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# Analytical method development by using QbD - An emerging approach for robust analytical method development

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**Abstract:**

Quality by Design (QbD) is a methodology of Pharmaceutical development, recommended by regulatory agencies like USFDA. It has gained more importance in recent times due to the rise in the number of quality issues in pharmaceutical products. QbD helps in building the quality of products by design through risk assessment at the early stage and defining the design space at the later stage. QbD based product development enables the understanding of additional formulation aspects by using a scientific approach and quality risk management. QbD based product development also provides additional assurance to regulatory agencies. The analytical methods which are used for testing of Pharmaceutical drug products are equally important and any design-related issue in the analytical method may create a quality risk for the patients. Even though there is no specific guideline from regulatory agencies on Analytical Quality by design (AQbD), extensive work has been done on this front in the recent past. Application of AQbD in method development aids in ensuring the robustness of the method. This article elaborates on the key elements of Analytical Quality by Design (AQbD) such as the Quality target method profile (QTMP), understanding the critical method parameters (CMP), performing design of experiments (DoE), establishing method sensitivities and control strategies. The analytical methods, developed based on the QbD concept are more robust and reduce the number of Out of trend (OOT) and Out of specification (OOS) results during the actual usage in quality control.

**Keywords:** AQbD, Method development, DoE, Pharmaceutical development, Control strategy

## INTRODUCTION

Quality, safety, and efficacy of pharmaceutical products have been the prime focus for regulatory agencies such as the United States food and drug administration (USFDA), and Medicines and Healthcare Products Regulatory Agency (MHRA). The recent recalls and warning letters have amplified the surmise on the quality of the drug products and resulted in a higher level of scrutiny by the regulators. Various guidelines (Q8, Q9, Q10, Q11, and Q12) have been introduced by ICH on the implementation of Quality by design (QbD) and PAT tools [1]. The quality of the pharmaceutical products can not solely be controlled by testing, instead it is expected to be built in by design. As per ICH guideline, Pharmaceutical Development Q8 (R2), "Pharmaceutical development is aimed at designing a quality product and its manufacturing process to consistently deliver the intended performance of the product. The information and knowledge gained during the product development give scientific understanding to define the design space, specifications, and manufacturing controls" [2].

QbD is an expectation from regulatory agencies to increase process and product understanding and thereby decreasing the risk for patients. From a manufacturer's perspective, it gives a better understanding of the product/process, and reduced regulatory burden. It gives regulatory flexibility to the regulators without sacrificing quality and to the patients, it gives increased assurance of product quality. Hence QbD implementation is a win-win-win situation for manufacturers, regulatory agencies, and patients.

Analytical testing is one of the important aspects of pharmaceutical development. Having the right analytical method is vital in ensuring the quality of the drugs. Various analytical techniques are used to test the physical, chemical, and biological parameters of the subjected pharmaceutical product. Chromatographic techniques (HPLC, UHPLC, etc.) are the most widely used techniques in the pharmaceutical industry due to its advantages over the other techniques. The key challenge in front of the analytical chemist is to develop a robust and rugged analytical method with optimum separation with shorter run time. The traditional approach for analytical method development is based on 'trial and error'. In this approach analytical chemist optimizes one factor at a time by using his prior knowledge. This approach may result in getting stable method conditions but these may not the optimal conditions. The methods developed based on a traditional approach may have robustness related issues.

Another approach for analytical method development is based on quality by design. It is based on sound scientific knowledge and starts with defining the separation goals, performing the risk assessment, conducting the design of experiments, and defining the MODR and control strategy. There are no specific guidelines on QbD based analytical method development, however, there are multiple methods reported that are developed based on the QbD principle [3-18]. The reported analytical methods utilized QbD application for various objectives such as method development, method optimization, robustness studies, etc. There are few review articles published on Analytical Quality by design [19-26]. Every author has represented

the analytical quality by design in his unique way however there is no uniformity in the terminology used for Analytical Quality by design (AQbD) elements. The current review article summarizes the basics of AQbD, various elements of AQbD, regulatory perspective on AQbD, implementation of AQbD in analytical method development for a generic product, in a much simpler way.

