# DESIGN OF EXPERIMENTS APPROACH IN ANALYTICAL CHEMISTRY- A COMPREHENSIVE REVIEW

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***ABSTRACT:*** *The review is a compilation of application of Design of Experiments (DOE) approach to characterize different pharmaceutical dosage forms, natural productsas well as biological samples by diversified analytical techniques including Ultraviolet- Visible (UV- Vis) spectroscopy, High Performance Liquid Chromatography (HPLC), LC- MS/MS etc. Emphasis is also given to novel extraction techniques employed for sample preparation in bio-analysis.The merits of chemometric approach over trial and error approach have been clearly stressed. Application of multivariate approach including Screening designs and response surface designs in analyzing different pharmaceutical dosage forms have been summarized in the review. This could form a source to systematically analyze novel pharmaceuticals and extract chemical constituents from natural sources including microbes byevaluating the effect ofdifferent factorsat different levels(Flow rate, mobile phase composition, solvent) on the response (Absorbance, Assay percentage, extraction yield) and optimize the developed method.*

***Key Words:*** *Chemometrics, Design of Experiments, Factor, Level, Multivariate approach, Pharmaceuticals.*

## Introduction

### STATISTICS

**Statistics** is a branch of mathematics dealing with data collection, organization, analysis, interpretation and presentation. In applying statistics to, for example, a scientific, industrial, or social problem, it is conventional to begin with a statistical population or a statistical model process to be studied.

### ROLE OF STATISTICS IN PHARMACEUTICAL DEVELOPMENT

Statistical methods are increasingly applied to accelerate the characterization and optimization of new drugs created via numerous unit operations well known to the chemical engineering discipline. In the implementation of design of experiment techniques, the increased incorporation of latent variable methods in process and material characterization, and the adoption of Bayesian methodology for process risk assessment.

These new approaches to regulation, compliance, and quality were embodied in a series of guidelines issued to the industry by the FDA and The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The ICH is a unique project that brings together the regulatory authorities of Europe, Japan, and the United States.[1]

### CHEMOMETRICS

**Chemometrics**is the chemical discipline that uses mathematical and statistical methods to improve the understanding of chemical information and to correlate quality parameters or physical properties or select optimal measurement procedures and experimentsto analytical instrument data.The concept of quality by design (QbD) has been recently adopted in the pharmaceutical industry involves many quality and statistical tools and methods (e.g., statistical designs of experiments, multivariate statistics, Six Sigma methodologies, and statistical quality control). Data analysis is done by considering multivariables at a time rather than conventional One- Variable at a Time (OVAT) approach. It ensures building of quality into product rather than establishing quality product.

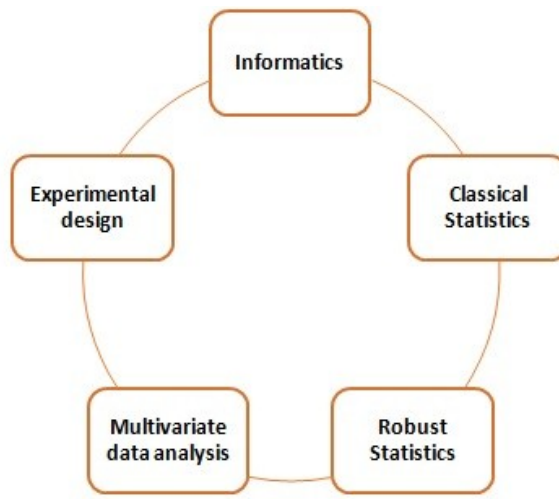
### OBJECTIVES OF USING CHEMOMETRICS IN PHARMACEUTICAL DEVELOPMENT

- To improve the understanding of chemical information and to correlate quality parameters or physical properties or select optimal measurement procedures and experimentsto analytical instrument data.

- Chemometric techniques are particularly heavily used in analyticalchemistry and metabolomics, and the development of improved chemometric methods of analysis also continues to advance the state of the art in analytical instrumentation and methodology.

