RESEARCH ARTICLE

Analytical Quality by Design (AQBD) Approach for HPLC Method Development, Method Optimization and Validation

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ABSTRACT

Two approaches to develop an analytical method are "one factor a time (OFAT)" and "analytical quality by design (AQbD)". In OFAT approach, only one parameter can be optimized at a time this have chances of method failure. Our aims is to give insight to the analytical method development and validation by AQbD approach. The present review discusses usefulness of AQbD tools in the high performance liquid chromatography (HPLC) method development, method optimization and validation. HPLC method development by using AQbD tools involves establishment of general analytical target profile (ATP) for analytical procedure, selection of critical quality attributes and risk assessment of the developed chromatographic method. This review gives information of various parameters of method validation like specificity, linearity, accuracy, precision, limit of detection (LoD), and limit of quantitation (LoQ), robustness and sensitivity as per International Council for Harmonization (ICH), United States Pharmacopoeia (USP) guidelines, and Food and Drug Administration (FDA).

Keywords: Analytical procedure, Critical quality attributes, Method optimization, Specificity, Risk assessment.

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INTRODUCTION

High pressure/performance liquid chromatography (HPPLC) is a form of column chromatography; used significantly in analytical chemistry.[1,2] It is widely used for qualitative and quantitative analysis of the components in a mixture, analysis of drugs and pharmaceutical products, and determining the stability of pharmaceutical products.[3,4] The basic components of a HPLC system are a column, required for holding packing material called stationary phase, a high-pressure pump needed for the movement of mobile phase through the column at a specified flow rate and a detector that detects the compound of interest and shows the retention time.[4,5]

In the year 1980, HPLC methods were used initially in the assay of bulk drug materials (USP, 1980) but nowadays, among the chromatographic techniques, HPLC is the highly used technique and approximately 80% industries over the world are using the HPLC method for analysis of drugs and drug products. Now, almost every pharmacopoeia is trying to change the monographs of Drugs and Drug products as per HPLC technique from titration.[6]

HPLC Method Development

Selection criteria for new method development.[7-9]

- Methods are developed for new products.
- A suitable analytical method for a drug is not found in the literature because of patent-related policies and regulations.
- Analytical procedure may not be available for a drug formulation due to the presence of excipients that may cause interference.
- Analytical procedure may not be available for the quantitative analysis of the drug in biological fluids.
- The existing analytical procedures are expensive due to the use of costly reagents and solvents.
- The existing procedure may include massive extraction and separation procedures, which may not be dependable.
- Alternate method for existing (Non-Pharmacopoeial) products is to lower the expense and time and for improving precision and robustness.

For the replacement of existing method with proposed method comparative laboratory data including pros and cons are made available.

The objective of the HPLC method is the separation and quantification of main active drug, reaction impurities, synthetic intermediates and degradent present.[10]

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ANALYTICAL QUALITY BY DESIGN (AQbD) APPROACH FOR METHOD DEVELOPMENT AND VALIDATION

These are developed at each stage of the product life considering the designated purpose and utility.[11,12]

The OFAT depends upon trial and error, where one parameter alone is optimized for the expected response while others remains constant, developing a narrow robust behavior of the method towards the industrial variables employed in method development phase.[13] Hence the design of analytical method i.e., OFAT development has ample chances of method failure and always needs revalidation step.[14]

Another approach is AQbD. According to ICH guidelines, QbD is a "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" (Table 1).[15]

Design of experiment (DoE) is a component of AQbD, and it shows the relationship among the input variables that eventually influence the method response and results. AQbD approach is a preferred and recommended planning to be followed in analytical method development to achieve regulatory flexibility and decrease Out of specification (OOS), Out of term (OOT) and Out of control (OOC) results.[13,14,16]

AQbD has different mechanisms during its life cycle such as ATP, CQA, risk assessment, method optimization and development with DoE, method operable design region (MODR), control strategy and risk assessment, AQbD method validation and continuous method monitoring (Figure 1).[14-18]

Method Design

Choosing or developing an ideal analytical method is the primary step in method lifecycle.[13] During an appropriate analysis method many elements must be considered. Method design by AQbD approach involves space generation, defining ATP, experimental design screening and establishing CQA's (Figure 1 and Table 1).[13-17]

