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Original Article

Quality by Design in HPTLC: A Review of Method Development Approaches

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ABSTRACT

A methodical approach to development, Quality by Design (QbD) starts with predetermined goals for a product, process comprehension, and process control based on knowledge and quality risk management. When developing and validating a traditional approach, it is possible that it will not achieve the desired outcome. AQbD is the application of the QbD idea to the validation of analytical methods. A systematic and trustworthy strategy for developing analytical processes that cover all stages of a product's lifecycle is offered by AQbD. The AQbD technique for creating the HPTLC method is predicated on changing one parameter while maintaining the other parameters constant in order to obtain the desired result. The development of the conventional HPTLC method necessitates numerous tries and errors, which impacts the accuracy,precision and resilience of the process.Therefore, AQbD is used for analytical method validation in order to reduce time, complexity, and—most importantly—validation failure. Method intent definition, experimental design, experimental result evaluation, method condition selection, and risk assessment with varying analytical parameters and evaluation conditions are some of the phases that make up the QbD Approach to method development. AQbD in HPTLC is essential to understand different factors showing significant impact on method outcome. The HPTLC method should display robustness to facilitate use for a longer period along with very low potential of failure.

Keywords: Design in HPTLC, Approaches, analytical methods

INTRODUCTION

Introduction to HPTLC

HPTLC is a more complicated and automated version of thin-layer chromatography (TLC) that has better detection limits and separation efficiency. It is also known as flat-bed chromatography, planar chromatography, or high-pressure thin layer chromatography. It is a strong analytical instrument that works well for both qualitative and quantitative issues. [1,2] Depending on the kind of adsorbents used to the plates and the solvent system utilized during development, separation may arise from partitioning, adsorption, or both. Phytochemical analysis, biomedical analysis, quantification of herbal medications, analytical analysis, fingerprint analysis, and the possibility of hyphenation (HPTLC-MS, HPTLC-FTIR, and HPTLC- Scanning Diode Laser) are among the applications cited. [3] High resolution sorbents with specific particle sizes or chemically altered surfaces, more effective elution methods, the capacity to integrate with other instrumental techniques, the development of computer programmes for method optimization, and the application of modern tools such as chromatographic chambers, densitometers, and video scanners. [4] HPLC, a very sophisticated chemical standardization technique, is far more reliable and reproducible when used to standardize herbal compositions, both single and compound. The separation method called high-pressure liquid chromatography uses a stationary phase and liquid mobile phase. The size of stationary phase utilized determines whether ion exchange, adsorption, or partitioning is employed for separations. [5].

Principle:

1. Separation: Components are separated by HPTLC. based on affinity between stationary and mobile phases

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2. Adsorption: Components interact with the adsorbent surface, resulting in separation.
3. Components split into mobile and stationary phases.

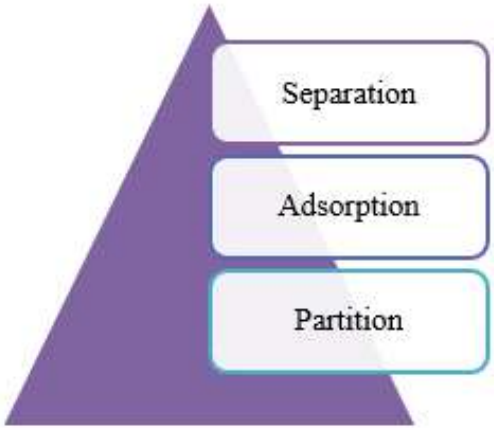


Fig No.1. Principles of HPTLC

Benefits and drawbacks of HPTLC in comparison to TLC: In recent years, HPTLC has become a reliable identification technique and has replaced traditional TLC.The device is managed by software. The most efficient silica gel hydrophilic phasethat meets the requirements of most pharmacopeias is being utilized for HPTLC pollutants identification. [6]

Table No. 1: Difference between TLC and HPTLC [6]