**TYPES OF CHEMOMETRICS**

- ❖ Informatics
- ❖ Classical Statistics
- ❖ Robust Statistics
- ❖ Multivariate data analysis
- ❖ Experimental design




**Classical statistics**

Classical Statistical Model Selection is to determine which variables are important in a regression model (equivalently a linear model) for the dependent response variable *y*, in terms of the auxiliary variables *x* in the model. However, the Classical Statistical Model Selection rules can also be used for problems of paradigm shift.

**Robust statistics**

Robust statistics that aim to construct models and estimates describing well data majority. Moreover, construction of robust models allows identifying outlying observations.

**Multivariate data analysis**

Multivariate regression techniques involves two or more variables. Before embarking on an analysis involving large number of variables, it is significant to first examine if there are any underlying data structure or patterns that we can exploit to improve and sometimes simplify the analysis. A common approach will be to graphically visualize the data cloud that is limited to three variables. Often, a fourth dimension can be added by varying the type and size of symbols, but that is our limit for graphic visualization. For high-dimensional datasets, an alternative approach is to reduce the dimensionality of the data with minimum loss of important attributes, for example, data variance. Multivariate data analysis techniques allow us to accomplish these goals.[2]

**DESIGN OF EXPERIMENTS**

Design of Experiments is a statistical approach, termed as Chemometrics used for multivariate analysis. This concept is used to organize, conduct, interpret results of experiments in an efficient way, making sure that as much useful information is obtained by performing a small number of trials eliminating conventional trial and error approach where independentvariables are varied by chance.

**Demerits of One- Variable at a Time Approach (OVAT)**

- Does not lead to real optimum
- Isolated, unconnected experiments
- Ignores interactions
- Variability in response
- No mapping of experimental space

**Goal of Experimental Designs in Pharmaceutical Technology**

- To establish and determine the relationship between independent variables (factors) and dependent variables (response).
- To study the effects of multiple independent variables (factors) on response.
- Define levels of analyzed factors

**Table 1 Important Terminology of Experimental Designs**

| | |
|---|---|
| ***Design Space*** | Multidimensional combination and interaction of input variables and process parameters of assured quality. |
| ***Factor domain*** | Variation domain with all the values between low and high level of factors (in coded variables). |
| ***Study domain*** | Region of experimental space where study is carried out. |
| ***Response Surface*** | Collection of all responses of points on the study domain. |

**Types of Experimental Designs**

Based on the need of study, Experimental designs are classified as follows as shown in fig 1.

***Screening Designs***

Screening designs are used to identify the most influential factors that have effect on studied responses. A huge number of factors *f*,can be screened by varying on two levels in relatively small number of experiments.

$$N \geq f+1$$

N = No. of experimental runs

The total no. of experimental runs is given by the formula $\mathbf{e^f}$where e is no. of levels

***Response Surface Designs***

The goal of applying Response Surface Methodology is to optimize the response (dissolution rate, assay %) by a response surface curve.[3]

