental design, sometimes referred to as "Taguchi Methods." Early in the 1980s, it came to be in the United States. It has recently proven to be a useful technique in engineering fields. Setting up the experiment is the most crucial step before any analysis, in Taguchi's opinion. The process quality can only be raised in this manner. This approach could reach the desired result while minimizing variation around it at the lowest possible cost [17].

Full Factorial Design

Probably the most popular and practical experimental design technique is the full factorial. An orthogonal approach of the design of experiments is the full factorial. Eight experiments plus central point replications are needed for the 2x3 full factorial design, with every variable being evaluated on two levels(+1,-1) [9].

Fractional Factorial Design

Screening design finds the most important main effects among many study lower order effect. The 2×4 fractional factorial requires eight experiments, with every variable evaluated at two levels (+1, -1) [18].

Plackett-Burman Design

R.L. Plackett and J.P. Burman created the Plackett-Burman design in 1946, which is a well-known class of screening designs. Its purpose was to enhance the control of quality procedures that could be utilized to investigate the effects of design variables on the system's current state in order to make informed decisions. For screening, orthogonal arrays produce an objective evaluation of multiple primary effects with a small design that is feasible. Two-level fractional factorials are shown by the Plackett-Burman Screening Design (PBSD).It makes the effective estimation of each factor's primary effects possible. A PBD example with twelve runs and eleven factors [5,19].

Latin Square Design

Similar in concept to the RCBD (Randomized complete block design), the Latin square experimental design seeks to minimize the number of samples needed while maintaining the significance of the key factor. Performing a single experiment in each block instead of an RCBD is the fundamental principle. Three levels and three factors of study were conducted on each of the nine runs in the Latin square design [20].

DESIGN OF EXPERIMENTS USES

- ➢ An effective method for identifying significant input components (input variables) and their relationships to outputs (response variables) in a variety of contexts is experiment design.
- ➢ DOE mostly employs "hard tools". Furthermore, DOE is essentially a versatile form of regression analysis.
- ➢ The following design types are frequently utilized
- ▪ comparisons: This is one of the comparisons that are used to identify the optimal option utilizing the t, Z, or F tests.
- ▪ Factors screening: comprises the process of choosing important variables from a wide range of parameters that affect how well a system, process, or product performs. These are usually factorial designs with two levels.
- ▪ Optimization: The transfer function can be used to optimize by moving the experiment to the optimal variable setting. Process, product, or system performance can be improved in this way.
- ▪ Robust design: Reducing variation in a system, process, product, or system without addressing the fundamental causes is known as resilient design. Dr. Genichi Taguchi created a strong design that prevented noise from entering the system [1].

DESIGN OF EXPERIMENTS IN ANALYTICAL METHOD DEVELOPMENT FOR PHYTOCONSTITUENTS

This review compiles the application of the design of experiments in analytical method development for the estimation and quantification of phytoconstituents in various medicinal plants, herbal formulations, and ayurvedic formulations. The summary of the various designs used and their corresponding dependent and independent factors identified for the analytical method development is described in **Table 1**. According to the reported articles, box-behnken design is used for the development and validation of an analytical method for the estimation of phytoconstituents. The usual independent factors considered are wavelength, chamber saturation time, solvent front, mobile phase ratio, temperature, particle size, and solvent composition; the common dependent variables are peak area, retention factor, retention time, and resolution. For box-behnken, the high, middle, and low levels are commonly employed for analytical method development and validation. Similarly, for a full factorial design, common independent variables are temperature, addition rate, stirring speed, concentration, mobile phase, solvent-to-drug ratio, stirring temperature, and stirring time, and dependent factors are drug efficiency, area, resolution, retention time, particle size, and entrapment efficiency. Further, in central composite design, the common independent factors used are compositions in mobile phases, chamber saturation time, wavelength, developing distance, band size, and mobile phase content, and the dependent factor is retardation factor.