Sr.No.	Feature	TLC	HPLC
1	Technique	Manual	Instrumental
2	Plates	Lab-made	Pre-coated
3	Plate height	30 µm	12 µm
4	Layer of Sorbent	250 µm	100 µm
5	Stationary Phase	Silica gel, alumina, &Kiesulgur	Numerous stationary phase options, such as C8, C18 for reversed phase and silica gel for normal phase
6	Separations	10-15 cm	3-5 cm
7	Analysis time	20-200 min	1-3 min
8	Average Size of Particles	10-12 µm	5-6 µm
9	Efficiency	Less	High
10	Sample Holder	Capillary/Pipette	Syringe
11	Sample Spotting	Manual Spotting	Autosampler
12	Size of Sample	Uncontrolled	Controlled solvent Independence
13	Sample Shape	Circular	Rectangular
14	Sample tracks for each plate	<10	<36
15	Volume Range	1-10 µl	0.1 to 500µl
16	Development Chamber	More amount	Less amount
17	Wavelength range	254 or 366	190 or 180
18	Detection limit	1-5 pg	100-500 pg
19	Limit of detection (fluorescence)	50-100pg	5-10 pg
20	PC Connectivity	No	Yes
21	Quantitative Analysis	No	Yes
22	Scanning	No	UV/Visible/Fluorescence scanner
23	Analysis Judgement	By Analyst	By machine

Automation for HPTLC:

Modern TLC, often referred to as HPTLC, is primarily used for quantification, requires tools, and can only be carried out on precoated layers. Consequently, the terms TLC and HPTLC are used interchangeably. TLC is widely used around the world to teach the principle of chromatography. The sample's visibility during chromatography, the demonstration apparatus's extremely low cost, and its user-friendliness are the main justifications for this method. It employs a multifaceted technique to improve resolution under capillary flow-controlled circumstances. Planar chromatography can separate molecules in one or two dimensions. External control of mobile-phase velocity is also possible, as seen in forced-flow development. [7] HPTLC is the quickest chromatographic method. The samples are chromatographed in parallel. Each stage of the technique is completed individually, making In addition to being quicker, HPTLC is adaptable enough to evaluate several samples at once. The amount of stationary and mobile phase used depends on the number of samples being analyzed. [8]

Steps involved in HPTLC [9]:

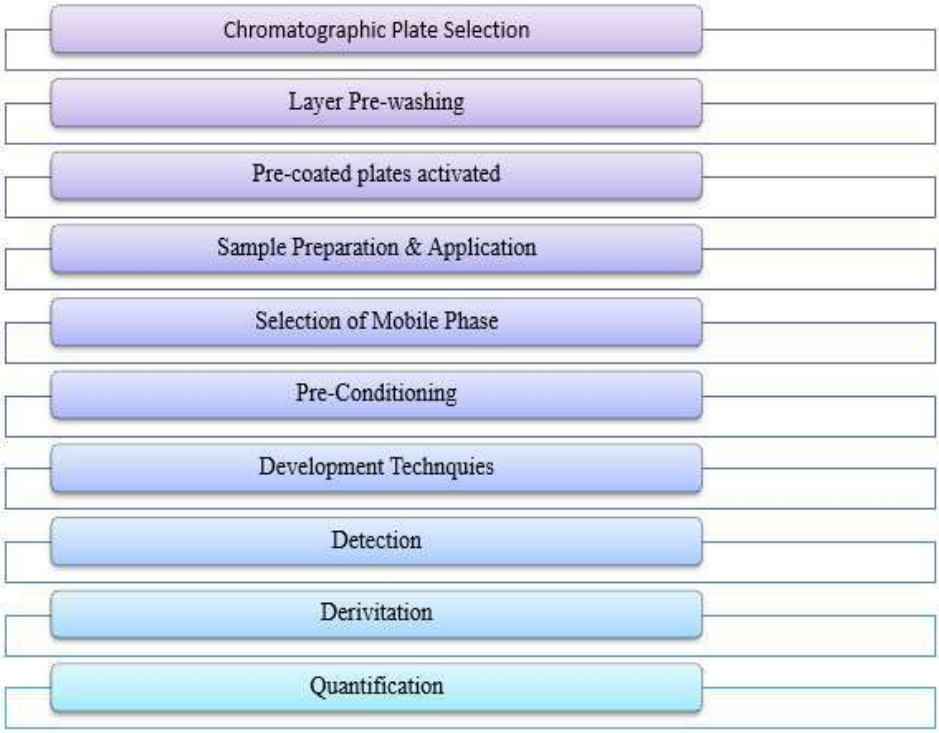


Fig. No.2 Steps Involved in HPTLC

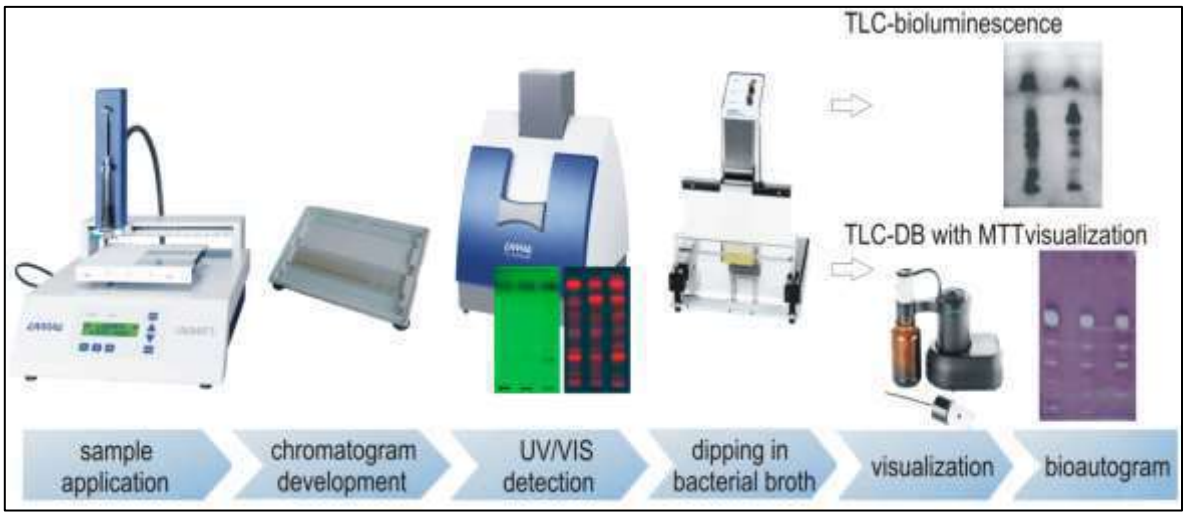


Fig.No.3. Instrumentation of HPTLC

Chromatographic Plate Selection:

- Think of utilizing handcrafted plates composed of cellulose or other materials thataren't as popular these days.
- **Pre-coated plates:** For both qualitative and quantitative analysis, pre-coated plates with sorbent layers and support materials are used.
- Plate support materials include glass, polyester/polyethylene, and aluminum. Sorbents include silica gel 60F, aluminum oxide, cellulose, and Silica gel that has been chemically altered with an amino group (NH2) or CN group.
- Resolution and sensitivity are enhanced by silica particles with a smaller size.

Layer pre-washing:

- The primary goal of pre-washing in this purification stage is to eliminate pollutants from the atmosphere, such as water vapors and other volatile compounds, when they are exposed in the lab environment.
- Several typical techniques for
- pre-washing are –Ascending, Dipping, Continuous
- Solvents for pre-washing:

1. Methanol
2. Chloroform: Methanol (1:1)
3. Chloroform: Methanol: Ammonia (90:10:1)

Activation of Pre-Coated Plate

- Activation is not necessary for recently opened HPTLC plates.
- It could be necessary to activate exposed plates to high humidity for a long time.
- To activate the plates, place them in an oven set between 110 and 1200 degrees Celsius for 30 minutes.
- Water that has been physically adsorbed on the sorbent layer's surface is removed using this method.

Sample Preparation and Application

Sample Preparation:

- To achieve comparable distribution in the starting zones, dissolve the sample and reference compounds in the same solvent.
- It requires a small amount of sample and a highly concentrated solution to be administered.
- After that, the plates were dried and stored in a dust-free environment.

Sample application:

- The typical concentration range for HPTLC is 0.5-5µL.
- The sample spot applied must be no larger than 1mm in diameter.
- To avoid overloading, apply samples in the form of a band.
- Choose the appropriate applicator based on sample volume and quantity.
- Micro syringes, Linomat, and other applicators are used to apply samples.

Fig.No. 4: Linomat Applicator

Selection of mobile phase

- Mobile phase selection is influenced by the analyte's physical and chemical properties as well as the adsorbent material utilized as the stationary phase.
- The peak of interest should be resolved between Rf values of 0.15 and 0.85.
- A characteristic called eluent strength, which is connected in relation to the polarity of mobile phase components, determines the power of elution in the mobile phase.
- The more nonpolar the molecule, the quicker it elutes (or the less time it will spend on the stationary phase), while the morepolarize the substance, the slower it will elute.
- Less mobile phase is required than in TLC. [10,11]

Preconditioning (Chamber Saturation)

- Unsaturated chambers provide high Rf values.

Fig.No. 5: Horizontal Chamber

Detection

- Detection of UV light-induced (ranging normally 200-400) fluorescence quenching improves the detection of isolated chemicals on absorbent layers.
- This phenomenon is frequently referred to as fluorescence quenching.