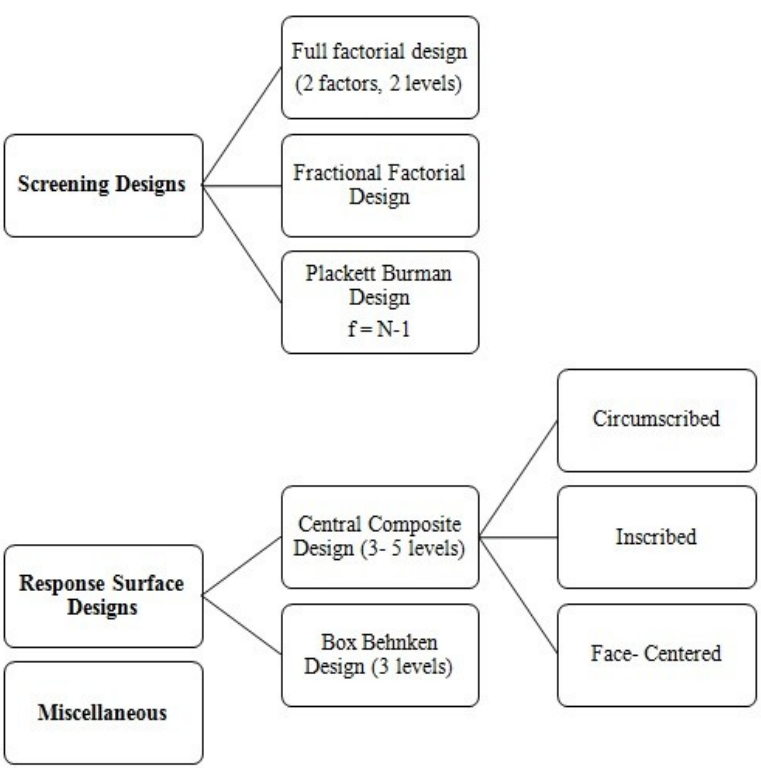



**Fig 1.Classification of Experimental Designs[4]**

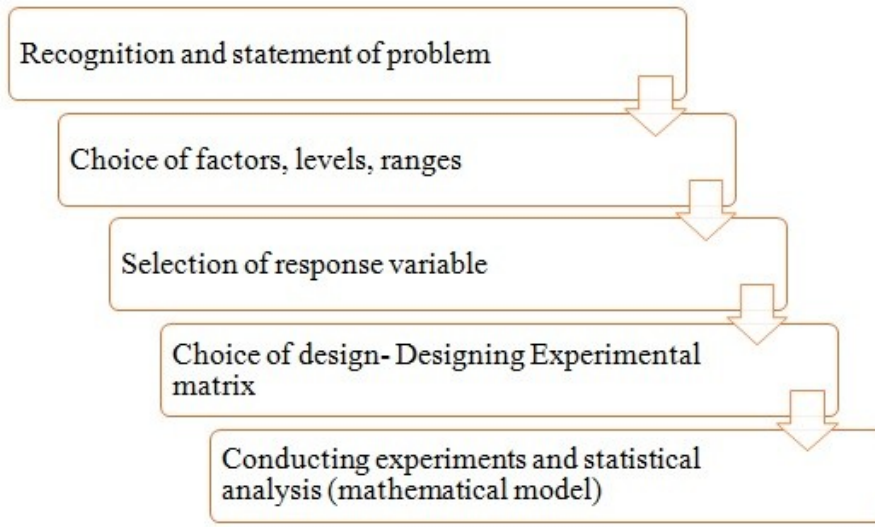



**Fig 2. Steps involved in Experimental Design approach[5]**

**Table 2 Sample Experimental Matrix for Circumscribed Central Composite Design**

| Experimental Run | Factor 1 | Factor 2 |
|---|---|---|
| **Full Factorial Design** | -1 | +1 |
| | +1 | -1 |
| | -1 | -1 |
| | +1 | +1 |
| **Central point** | 0 | 0 |
| **Axial Design** | -α | 0 |
| | 0 | -α |
| | +α | 0 |
| | 0 | +α |

The parameters/ factors are in coded variables represented as -1,+1 etc. α values are the extreme values away the study domain.

After the choice of factors and design and experimental matrix is set, the experiments are run and the output is recorded. Using suitable software, the design is run and the data is analyzed mainly using pareto chart (Significance of each factor along with interaction at chosen p value) and response surface curve along with one way ANOVA. The linear/ quadratic models determine mathematically the influence of each factor change on the response change. Desirability charts are helpful to depict multiple responses.