Establish CQA's

To enhance the method performance, one needs to identify the Critical Quality Attributes for a particular method. To identify these parameters a deep knowledge of the method is required. This can be acquired by an extensive literature survey or by referring to found retrospective data of the analyte. CQA's are divided into three types, i.e., analyte parameters related, instrument-related parameters and parameters related to operation conditions. CQA's for chromatographic analysis are sampling, sample preparation, standards, reagents, column chemistry, mobile phase composition, pH, mobile phase flow rate, column temperature, detector and its selection etc. Responses for the above parameters would be resolution retention time, tailing factor detection limit and robustness etc.[23-25]

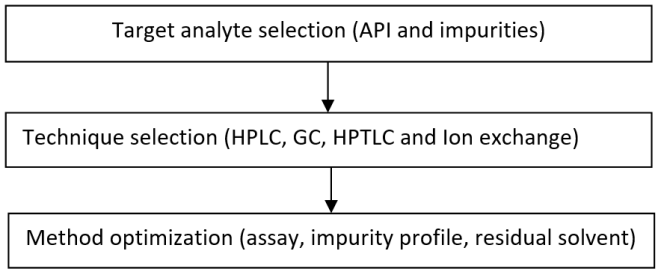

Figure 1: General ATP for Analytical Procedure.[15,19-22]

Perform Risk Assessment

Critical method parameters are evaluated and their risk on method performance is estimated. Risk analysis can be done by using various risk assessment tools such as Fishbone diagram and failure mode effect analysis (FMEA's).[26] Risk matrices are used to evaluate parameter risk with respect to relevant attributes (e.g., accuracy, precision, resolution, tailing).[25] To perform risk assessment study of the proposed analytical methods a deep understanding of physicochemical properties of analyte is needed.[13,27]

Generate Design Space

As per ICH guidelines, design space or method operable design region (MODR) is defined as "the multidimensional combination and interaction of input variables (material attributes) and process parameters that have been demonstrated to assure quality". The chromatographic method involves all parameters and their interactions evaluated by some statistical design.[28,29] This design combines varied ranges of mobile phase combination, pH, columns, flow rate etc., providing expected outcomes.[13,20,24,30] The optimized method parameters are found around this region. MODR ensures a robust and rugged area around the method conditions. The results of various trials for initial optimization must be noted and the best suitable combination is statistically evaluated.[13,30,31]

Using Design of Experiment for Method Screening and Optimization

- *Design of Experiment*

The Design of Experiment (DoE) approach was recently employed to develop and optimize the analytical methods.[39] Implementation of DoE approach provides a risk-based understanding of the analytical procedure and major pointers influencing the feasibility of the analytical method. Based on the Design of Experiment principles, it helps understand the plausible risk (s) and association interaction (s) among the method variables. DoE can be employed as multivariate optimization stratagem (Figure 2).[30,32-34]

- *Critical Factors as Independent Variables and their Selection*

Factors affecting the chromatographic analytical method can be considered operational or environmental factors. Operational factors are decided based on the operating procedure adopted during chromatographic analysis, but

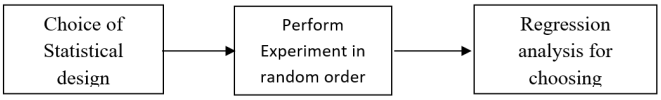

Figure 2: Showing steps involved in DoE as Multivariate Optimization Stratagem

the environmental factors are not necessarily mentioned clearly in the analytical procedure. The selected factors can be quantitative (continuous), qualitative (discrete) or mixed factors. Qualitative and/or quantitative factors influencing the end results of the analysis are called critical factors or independent variables.[30,36,37]

- *Categorical Variables:* or qualitative variables are those variables that cannot be measured numerically. eg. type of columns eg. C8, C18, phenyl and cyano columns.[30,38]
- *Continuous Variables:* These are also called as quantitative variables. These variables can be measured, e.g. type of buffers, nature of buffers, pH, temperature and buffer concentration. These variables can be controlled.[30,38]

Design of experiment approach for method optimization has various advantages such as factors and interactions assessment with maximum efficiency, conclusions applied to various conditions and maximum use of the data for calculating main effects and interactions. Most common experimental designs applied to chromatographic techniques are full factorial, fractional factorial, Plackett- Burman and Box- Behnken design.[13,30,40]

Control Strategy Design/Life Cycle Management

Control strategy is a planned controls, derived through analyte nature and MODR understanding. The method control strategy is based on the complete statistical data sourced during the DoE and MODR stages. This strategy can resolve the method parameters with inconsistency (e.g., reagent grade, instrument brand or type and column type).[41-43]

AQbD Method Validation

Method validation for all kinds of API manufacturing changes without revalidation. The AQbD tools define the required ICH validation elements statements on interactions, measurement uncertainty, control strategy and continuous improvement.[15,44,45]

Continuous Method Monitoring and Continual Improvement

CMM is the last step in AQbD life cycle, and a continuous process to learn during design space development and implementation. It consists of results of risk assessments, pre-concieved assumptions, statistical design considerations, and a link connecting the design space, MODR, control strategy, CQA and ATP. After method validation, method may help in routine purpose and continuous method performance may be observed. CMM permits the analyst to recognize and address any out-of-trend performance,[46-49] by themselves (Table 1).