Ultraviolet 254 nm visualization:

- One could consider F254 to be phosphorescence quenching. In this instance, the fluorescence lasts for a little while after the excitation source is
- Saturation of the mobile phase ought to be done in the chamber by lining it with 30 min by filter paper before development to ensure uniform distribution of the solvent vapor's and low Rf values.
- Saturation is only necessary in high polarity mobile phases; it is not necessary in low polarity mobile phases.

Developmental Techniques

- The plates are dotted with sample, air dried, and then placed in the developing chambers.
- The various development techniques employed are:

1. Ascending
2. Decsending
3. Horizontal

- For optimal reproducibility, use saturated twin-through chamber with filter paper. [12]

Fig. No. 6: Twin-through Chamber

eliminated. The duration exceeds ten seconds. It is longer than ten seconds.

- Green fluorescence is produced by F254 fluorescent indicators when they are activated by 254 nm UV light [13].
- The layer's emission is limited by compounds that absorb light at 254 nm, which causes the compound zones to appear as a dark voilet with patch green background [14].
- Conjugated double bonds are among the compounds that produce this quenching. At 254 nm, one should be able to detect anthraglycosides, coumarins, flavinoids, polyphenols in essential oils certain alkaloid types such as indole,

isoquinoline, quinoline alkaloids, and so forth. [15].

Visualization at UV 366 nm:

- One could consider F 366 to be fluorescence quenching. In this instance, removing the excitation source causes the fluorescence to stop. [13].
- All anthraglycosides, coumarins, flavonoids, phenolcarboxylic acids, and several alkaloid types (Rauwolfia and Ipecacuanha alkaloids) exhibit this quenching effect. [16]
- **Visualization of white light:**
- The zone comprising separated chemicals can be identified by observing their natural color in daylight.

Fig.No.7: Visualizer

Derivatization:

- Derivatization is a process that changes analytes to allow for chromatographic separations.
- Derivatization can be conducted by submerging the plates or spraying them with a suitable reagent.
- Immersion is the favored derivatization procedure due to its higher repeatability. [17-18]

Colour Reagents used in Derivatization:

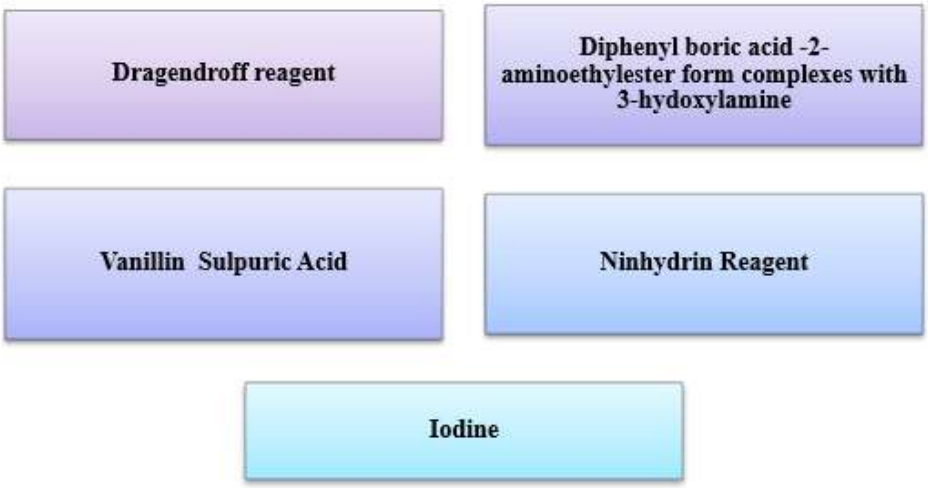


Fig. No. 8: Colour Reagents used in HPTLC

Quantification:
Scanning densitometry:

- Measures the absorption and fluorescence of underivatized or derivative compounds at 200-800 nm.

- The system can analyze up to 31 wavelengths and record spectra for any peak. Biological testing can be done directly on the HPTLC plate. [19]

Fig. No. 9: Scanner

Digital Camera-Based Image Documentation

- Better-designed UV cabinets that can accommodate a digital camera for taking plate photos are now replacing UV cabinets.
- Small labs choose this device, despite the fact that it does not meet GLP standards. HPTLC is now a must-have for any laboratory working in herbal analysis.
- It is used for formulation studies and to identify plant extracts by comparing them to extracts from Botanical Reference Materials (BRM) to identify adulterants or replacements. It has long been asserted by forensic analysts that they begin with a microscope for physical examination and TLC for chemical examination. [20].