**REGULATORY ASPECTS TO QBD**

As per ICH Q8 (R), Step 2 "QbD is A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". The key expectation from regulatory agencies is to design a quality product by using the manufacturing process which consistently delivers the intended performance of the product. The regulatory agency expects that aspects of drug substances, excipients, container closure systems, and manufacturing processes that are critical to product quality should be determined and control strategies should be defined. Critical formulation attributes and process parameters should be identified through an assessment of the extent to which their variation can have an impact on the quality of the drug product. The information and knowledge gained during pharmaceutical development studies and manufacturing experience should provide scientific understanding to support the establishment of the design space, specifications, and manufacturing controls. Information from pharmaceutical development studies should be the basis for quality risk management. It is important to recognize that quality cannot be tested into products; i.e., quality should be built in by design. Changes in formulation and manufacturing processes during development and lifecycle management should be looked upon as opportunities to gain additional knowledge and further support the establishment of the design space [2].

Similarly, the inclusion of relevant knowledge gained from experiments giving unexpected results can also be useful. The design space proposed by the applicant is assessed by the regulatory agency and post-approval of the proposed design space, working within the design space is not considered as a change. Even though ICH Q8(R) does not mention explicitly about implementation about QbD in the analytical method, however, the basic concept of QbD can be extrapolated to analytical method development as well. Defining the analytical method profile, finding the critical method parameters, establishing the design space, and putting the right control strategy could be considered the key elements of AQbD in parallel to formulation QbD. FDA has also approved some NDA applications applying the QbD approach to analytical methods. Regulatory flexibility has been granted for movements within the defined analytical method "Design Space".

**ANALYTICAL QUALITY BY DESIGN (AQBD)**

Analytical Quality by Design (AQbD) is a systematic approach to design the methods that start with defining the separation goals and target method profile (Figure-1).

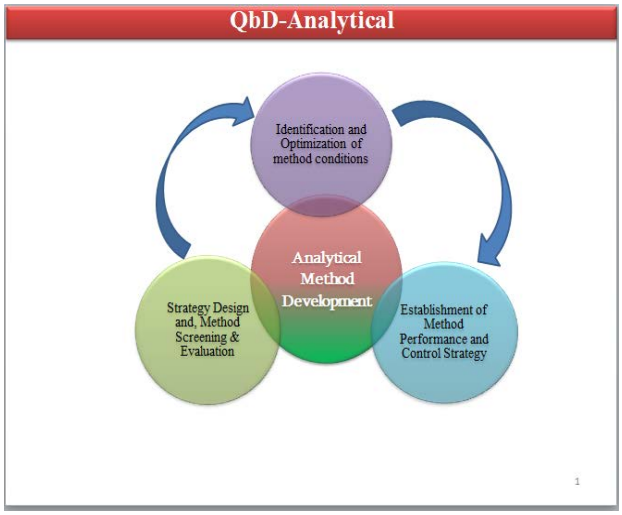


**Figure-1:** AQbD overview

Understanding of method parameters and controls, based on sound science and quality risk management are the key focus areas in AQbD. AQbD is also an integral part of the product development control strategy along with other parameters such as process parameters, material attributes, equipment operating conditions, in-process controls, and finished product specifications. Regulatory agencies do not define any specific process of AQbD, however, a parallel approach can be drawn based on product QbD e.g. Quality target product profile (QTTP) can be inferred as Quality target method profile (QTMP), CQA can be interpreted as critical quality attributes such as tailing factor, the resolution between adjacent peaks, and plate count, etc. Design space can be called method operable design range (MODR) [27, 28].