STEPS INVOLVED IN METHOD DEVELOPMENT[50]

Physicochemical properties of drug molecule play very significant role in method development. These properties help an analyst to decide the solvent and mobile phase composition.[51-55] For method development following properties of drug molecule are taken into consideration (Figure 3).

Selection of Chromatographic Conditions

During initial method development, a set of initial conditions (detector, column, mobile phase) is selected to obtain the sample's first "scouting" chromatogram. Next step is to decide on developing either an isocratic or a gradient method. Column chemistry and column physics play a significant role concerning analyte during the development of the method (Figure 4).

For selecting chromatographic conditions, the first step is determining the analyte's polarity. This sets the basis for choosing the right solvent of either less or more polarity than analyte of under-determination. The stationary phase greatly affects capacity factor, selectivity, efficiency and elution.[46,56]

Non Polar Moderately Polar Polar

C18<C8<C6 Phenyl<Amino Cyano<Silica

Several types of matrices for stationary phase are used like silica, alumina and polymers. Out of this silica is used most commonly.[57]

Selection of Column

The column selection depends on five fundamental parameters: hydrophobicity, stearic activity, ion exchange, silanolol activity, carbon load and hydrogen bonding.[31,58-62]

Column Physics

Chromatographic column parameters such as column bed dimensions, particle size, surface area and pore size, bonding type, base material, particle shape and end-capping need to

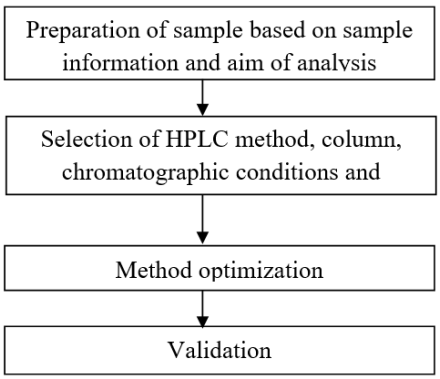

Figure 3: An illustrative image showing steps involved in method development 3.1 understanding physicochemical properties of drug molecule

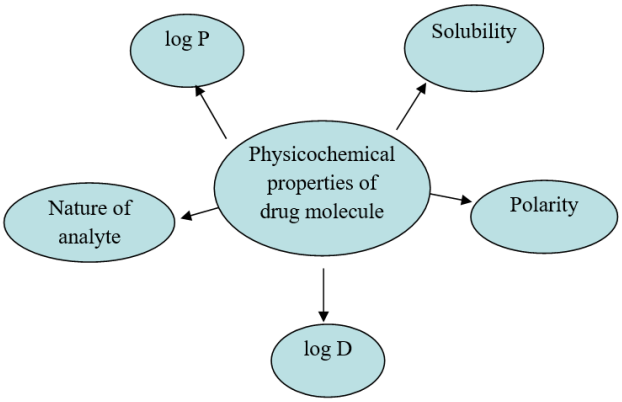

Figure 4: Physicochemical properties of the drug affecting HPLC method Development

Table 1: Validation Parameters Recommended by FDA, USP and ICH[7,46,78-79]