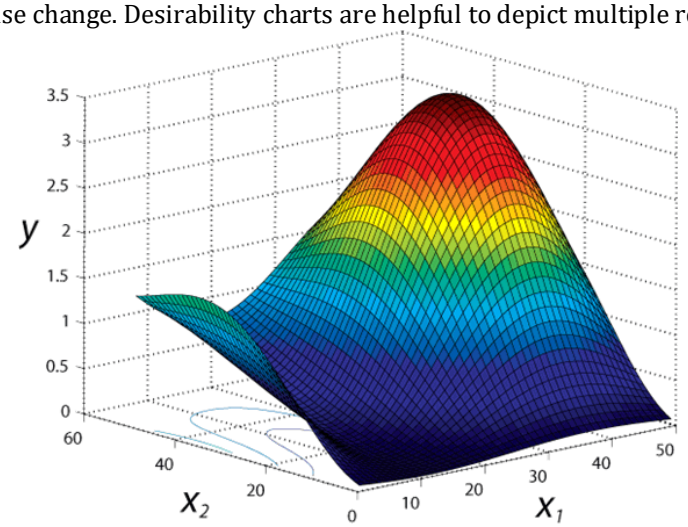



**Fig 3. Sample Response curve with x1 and x2 factors and y response**
**The red pixel depicts maximum response.**

**Application of Experimental Design Approach to analyze pharmaceutical dosage forms by Liquid Chromatography**

| Analytical Technique | Analyte(s) of interest | Sample Preparation | Experimental Design | Independent Variables (factors) | Response | Reference |
|---|---|---|---|---|---|---|
| RP- HPLC | Moxifloxacin HCl and Ketorolac tromethamine | - | Face-centered Central Composite design | Methanol content in the mobile phase composition, Buffer pH and flow rate | Retention time, Tailing factor | 6 |
| HPLC- UV | Melamine from soil samples | Surfactant-enhanced hollow fiber liquid phase (SE-HF-LPME) microextraction | Screening- Fractional factorial design, Optimization- Central Composite design | Type of extraction solvent | Extraction yield | 7 |
| HPLC | Separation of amino acids | Precolumn derivatization with phenylisothiocyanate | Fractional Factorial and Central composite designs | triethylamine content of the aqueous buffer, pH of the aqueous buffer, separation temperature, methanol/acetonitrile concentration ratio in the organic eluant, and mobile phase flow rate | Resolution of amino acids | 8 |
| HPLC | Stability of Eberconazole nitrate | - | QbD | Hydrolytic (acid, base, neutral), oxidative, thermal and photolytic stress | Stability | 9 |
| HILIC | Separation of four monophosphate nucleotides | | | Buffer concentration (ammonium acetate concentration), gradient time, and temperature | Different chromatographic parameters | 10 |
| RP- HPLC-UV | Separation of Curcumin in human plasma and food samples | Solid Phase Microextraction | Central Composite Design | Role of sorbent mass, volume of eluent and sonication | Extraction yield of sample by SPME | 11 |

| | | | | | | |
|---|---|---|---|---|---|---|
| RP- HPLC | Temozolomide (TMZ) in bulk sample and nanostructured lipid carriers | | Box–Behnken design | | | 12 |
| HPLC- UV-MS | Losartan and valsartan in human plasma samples | Stir bar sorptive extraction (polydimethylsiloxand and polyacrylate) followed by Ultrasound assisted liquid desorption | Box–Behnken design | | Extraction efficiency by stir bar | 13 |
| HPLC | Valsartan in nanoparticles | | Full factorial design | Flow rate and wavelength, pH of buffer | Peak area, tailing factor and number of theoretical plates | 14 |
| HPLC- UV | Eprosartan from human plasma samples | Solid Phase Extraction | Screening- Fractional factorial design, Optimization- Central Composite Design | | Corrected area, the separation of eprosartan chromatographic peak from plasma interferences peaks and the retention time of the analyte | 15 |
| HPLC- ECD | Captopril | | Central Composite design | Mobile phase pH, molarity and concentration of acetonitrile | Retention time | 16 |
| HPLC-DAD | Orientin, Isoorientin, Vitexin, Isovitexin and Rutin flavonoids in the leaves of seventeen Passiflora species | Accelerated Solvent Extraction | Box-Behnken design | Extraction temperature, % Ethanol, Extraction cycles | Extraction yield | 17 |
| HPLC | Sesquiterpenes in Nardostachyos Radix et Rhizoma | Reflux | Box-Behnken design | | Extraction yield | 18 |