In AQbD, critical method parameters (CMP) are defined based on the technique involved and the method intent. Risk assessment is done based on prior knowledge, to shortlist the CMPs. Design of Experiment (DoE) is used to optimize the CMPs. DoE helps in understanding the interactions among the input variables and their effect on selected responses (Figure-2). AQbD paradigm is a preferred and recommended strategy to be followed in analytical method development to attain regulatory flexibility and to reduce Out of specification (OOS) and Out of trend (OOT) results.

**Elements of AQbD**

**Critical Quality Attributes (CQA)**

CQAs are the parameters which influence the method performance and can impact the results. CQAs are selected based on the techniques used (e.g. High performance liquid chromatography, and Gas chromatography) and the method intent (e.g. Assay, impurity estimation, drug release determination). Tailing factor, plate counts, % relative standard deviation of replicate injections of the reference standard, and extraction efficiency (% recovery) are the CQAs for the assay determination method. In addition to these CQAs, the resolution between adjacent peaks could be an additional CQA for the impurity estimation method.

**Quality target method profile (QTMP)**

The quality target method profile is the target profile of CQAs, which is decided based on the intended use of the method and regulatory requirements. Pharmaceutical products are analyzed to ensure that the product meets its intended performance. Product performance comprises of drug safety and efficacy. To assess the drug efficacy, usually, pharmaceutical products are tested for assay and drug release. Similarly for safety assessment, impurities are estimated in pharmaceutical products. Hence while developing the analytical method, the most common goals are assay estimation, determination of drug release, and quantification of impurities in pharmaceutical products. A typical example of QTMP for the different methods is given in Table-1.