Necessary Validation Parameters	*ICH Guidelines*	*USP 23*	*Recommendation*
Selectivity (Specificity): It is defined as the ability to differentiate and quantify analyte in other components' presence.	Yes	Yes	No interference
Linearity: A linearity study for analytical technique verifies the response which is linearly proportional to the analyte concentration in the range of sample solutions. It is determined by a series of 5-6 injections of 5 or more standards whose concentration range 80-120% of the expected concentration [80,81].	Yes	Yes	Correlation coefficient (r^2) >0.998
Range: It is the interval amid the upper and lower concentration values of drug active compounds [80,81].	Yes	Yes	**Assay of drug/Finished product:** 80-120% of test conc. **For content uniformity:** 70–130% of test concentration. **For dissolution testing:** ± 20% over specified range **For impurity:** From reporting level to 120% of specification
Accuracy: The closeness of agreement between a measured quantity value and a true quantity value [80,81,82,84].	Yes	Yes	Recovery 98- 102% with 50, 100, 150%
LOQ: The lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ= 10 σ/s s = Slope of calibration curve σ = S.D. of response [83,**85, 86]**	Yes	Only for quantitative studies	Determination of signal- to- noise (S/N) ratio (10:1) S/N> 10
LOD: It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitiated as an exact value. LOD = 3.3 σ/s s = Slope of calibration curve σ = S.D. of response [83,85,87]	Yes	For limited test only	LOD can be based on a signal- to- noise (S/N) RATIO (3:1) S/N > 2 or 3
Precision: The degree of an agreement between a series of measurements obtained from multiple sampling of the same homogenous samples under the prescribed conditions. It depends not only on the distribution of random errors and does not relate to the true value. Precision usually expressed as standard or relative standard deviation of the replicate analysis. Precision may be considered at three levels: Repeatability, intermediate precision and reproducibility [88,89,90]	Yes	Yes Not	The **repeatability** criteria for an analytical assay method will be 1% (RSD). A precision criteria for an assay method is that the intermediate precision will be 2.0%. However for any impurity or trace amount assay, this precision will be 5.0%. A precision criterion for an assay method is the reproducibility will be 2.0%. For an impurity or trace amount assay, this precision parameter can be 10.0%.
Robustness: The reproducibility of experimental results when the method is performed under actual used conditions. This includes different laboratories, instruments and analysts, sources of reagents, chemicals, solvents and different elapsed assay times and so on. These experiments and results are similar within reproducibility [91].	Recommended	Yes	RSD < 2%
Ruggedness: It is the value below which the absolute difference between two single tests results obtained by the same method on investigated test compound under different conditions such as different analyst, different equipments, different laboratories etc. [92].	Recommended	Yes	RSD< 2%
Solution Stability [93]	Not specified	Recommended	>24 h or >12 h
Sensitivity: It is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The specificity of a test method is determined by comparing test results from an analysis of samples containing impurities, degradation products, or placebo ingredients with those obtained from an analysis of samples without impurities [92,93]	Recommended	Not specified	No interference

be considered while choosing a column (stationary phase) for separation of assay, impurities, and degradation products.[31,64]

Selection of Chromatographic Mode

The selection of isocratic or gradient mode depends on the number of active components to be resolved. An initial gradient run is performed to conclude the choice of isocratic or gradient, and the ratio between total gradient time and the difference in first and last gradient time components are noted. If calculated ratio is < 0.25, isocratic mode is sufficient, whereas if ratio is > 0.25, gradient mode is beneficial.[64,65]

Optimization of Mobile Phase

- *Selection of Solvent (Methanol, Acetonitrile, THF):* Choice between methanol and acetonitrile may depend on the analyte's solubility and buffer used. THF is the least polar among three solvents, responsible for large changes in selectivity and compatible with low wavelength detection. The mobile phase and gradient conditions' selection relies on ionogenic nature and the hydrophobicity of analyte.[66,67]
- *Buffer Selection:* The selection of buffer helps in deciding peak symmetries and peak separation. The correct choice of buffer reduces the tailing factor for each peak separately. The buffer strength from 0.05 to 0.20M. Buffer has a pKa close to the expected pH because buffer controls pH best at their pKa. Selecting a buffer with pKa value < 2 units of the expected mobile phase is ideal.
- *Effect of pH:* pH helps to achieve chromatographic separations as it controls elution properties through ionization characteristics.

pH of buffer and mobile phase selection based on the pKa of analyte or test mixture and structure of drug molecule pH.[68]

If pKa of the compound is high, lower pH or acidic mobile phase is choosen. For basic compounds high pH or basic mobile phase is choosen. For neutral compounds neutral mobile phase is selected.

When pH= pKa-2	The acid is 1% dissociated (mostly unionized)
When pH= pKa	The acid is 50% dissociated (equal amount of ionized and unionized)
When pH= pKa+2	The acid 99% dissociated (mostly ionized)
When pH= pKa-2	The base is 99% dissociated (mostly ionized)
When pH= pKa	The base is 50% dissociated (equal amount of ionized and unionized)
When pH= pKa+2	The base is 1% dissociated (mostly unionized)

The table explains about a direction to decide the pH of the mobile phase based on the pKa value of the analyte. The mobile phase pH should be 2 units above or below the pKa to obtain optimum analyte neutralization.