| | | | | | | |
|---|---|---|---|---|---|---|
| RP- HPLC-UV | 15 phenolic compounds and caffeine in green tea, oolong tea, black tea and mate | Heat flux extraction | Central Composite Design | Extraction time, extraction temperature, Ethanol concentration | Extraction yield | 19 |
| UV, HPLC- UV | ondansetron, dexamethasone and aprepitant in new organogel formulation | | Partial Least Squares Regression model | | Quantification | 20 |
| RP- HPLC | Separation of six alkaloids in Huanglian | | QbD- Plackett Burman design to identify HPLC parameters, Box-Behnken design for optimization | Acetonitrile, concentration of sodium dodecyl sulfate, and concentration of potassium phosphate monobasic, | | 21 |
| HPLC- UV | Levothyroxine in human urine and serum samples | Ultrasound assisted-dispersive solid liquid microextraction based on solidification of floating organic droplet | RSM | pH, volume of extraction solvent, amount of solid disperser, ionic strength and time of ultra-sonication | Extraction effiency | 22 |
| UV- Visible spectroscopy | Polyphenols from different seeds including basil seed, red seed, Sesame seeds and ajwan seeds | | RSM | Concentration and volume of methanol and NaOH solutions as well as the temperature and time of extraction | Phenolic extraction efficiency | 23 |
| HPLC-MS/MS | R- and S-ibuprofen (IBU) in human plasma | LLE, SPE | LLE- Full Factorial 32 design, SPE- D-Optimal Design | | Assay% | 24 |

| | | | | | | |
|---|---|---|---|---|---|---|
| UV | Degradation of tolfenamic acid(TA), a common anti-inflammatory drug used in both human and veterinary medicine | | 23 factorial design with added center point | H2O2 concentration, Tolfenamic acid concentration and experiment time | Tolfenamic acid degradation and H2O2 photolysis | 25 |
| HPLC | Wedelolactone from Wedelia calendulacea Less | Supercritical Fluid assisted Extraction | Taguchi experimental design | Operating pressure, temperature, modifier concentration and extraction time | Extraction yield | 26 |
| UV-spectrophotometry | Pregabalin content in bulk and in capsule dosage | | | | Assay% | 27 |
| Pseudo-stationary phase micellar liquid chromatography | Simultaneous isocratic isolation of hydrochlorothiazide, triamterene and losartan potassium | | Central Composite Design | Concentration of sodium dodecyl sulphate (SDS), as well as volume percentages of ACN, DES, and ACA | Chromatographic behavior of drugs | 28 |
| RP-HPLC-PDA | Zileuton racemate in bulk and in tablet | | Central Composite Design | Methanol content, flow rate, concentration of orthophosphoric acid | Assay% | 29 |
| Stability indicating HPLC | Luliconazole in bulk and cream formulation | | Experimental design | | | 30 |
| HPLC | Voriconazole in pharmaceutical dosage form | | Experimental design | Percentage of acetonitrile in mobile phase, flow rate and pH | Robustness | 31 |

| | | | | | | |
|---|---|---|---|---|---|---|
| HPLC | UV filters determination using 1-hexyl-3-methylimidazolium hexafluorophosphate as extractant | In-syringe magnetic stirring assisted dispersive liquid–liquid microextraction | Multivariate optimization | Quantity of extraction and dispersive solvents, extraction and sedimentation time, ionic strength and pH | Extraction efficiency | 32 |
| | | | Partial least squares (PLS) | | | |
| HPLC | Metacrate in water samples | Dispersiveliquid–liquidmicro extraction (DLLME) | Central Composite design | Volume of extraction solvent and disperser solvent, salt effect, sample volume, and extraction time in the DLLME | Extraction efficiency | 33 |
| HPLC- UV | Cyanidin chloride and pelargonidin chloride anthocyanins in cherry, sour cherry, pomegranate and barberry | Magnetic solid phase extraction | Central Composite design | | | 34 |

## CONCLUSION

The systematic approach of analyzing drugs either as single or multicomponents in different pharmaceutical dosage forms by different analytical techniques using chemometrics will accelerate the research including extraction of different natural constituents. Quality, Cost and Time being three significant parameters could be effectively monitored to achieve optimum output (Assay, Extraction yield etc).

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