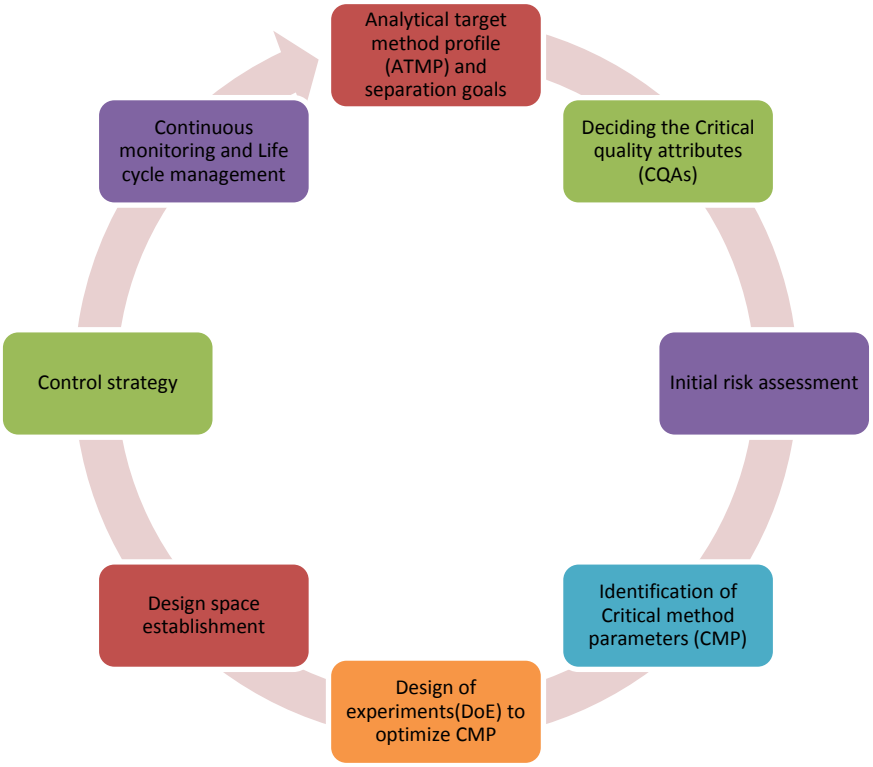


**Figure-2:** Analytical Quality by design (AQbD) elements

Table-1 **Quality target method profile**

| **Test** | **Critical quality attribute** | **Regulatory Requirement** | **Quality target method profile** |
|---|---|---|---|
| Assay method | Tailing Factor | NMT 2.0 | NMT 1.5 |
| | %RSD[1] | NMT 2.0 | NMT 2.0 |
| | Plate Counts | NLT 2000 | NLT 4000 |
| | Recovery | 97.0% to 103.0% | 97.0 % to 103.0 % |
| | Run time | - | < 10 Minutes |
| Drug release method | Tailing Factor | NMT 2.0 | NMT 1.5 |
| | %RSD[1] | NMT 2.0 | NMT 2.0 |
| | Plate Counts | NLT 2000 | NLT 4000 |
| | Recovery | 95.0 % to 105.0 % | 95.0 % to 105.0 % |
| | Run time | - | < 7 Minutes |
| Impurity estimation method | Tailing Factor | NMT 1.5 | NMT 1.5 |
| | %RSD[1] | NMT 10.0 | NMT 10.0 |
| | Plate Counts | NLT 2000 | NLT 4000 |
| | Resolution | NLT 1.5 | NLT 2.0 |
| | Recovery | 85.0 % to 115.0 % | 85.0 % to 115.0 % |
| | Run time | - | < 30 Minutes |

[1] % Relative standard deviation of peak area from five replicate injections of reference standard

Table-2 **Categorization of Critical method parameters (CMP)**

| S.No. | Category of CMP | CMP |
|---|---|---|
| 1. | Material attributes | Make and grade of reagents used for analysis e.g. buffers and ion pair reagents used in mobile phase preparation<br>Quality of reference standard e.g. purity of standard<br>HPLC columns of various lots<br>Type of glassware used for analysis e.g. amber coloured or clear<br>Type of filters used for sample filtration |
| 2. | Instrument related aspects | Dimensions and stationary phase of HPLC column<br>Different HPLC detectors e.g. UV/PDA<br>Make of HPLC e.g. Agilent, Waters<br>HPLC system configuration e.g. diameter of tubing and size of injector loop |
| 3. | Instrument operating parameters | Column flow, column oven temperature, gradient program, detection wavelength, detector sampling rate, needle wash after injection |
| 4. | Method parameter | pH of buffer, concentration of buffer, organic modifier in mobile phase, diluent for sample preparation, sonication time |

Table-3 **Critical Method parameters for HPLC, GC and TLC methods**

| S.No. | Critical Method parameters | | |
|---|---|---|---|
| | **HPLC method** | **GC method** | **TLC method** |
| **1** | HPLC Column (dimensions, stationary phase, make, ageing) | GC Column (dimensions, stationary phase, make, ageing) | TLC plate stationary phase and coating thickness |
| **2** | Column Flow | Column Flow | Development distance |
| **3** | Column oven temperature | Column oven temperature | Temperature of solvent mixture (mobile phase) |
| **4** | Buffer for mobile phase | Carrier gas e.g. Hydrogen, Nitrogen | Composition of solvent mixture |
| **5** | Buffer concentration | Split flow | pH of solvent mixture |
| **6** | Concentration of additives (ion pair etc.) | Oven temperature program | Volume of sample solution spotted |
| **7** | pH of mobile phase buffer | Injector temperature | Size and shape of spot |
| **8** | Mobile phase gradient | Detector temperature | Drying time and conditions of TLC plate |
| **9** | Organic modifier in mobile phase | Type of injector liner | Technique used for visualizing the spot e.g. by spraying reagent, detection under UV light |

Table-4 **Cause effect relationship of CMP and CQA**

| S.No. | CMP | CQA |
|---|---|---|
| **1** | Column flow rate, pH of mobile phase buffer, concentration of organic modifier in mobile phase, column oven temperature | Retention time , Tailing factor and plate counts |
| **2** | pH of mobile phase buffer, organic modifier and its concentration in mobile phase, gradient program, column stationary phase, dimension of HPLC column | Resolution between adjacent peaks |
| **3** | Diluent, sample extraction methodology i.e. shaking or sonication, shaking/sonication time, temperature during sonication | Drug recovery from sample matrix |