- *Effect of Organic Modifier:* The usual choice is between acetonitrile and methanol (rarely THF) organic modifier type in reverse phase HPLC. Gradient elution is used along with complex multicomponent samples since possibly all components eluted between K (retention factor) 1 to 10 using a single solvent strength under isocratic conditions.[69,70]

Column Temperature

The column temperature has unpredictable effects on selectivity. Employing high temperature will reduce viscosity and back pressure. It may help to operate at high flow rates, or to use longer columns smaller particle sizes, reduce elution time and improve method reproducibility.[70] The target temperature 30 to 40 degrees is good for reproducibility.

Selection of Detector and Wavelength

After the chromatographic separation, the analyte of interest is detected using suitable detectors. The choice of detector depends on the sample and the purpose of analysis. Some commercial detectors used in LC are UV-visible, fluorescence, refractive index (RI), electrochemical, evaporating light scattering (ELSD) and mass spectrometer.[71-73]

Developing the Approach of Analysis

In the development of Reverse phase- HPLC, the first step involves the selection of various chromatographic parameters as discussed above. Typical system suitability parameters are retention time of more than 5 minutes, theoretical plates of more than 2000, tailing factor of less than 2, resolution between two peaks is more than 5, and %RSD of analyte peaks area in standard chromatogram must be more than 2%. Detection wavelength is usually an isobestic point in the simultaneous estimation of two components.[74]

Sample Separation

The analytical procedure must specify the manufacturer, type of filter and pore size of the filter media.[75] The prepared sample should be in aliquot relatively free from interferences that are compatible with the HPLC method development and will not damage the column.[74]

METHOD OPTIMIZATION

Firstly, primary control variables are optimized, such as mobile phase, gradient flow rate, temperature, sample amount, injection volume, diluents and solvent type. Then parameters like column dimensions, column-packing particle size and flow rate are optimized. [73,76]

METHOD VALIDATION

Here the laboratory finds that the performance characteristics meet the requirements for the desired analytical application. It helps to prove that the method can give reproducible and worthy results, employed by various operators with the same set of equipments in varied laboratory conditions. The validation results help to judge the quality, reliability and consistency.[77]

CONCLUSION

Analytical method development by AQbD approach ensures the development of a suitable method for desired purpose. Analytical method development by AQbD approach provides

regulatory flexibility and decreases OOS, OOT, and OOC results. AQbD approach provides more understanding of chromatographic parameters that can be monitored and controlled during the life cycle. AQbD tools are ATP, CQA, risk assessment, method optimization and development with DoE, MODR, control strategy and risk assessment, AQbD method validation and continuous method monitoring.