Table 1- Summary of various designs of design of experiment used for analytical method development and validation for quantification and estimation of phytoconstituents

Sr. No	Title	Design used in DoE	Dependent Variables	Independent Variables	Variable Level (Coded)	Ref.
1	Gallic acid, quercetin, and rutin were simultaneously estimated using the HPTLC method from ethanolic and aqueous leaf extracts of *Moringa oleifera*.	Two Level Factorial	Retardation factor, Peak area	Wavelength, Mobile Phase Ratio, Time from chromatography to scanning, Activation time, Time from spotting to chromatography	High, Low	[21]
2	Applying the HPLC Method Optimization of the extraction of piperine from *Piper longum L.* Fruits	Box-Behnken	Retardation time	Extraction temperature, Extraction time, Solvent to Drug Ratio	High, Middle, Low	[22]
3	Scopoletin estimation from a polyherbal formulation composition utilizing HPLC and a fluorescence detector in accordance with the DoE concept	Full-Factorial	Area, Resolution, Retention time	Mobile Phase, Solvent to Drug Ratio, Temperature	High, Low	[23]
4	Development and Optimization of Epigallocatechin-3-Gallate Nano-Phytosome via UHPLC.	Full-Factorial	Particle Size, Drug efficiency	Stirring temperature, Stirring time, Addition rate, Stirring speed	High, Low	[24]
5	The HPTLC method was employed in the optimization of the extraction of therapeutically active furanocoumarin Khellin from *Ammi majus L.* fruits	Box–Behnken	Peak area, Retardation factor,	Solvent to drug ratio, Extraction time, Extraction temperature	High, Middle, Low	[25]
6	HPTLC method for the simultaneous estimation of quercetin, kaempferol, and keto-β-boswellic acid.	Box–Behnken	Retardation factor, Resolution	Solvent front, Volume of n-hexane (Mobile phase ratio), Chamber saturation time	High, Middle, Low	[26]
7	Development and Validation of an HPTLC Method for the Simultaneous Estimation of Berberine Chloride and Galangin in *Tinospora cordifolia M.* and *Alpinia galanga L.* Herbal Formulations	Box–Behnken	Retardation factor	(Mobile phase ratio) Ethyl acetate Toluene Formic acid	High, Middle, Low	[27]
8	Development and validation of HPTLC method: Relevance in quantitative evaluation of protopine in *Fumaria indica*	Central Composite	Retardation factor	Compositions in Mobile phases (Toluene volume), Chamber saturation time, Wavelength	High(+2), Low(-2)	[28]
9	Simultaneous Estimation of Stigmasterol and Withaferin A in Union Total Herbal Formulation Using Validated HPTLC Method	Central Composite	Retardation factor	Mobile phase content (chloroform), Developing distance , Band size	High, Middle, Low	[29]

10	Analytical Quality by Design Assisted Development and Validation of HPTLC Method for Estimation of Rottlerin in Topical Patch Formulation	Box–Behnken	Retardation factor	Toluene content Saturation Time	High, Middle, Low	[30]
11	Design of Experiment Approach Based Formulation Optimization of Berberine Loaded Solid Lipid Nanoparticle for Antihyperlipidemic Activity	Full Factorial	Particle size, Entrapment efficiency	PVA concentration, Amount of lipid	High, Middle, Low	[31]
12	Optimization of diosgenin extraction from *Dioscorea deltoidea* tubers using response surface methodology and artificial neural network modelling	Box–Behnken	Yield	Temperature, Particle size, Solvent Composition, Extraction time, Solvent ratio	High, Middle, Low	[32]
13	Process optimization for the supercritical carbon dioxide extraction of *Foeniculum vulgare* Mill. seeds aromatic extract with respect to yield and *trans*-anethole contents using Box-Behnken design	Box-Behnken	Yield (oil) Content Comprehensive score	Extraction time, Extraction temperature, Pressure	High, Middle, Low	[33]

Variables Level - (+ High, Middle, - Low)

CONCLUSION

The present compilation of diverse experiments highlights the widespread application of Design of Experiments (DoE) in optimizing analytical methods for phytoconstituents analysis. Various designs, including two-level factorial, box-behnken, full-factorial, and central composite, have been employed to systematically enhance method performance. Each study focuses on specific dependent and independent variables, demonstrating the versatility of DoE in accommodating different analytical techniques like HPTLC, HPLC, and UHPLC. The incorporation of DoE principles ensures a methodical approach to method development, resulting in reliable and reproducible outcomes.

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CONFLICT OF INTEREST

Authors listed into the article suggest no conflict of interest.

AUTHOR'S CONTRIBUTION

Each author contributed in the work is mentioned.

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