Table-5 **Full factorial and fractional factorial designs**

| **Full Factorial design** | | | |
|---|---|---|---|
| | **Factor-1** | **Factor-2** | **Factor-3** |
| **Run-1** | L | L | L |
| **Run-2** | L | L | H |
| **Run-3** | L | H | L |
| **Run-4** | L | H | H |
| **Run-5** | H | L | L |
| **Run-6** | H | L | H |
| **Run-7** | H | H | L |
| **Run-8** | H | H | H |
| **Fractional Factorial design** | | | |
| | **Factor-1** | **Factor-2** | **Factor-3** |
| **Run-1** | **L** | **L** | **H** |
| **Run-2** | **L** | **H** | **L** |
| **Run-3** | **H** | **L** | **L** |
| **Run-4** | H | H | H |

**Critical method parameters (CMP) and Risk assessment**

Critical method parameters are the sensitivities associated with the analytical method. CMP has a cause-effect relationship with CQA and can impact the defined CQAs. CMP can be categorized into multiple categories such as material attributes, instrument-related CMP, operating parameters of instrument, and other method parameters. An example of typical CMPs of a HPLC method is given in Table-2.

Critical method parameters (CMP) can be classified based on the technique also (High-performance liquid chromatography, Gas chromatography, and Thin layer chromatography, etc.). For a HPLC method, pH of the mobile phase, organic modifier in the mobile phase, and column oven temperature are the critical method parameters whereas for a Gas chromatography (GC) method, injector temperature, detector temperature, type of carrier gas, and split ratio could be the critical method parameters. Categorization of CMP based on technique is given in Table-3.

Critical method parameters have a direct relation with CQA (Table-4). For a HPLC method, the column aging (CMP) can impact the tailing factor and plate counts (CQA). Similarly, during sample preparation, sonication time (CMP) has an impact on drug extraction efficiency (CQA). After finalization of CMP and CQA, risk assessment is performed based on prior knowledge to shortlist the CMPs for further evaluation through Design of experiments (DoE).

**Design of Experiments (DoE)**

Design of experiments (DoE) is a series of tests, in which changes are made to input factors so that the causes for significant changes in the output responses can be identified. DoE is a statistics optimization tool, which helps in achieving a predictive knowledge of a complex, multivariable process with the fewest trails possible. Key steps of DoE are summarized in Figure-3.

**Selection of input variables and responses**

Based on initial risk assessment, CMPs are shortlisted and are subjected to the Design of experiments (DoE) for further optimization. CMPs are input variables (factors) in DoE and could be qualitative or quantitative in nature. Qualitative variables are different columns, the grade of the buffer, and ion pair reagent for the mobile phase. Quantitative variables are column flow rate, column oven temperature, and concentration of organic modifier in the mobile phase, etc. After the selection of input variables, responses are finalized. Critical quality attributes (CQA) are the responses in DoE. Again, the response could be quantitative or qualitative in nature. A qualitative response could be acceptable/not acceptable (1/0) e.g. when the impact of the grade of buffer in the mobile phase on the interference at the retention time of analyte peak, is studied, the response would be either Yes or No. Quantitative responses are resolution between adjacent peaks, tailing factor, recovery of drug from the sample matrix, etc.

The selection of levels for the input variables is the next step in DoE. Levels are selected based on the normal operating ranges (NOR) of the selected variables e.g. for column flow rate in HPLC, NOR is ±2%, hence the levels selected for DoE for column flow rate should be broader than ±2% (e.g. ±10%) from the centre point. Usually, 3 levels (including center point) are selected for DoE.