REFERENCES

1. Gupta V, Jain ADKJ, Gill NS, Guptan K. Development and validation of HPLC method-a review. International research journal of pharmaceutical and applied sciences 2012; 2(4): 17-25.
2. Thamman M. A review on high-performance liquid chromatography (HPLC). Res Rev: J Pharm Anal. 2016; 5: 22-8.
3. Malviya R, Bansal V, Pal OP, Sharma PK. High performance liquid chromatography: a short review. Journal of global pharma technology 2010; 2(5): 22-26.
4. Liu Y, Lee ML. Ultrahigh pressure liquid chromatography using elevated temperature. Journal of Chromatography 2006; 1104 (1-2): 198–202.
5. Abidi SL. High-performance liquid chromatography of phosphatidic acids and related polar lipids. J. Chromatography 1991; 587: 193-203.
6. Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. Arabian Journal of chemistry 2017; 10: S1409-S1421.
7. FDA Guidance for industry Analytical Procedures and Method Validation. Chemistry, Manufacturing, and controls Documentation. Center for Drug Evaluation and Research (CDER) and center for Biologics Evaluation and Research (CDER) 2000.
8. Ravisankar P, Navya CN, Pravallika D, Sri DN, A review on step-by-step analytical method validation, IOSR J Pharm, 2015, 5(10), 7-19.
9. Breaux J, Jones K, Boulas P. Analytical methods development and validation. Pharm. Technology 2003; 1: 6-13.
10. Reversed Phase Chromatography, Principles and methods handbook. Smentic Scholar 1999, 66-8.
11. Kumar N, Jha A. Pharmaceutical quality during product life cycle–A need of holistic approach. European Journal of Pharmaceutical and Medical Research 2015; 2(7): 370-374.
12. Chauhan A, Mittu B, Chauhan P. Analytical method development and validation: a concise review. J Anal Bioanal Tech 2015; 6(1): 1-5.
13. Thakor NS, Amrutkar SV. Implementing quality by design (QbD) in chromatography. J Anal Pharm Chem 2017; 4(1): 1078.
14. Peraman R, Bhadraya K, Padmanabha, Reddy Y. Analytical quality by design: a tool for regulatory flexibility and robust analytics. International journal of Analytical chemistry 2015.
15. Raman NVVSS, Mallu UR, Bapatu HR. Analytical quality by design approach to test method development and validation in drug substance manufacturing. Journal of chemistry 2015.
16. Kumar N, Sangeetha D. Analytical method development by using QbD-An emerging approach for robust analytical method development. Journal of Pharmaceutical Sciences and Research 2020; 12(10): 1298-1305.
17. Bhutani H, Kurmi, M, Singh S, Beg S, Singh B. Quality by design (QbD) in analytical sciences: an overview. Quality Assurance 2004; 3: 39-45.
18. Das P, Maity A. Analytical quality by design (AQbD): a new horizon for robust analytics in pharmaceutical process and automation. International Journal of Pharmaceutics and Drug Analysis 2017; 324-337.
19. Barnet K, Mc Gregor P, Martin GP, Le Blond DJ, Weitzel MLJ, Ermer J, Walfish S, Nethercote P, Gratzl GS. Kovacs E Analytical target profile: structure and application throughout the analytical lifecycle. In Pharmacopeial Forum 2017; 42(5).
20. Ganorkar AV, Gupta KR. Analytical Quality by Design: A Mini Review. Biomedical journal of scientific and technical research 2017; 1(6): 1555-1558.
21. Gupta K. Analytical quality by design: A mini review. Biomed. J. Sci. Tech. Res 2017; 1(1-439): 5.
22. Schweitzer M, Pohl M, Hanna-Brown M, Nethercote P, Borman P, Hansen G. et al. Implications and opportunities of applying QbD principles to analytical measurements. Pharmaceutical Technology 2010; 34(2): 52-59.
23. Kumar N, Sangeetha D. Analytical method development by using QbD-An emerging approach for robust analytical method development. Journal of Pharmaceutical Sciences and Research 2020; 12(10): 1298-1305.
24. Reid GL, Morgado J, Barnett K. et al. Analytical quality by design (AQbD) in pharmaceutical development. Am. Pharm. Rev 2013; 144191.
25. Nadpara NP, Thumar RV, Kalola VN, Patel PB. Quality by design (QBD): A complete review. Int J Pharm Sci Rev Res 2012; 17(2): 20-8.
26. Mascia A, Cirafici AM, Bongiovanni A. et al. A failure mode and effect analysis (FMEA)-based approach for risk assessment of scientific processes in non-regulated research laboratories. Accreditation and Quality Assurance 2020; 25(5): 311-321.
27. https://database.ich.org/sites/default/files/Q8_R2_Guideline.pdf
28. Sangshetti JN, Deshpande M, Zaheer Z, Shinde, DB Arote R. Quality by design approach: Regulatory need. Arabian Journal of Chemistry 2017; 10: S3412-S3425.
29. Lawrence XY, Amidon G, Khan MA. et al. Understanding pharmaceutical quality by design. The AAPS journal 2014; 16(4):771-783.
30. Ganorkar SB, Shirkhedkar AA. Design of experiments in liquid chromatography (HPLC) analysis of pharmaceuticals: Analytics, applications, implications and future prospects. Reviews in Analytical Chemistry 2017; 36(3).
31. Lakka NS, Kuppan C. Principles of chromatography method development (Vol. 10), Intech Open, 2019.
32. Tome T, Žigart N, Časar Z, Obreza A. Development and optimization of liquid chromatography analytical methods by using AQbD principles: Overview and recent advances. Organic Process Research & Development 2019; 23(9): 1784-1802.
33. Fukuda IM, Pinto CFF, Moreira CDS, Saviano, AM, Lourenço FR. Design of experiments (DoE) applied to pharmaceutical and analytical quality by design (QbD). Brazilian Journal of Pharmaceutical Sciences 2018; 54.
34. N Politis, S Colombo P, Colombo GM, Rekkas D. Design of experiments (DoE) in pharmaceutical development. Drug development and industrial pharmacy 2017; 43(6): 889-901.
35. Ferreira SL, Junior MMS, Felix CS. et al. Multivariate optimization techniques in food analysis–A review. Food chemistry 2019; 273: 3-8.
36. Marson BM, Concentino V, Junkert AM, Fachi MM, Vilhena RO, Pontarolo R. Validation of analytical methods in a