**Selection of design**

The selection of design is an important aspect of DoE and is made based on the purpose of DoE. Screening DoE is used to find out the most critical variables among multiple variables. More variables can be studied by using this category of designs and only the main effects can be understood e.g. Fractional factorial and Placket-burman design. Advanced screening designs are used to study the main effects and interactions among variables e.g. Full factorial design. Optimization designs are used to optimize critical variables e.g. Full factorial, Box Behanken, and central composite designs [29].

**Factorial Designs**

Factorial designs are of two types i.e. full factorial and fractional factorial design.

**Full factorial design**

All the possible combinations of all the levels for factors (2 or more) are considered in a full factorial design. This is the simplest design to create but highly inefficient. Key disadvantages are high cost, materials, and resources

$r= a^b$

Where r is the number of runs, a is the number of conditions and b is the number of input factors.

For 3 input factors, the total number of runs will be 8. (Table-5)

**Fractional Factorial design**

In a fractional factorial design, a fraction of experimental runs from full factorial design is chosen. This is a more efficient design but there is a risk of missing interaction between input factors. For 3 input factors, the total number of runs will be 4. (Table-5)

**Placket burman design**

Placket burman designs belong to class resolution III. These designs are used to understand the main effects and are considered to be economical. These are also useful for eliminating insignificant factors and selecting critical factors for full factorial or response surface designs. The interaction of factors cannot be estimated by using these designs. Placket burman design is a good alternative to fractional factorial design for screening purposes. The number of runs from this design will always be not more than the number of factors + 4.

**Response surface designs**

Response surface designs use regression analysis to calculate a system model, to test its validity, and to analyze the model. These designs are most suitable for optimization for critical factors, selected based on screening experiments. There are two types of response surface designs i.e. *central composite design* and *box-behanken designs*

**Central composite designs (CCD)**

CCD models the response surface precisely by studying 5 levels for each input factor. These designs can be built as an extension of the full factorial design and by using these designs, we can study center points, axial points and can

estimate pure quadratic effects also. Central composite designs are of two types; uniform precision and orthogonal.

**Box-behanken designs:**

Box-behanken designs are constructed by combining two-level factorial designs with incomplete block designs. Even though these designs have complex confounding of interactions, still these are economical designs and used where experimentation is very expensive.

After design selection, the design is constructed by using the input factors and responses, and experimental runs are performed. Data is collected from each experimental run and is evaluated by using statistical tools to find out the method operable design range (MODR).

**Method Operable Design Range (MODR)**

Method Operable design range is the design space in which the analytical method is expected to meet the defined Quality target method profile (QTMP). It helps in identifying critical method variables and their optimal ranges, where a robust region for the critical method parameters could be obtained. MODR should always be broader than Normal operating ranges (NOR) to ensure the robustness of the method.

**Control Strategy**

Based on Method Operable design range and method sensitivities found during DoE studies; control strategies are defined. The control strategy can be in form of system suitability, specifying any grade of reagents used for analysis or any other specific precaution to be followed during analysis. Few examples of control strategy are given below

- Based on DoE, if the separation between two adjacent peaks is found to be critical, then resolution criteria between these two peaks should be added as part of system suitability for routine analysis.
- Similarly, if the grade of reagent/chemical used for analysis is critical for chromatographic separation, then the most suitable make/grade of reagent should be specified in the standard testing procedure.
- For sample preparation, a sonicator is used to extract the drug from the sample matrix. Few drugs are susceptible to heat stress and temperature during sonication could be the critical method parameter. Hence precaution related to control of temperature during sonication shall be incorporated in the standard test procedure.

Basically, all the knowledge gained during various steps of QbD such as risk assessment, design of experiments, and method operable design range; is utilized to finalize the control strategy. It helps in putting necessary controls in the analytical method to avoid failures during analysis.

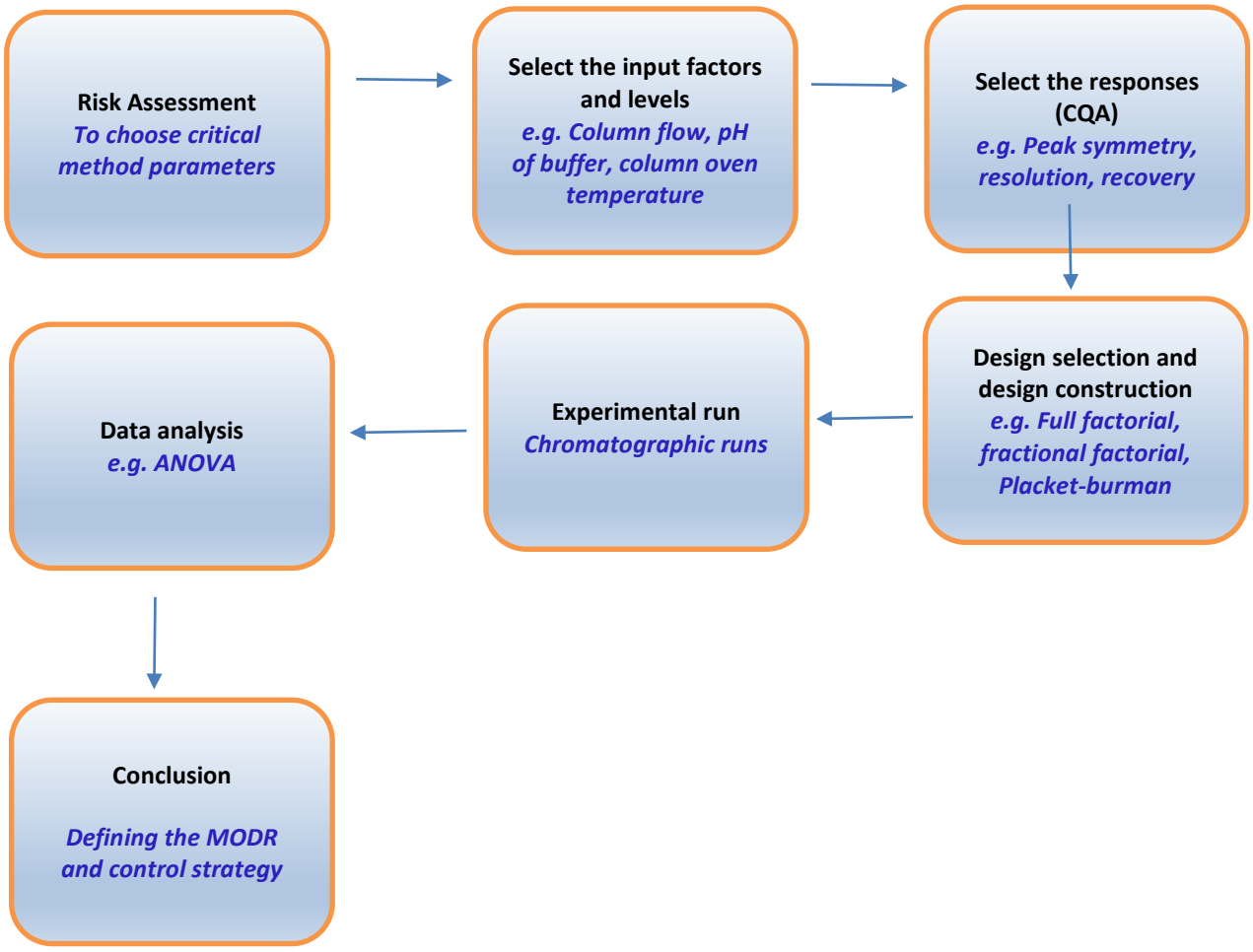


**Figure-3:** Different steps in Design of experiment (DoE)

**Method validation**

A finalized analytical method can be taken for method validation. Analytical method validation is performed based on ICH guidelines on Analytical method validation [30] to demonstrate that the developed analytical method fits for the purpose. Specificity, precision, accuracy, linearity, ruggedness, robustness, ranges, and limit of detection/limit of quantitation are the parameters, usually performed during method validation. Method validation parameters are selected based on method intent e.g. for testing for the impurities-limit test, specificity and detection limit are adequate whereas, in case of testing for the impurity-quantitative test, all the above-listed parameters are required. For the assay estimation method, detection limit and quantitation limit tests are not required. After successful validation, the analytical method can be implemented for regular analysis of Pharmaceutical products.

**Continuous monitoring and life cycle management**

After successful method validation, the analytical method is implemented in the quality control lab for routine testing. During this process, certain challenges may arise due to differences in the operating environment, the model of instruments, etc. These challenges need to be looked very carefully and accordingly, adjustments should be made in method conditions within a method operable design range. It is also expected that the analytical method may need some changes or improvements during the product life cycle due to continuous improvement, unplanned deviations, and operating in a different environment. As a part of continuous improvement, the performance of the analytical method is monitored by doing a trending of incidents, out of specification (OOS), and out of trend (OOT) occurred during a specified time. Based on the trending, if a trend emerges which indicates a specific concern in the analytical method, it is relooked and necessary improvements are done. If the improvements/changes are within the defined method operable range, it will not call for any additional method validation but when changes are beyond the MODR, these need to be validated appropriately. Sometimes based on the outcome of the investigation of out of trend or out of specification, the identified root cause is related to the analytical method. In such cases, necessary precautions are added in the standard testing procedure to prevent the reoccurrence of a similar incident.