pharmaceutical quality system: An overview focused on HPLC methods. Química Nova 2020; 43:1190-1203.
37. Creswell JW. The selection of a research approach, Research design: A qualitative, quantitative, and mixed method approaches. Sage 2014.
38. Mayya SS, Monteiro AD, Ganapathy S. Types of biological variables. Journal of thoracic disease 2017; 9(6): 1730.
39. Little TA. Design of experiments for analytical method development and validation. BioPharm Int 2014; 27(3): 40-45.
40. Jayagopal B, Shivashankar M. Analytical quality by design–a legitimate paradigm for pharmaceutical analytical method development and validation, Mechanics, Materials Science & Engineering Journal 2017; 9.
41. Davis B, Lundsberg L, Cook G. PQLI control strategy model and concepts, Journal of Pharmaceutical Innovation 2008; 3(2): 95-104.
42. http://www.ich.org/fileadmin/Public_Web_Site/Training/GCG_Endorsed_Training_Events/APEC_LSIF_JCCT_workshop_Beijing__China_Dec_08/Day_2/Control_Strategy_Case_Studies.pdf.
43. https://www.fda.gov/files/about%20fda/published/Control-Strategy-and-ICH-Q11--John-Smith--Ph.D.--October-18--2012--AAPS-Annual-Meeting.pdf
44. http://www.mournetrainingservices.co.uk/Preview_book_method-validation.pdf.
45. Scypinski S, Roberts D, Oates M, Etse J. Analytical method transfer. Pharmaceutical Technology 2002; 26(3): 84-88.
46. Swartz ME, Krull IS eds. Analytical method development and validation. CRC press 2018.
47. http://www.pharmtech.com/pharmtech/Article/Method-Validation-by-Design-to-Support-Formulation/ArticleStandard/Article/detail/811796.
48. http://sspcmsn.org/yahoo_site_admin/assets/docs/Analytical_approach_in_QbD_SSPC.44161808.pdf.
49. Phani RSKRSP, Prasad KRS, Mallu UR. Scientific approach for RP-HPLC method development: complete review. International Journal of Science Innovations and Discoveries 2012; 2(6): 218-228.
50. Xiaayang Zheng, Scott Saera, Mar F, Verhagen Claire Davies. An efficient analytical development strategy for rapid development of Biopharmaceuticals. American Pharmaceutical Review 2019.
51. Patil MPN. HPLC Method Development–A Review. Journal of Pharmaceutical Research and Education 2017; 1(2): 243-260.
52. Vidushi Y, Meenakshi B. A review on HPLC method development and validation. Res J Life Sci. Bioinform. Pharm Chem Sci. 2017; 2(6): 178.
53. https://www.cambridgemedchemconsulting.com/resources/physiochem/logD.html
54. https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/reversed-phase-chromatography
55. Bhal SK. Lipophilicity descriptors: Understanding when to use logP & logD. ACD/Labs Phys. Chem Software Application Notes 2007.
56. Sood S, Bala R, Gill NS. Method development and validation using HPLC technique – A review. Journal of Drug Discovery and Therapeutics 2014; 2 (22): 18-24.
57. Gocan S. Stationary phases for thin-layer chromatography. Journal of chromatographic science 2002; 40(10): 538-549.
58. Ahuja S, Rasmussen H. eds. HPLC method development for pharmaceuticals. Elsevier 2011.
59. https://web.vscht.cz/~schulzov/HPLC/Materialy%20pro%20studenty/MethodDevelopment.ppt
60. https://phenomenex.blob.core.windows.net/documents/4e38c0e4-ddf3-44df-aab7-da72332eef8d.pdf
61. Acikara ÖB. Ion exchange chromatography and its applications. Column chromatography 2013; 10: 55744.
62. Layne J. Characterization and comparison of the chromatographic performance of conventional, polar-embedded, and polar-endcapped reversed-phase liquid chromatography stationary phase. Journal of Chromatography A 2002; 957(2): 149-164.
63. https://m.ibric.org/miniboard/down.php?Board=exp_qna&filename=carbon-load[1].pdf&id=468593&fi dx=1
64. Boldura OM, Balta C, Awwad N. eds. Biochemical Analysis Tools: Methods for Bio-molecules Studies. BoD–Books on Demand 2020.
65. Sabir AM, Moloy M, Bhasin PS. HPLC method development and validation: A review. Int. Res. J. Pharm 2013; 4(4): 39-46.
66. Phenomenex; Part 3. The Role of the Mobile Phase in Selectivity (online) slide: 1-56.
67. Usman AG, Işik S, Abba SI. A novel multi-model data-driven ensemble technique for the prediction of retention factor in HPLC method development. Chromatographia 2020; 83: 933-945.
68. ZirChrom-EZ, Zir Chrom-MS. Method Development column use tip. www.Zirchrom.com: 1-26.
69. Kaushal CK, Srivastava B. A process of method development: A chromatographic approach. J. Chem. Pharm. Res. 2010; 2(2): 519-545.
70. Sowjanya P, Subashini D, Rakesh S. A Review on method development and validation by HPLC. RRJPPS 2015; 4(1): 42-49.
71. Willar DHH, Merritt LL, Dean JA, Settle FA. Instrumental Methods of Analysis. Seventh Edition, CBS Publishers and Distributors Pvt. Ltd. 1988; 118-148, 598-607.
72. Lindholm J. Development and Validation of HPLC method for Analytical and Preparative Purpose. Acta Universities Upsaliensis Uppsala 2004; 13-14
73. Nigovic B, Mornar A, Sertic M. Chromatography – The Most Versatile Method of Chemical Analysis. Intech. 2012; 385-425.
74. Toomula N, Kumar A, Kumar SD, Bheemidi VS. Development and Validation of Analytical Methods for Pharmaceuticals. J Anal Bioanal Techniques 2011; 2(5): 1-4.
75. Charde MS, Welankiwar AS, Kumar J. Method development by liquid chromatography with validation. International Journal of Pharmaceutical Chemistry 2014; 4(2): 57-61.
76. Kardani K, Gurav N, Solanki B, Patel P, Patel B. RP-HPLC Method Development and Validation of Gallic acid in Polyherbal Tablet Formulation. Journal of Applied Pharmaceutical Science 2013; 3 (5): 37- 42.
77. Prathap B, Rao GHS, Devdass G, Dey A, Harikrishnan N. Review on Stability Indicating HPLC Method Development. International Journal of Innovative Pharmaceutical Research 2012; 3(3): 229- 237.
78. Vichandran V, Shalini S, Sundram, KM, Harish Rajak. Validation of analytical methods- strategies and importance. International Journal of Pharmacy and Pharmaceutical sciences 2010; 2(3): 18-22.
79. ICH Q2(R1). Validation of Analytical procedures: Text and methodology. International Conference on Harmonization, IFPMA, Geneva, 2005.
80. European Medicines Agency, ICH Harmonised tripartite guideline - Validation of analytical procedures: text and methodology Q2 (R1), London, 2005.

81. United States Pharmacopoeial Convention, USP 41-NF 36, chapter 1225, Validation of compendial procedures, United States Pharmacopoeia, North Bethesda, 2018.
82. WHO, Guidelines on validation - Appendix 4 - Analytical method Validation, Geneva, 2016.
83. U. S. Food and Drug Administration; Analytical procedures and methods validation for drugs and biologics Guidance for Industry; Rockville, 2015.
84. Bhagyasree T, Injeti N, Azhakesan, A, Rao UMV. A review on analytical method development and validation. International Journal of Pharmaceutical Research & Analysis 2014; 4 (8): 444-448.
85. Shweta K, Anita S. A review on analytical method validation. International Journal of Pharm Research and Review 2016; 5(9): 30-36.
86. Jenke DR, Chromatographic method validation: a review of current practices and procedures, Part II. Guidelines for primary validation parameters, Instrumentation science & technology 1998; 26(1): 1-18.
87. Kumar V, Bharadwaj R, GG SK. An Overview on HPLC Method Development, Optimization and Validation process for drug analysis. The Pharmaceutical and Chemical Journal 2015; 2(2): 30-40.
88. Lavanya G, Sunil M, Eswarudu MM, Eswaraiah MC, Harisudha K, Spandana BN, Analytical method validation: An updated review. International Journal of Pharmaceutical Sciences and Research 2013; 4(4): 1280.
89. Swartz ME, Krull IS. eds. Analytical method development and validation. CRC press 2018.
90. Validation of Analytical Procedures: Text and Methodology. International Conferences on Harmonization, Draft Revised 2005; Q2 (R1).
91. Validation of Compendial Procedures. United State Pharmacopeia, USP 36 NF, 27 (2), 2010.
92. WHO, WHO Expert Committee on Specifications for Pharmaceutical Preparations, Geneva, 2018.
93. Marson BM, Concentino V, Junkert AM, Fachi MM, Vilhena RO, Pontarolo R, Validation of analytical methods in a pharmaceutical quality system: An overview focused on HPLC methods, Química Nova, 2020, 43, 1190-1203.