## DIFFERENCES BETWEEN THE CONVENTIONAL APPROACH AND QBD

QbD is a methodical approach to method development that is driven by deep scientific knowledge. It helps in building the robustness in the analytical method by design. Method intent and critical quality attributes are defined clearly. Each parameter of the analytical method is chosen based on scientific understanding. Risk assessment helps in identifying the critical method parameters which are further optimized by using the Design of experiments (DoE). Method operable design ranges are established based on DoE. AQbD also helps in identifying the method sensitivities which are controlled through control strategy.

In the conventional approach, usually, methods are developed based on the “trial and error” approach. During method development with a conventional approach, all the aspects are not studied in a systematic way hence there is a possibility that some of the key information (e.g. method sensitivities) may be missed. Due to which, adequate controls cannot be put to avoid the failures. The number of experiments, to reach the final conditions are also higher as compared to the QbD approach. The conditions finalized by using the conventional approach may be stable but not optimal.

## KEY BENEFITS OF QBD IN ANALYTICAL METHOD DEVELOPMENT

The key benefits of method development by using Analytical Quality by design approach are given below

- A systematic approach to method development.
- It is helpful in building insights into the critical attributes of the analytical method.
- DoE reduces the number of experiments to reach optimal conditions.
- Ensures the method robustness by design.
- It helps in reducing the Out of specification (OOS) and Out of trend (OOT) results during analysis
- It reduces the costly and time consuming investigations.
- It helps in eliminating batch failures due to analytical method variations.
- Avoid deficiencies from regulatory agencies.
- Adjustments within “Design Space” are not considered a change in method.
- It helps in gaining regulatory flexibility.
- Enhanced assurance on quality.

## CONCLUSION

QbD is a scientific approach, being extensively used in the pharmaceutical industry for product development because it reduces product variability and risks associated. AQbD is also emerging gradually and a lot more analytical methods are being developed now based on the QbD principle. The AQbD comprises defining the target method profile and critical quality attributes; performing risk assessment and optimization of critical method parameters by using DoE. MODR and control strategy is defined based on the outcome of the DoE. Moreover, it helps in gaining regulatory flexibility. The most common benefit is methods are more robust and rugged, and thus sustain through the challenges of long-term usage in the product life cycle.

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