Fig.No.10: Photo-documentation with Digital Camera

Software-Induced Scanning

- An "Entry Level" HPTLC system is already extremely advanced and can handle the majority of everyday tasks. It can scan for quantification in both absorbance and fluorescence modes, as well as record UV-Visible absorbance spectra in situ.
- Depending on the end-user's needs, a gradient chamber, picture documentation device, and bioluminescence detector may be incorporated, or a completely automatic system may be purchased.
- An appropriate commercially available interface allows for hyphenation with MS, IR, or NMR. A recently accessible device connects HPTLC and MS. This interface extracts the desired fraction from the layer and feeds it directly into the MS.
- This brings up many new possibilities for an analytical lab. When LC-MS analysis is

combined with TLC/HPTLC, the output can be significantly boosted.

- ❖ Any defined fraction of a plate can be studied. Other fractions can be ignored.
- ❖ TLC can help adjust MS parameters for a specific molecule (Table 1). LC-MS and TLC-MS are complementary methods. [21]

Introduction to QbD: -

Quality by Design (QbD) is a structured methodology in drug development that applies risk management and analytical techniques to ensure product quality throughout the design, development, and manufacturing processes. The main objective of QbD is to integrate quality into the process from the outset. In the initial phases of a project, the product's key characteristics and goals are established, and risk assessment and data analysis are employed to understand how processes impact the product's attributes. This enables the creation of robust procedures that sustain consistent quality, meeting predetermined specifications. Both the US FDA and the International Council for Harmonisation (ICH) support various strategies for the development and manufacturing of pharmaceutical products. QbD is defined as "a systematic approach to development that begins with predefined goals, focusing on both product and process." According to Janet Woodcock (2004), "Product and process performance characteristics should be scientifically designed to meet specific goals rather than being based solely on empirical test results." Quality by Design (QbD) focuses on creating the right process and comprehensively understanding how it performs to achieve the desired product outcome. The core principle of QbD is continuous improvement, driven by insights gained from process understanding. This approach aims for a 'desired state,' which allows for greater regulatory flexibility and emphasizes the importance of scientific knowledge development, superior design, performance validation, Quality Risk Management (QRM), Design of Experiments (DoE), Process Analytical Technology (PAT) tools, ongoing improvement and learning, and effective life cycle management. [26]

Use of QbD in HPTLC [27]:

1. Define: Specify the method's requirements and aims, such as separation, detection, and quantification.

2. Identify: Find the Critical Method Parameters (CMPs) that affect separation, such as temperature, development distance, and the composition of mobile phase.

3. Design: Conduct studies to optimize CMPs using statistical tools and Design of studies (DoE) concepts.

4. Optimize: Improve method conditions for robust separations, such as selecting the best mobile phase composition and development distance.

5. Validate the approach to meet regulatory criteria and demonstrate robustness and reliability.

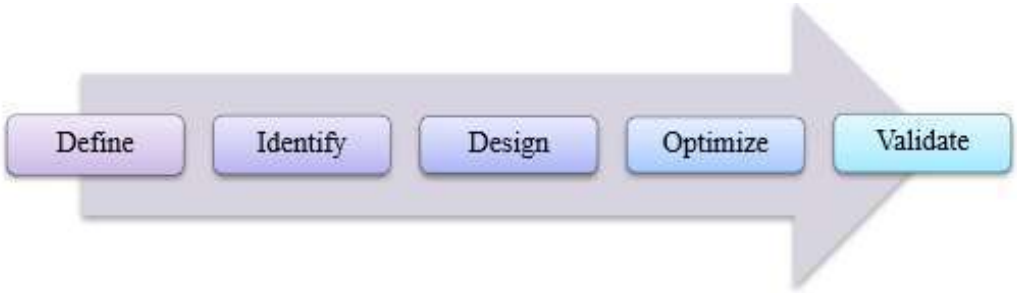


Fig. No. 11: Uses of QbD in HPTLC

Benefits of QBD for HP TLC Development [28]:

Enhanced Method Robustness: QBD helps discover and regulate crucial method parameters, resulting in more robust procedures.

Improved Method Sensitivity and Specificity: By improving method parameters, QBD can improve HPTLC sensitivity and specificity.

Reduced Development Time: QBD helps speed up method development by focusing on important aspects and avoiding superfluous tests.

Improved Method Performance: A well-designed and verified HPTLC method established utilizing QBD principles increases confidence in its performance and reliability.

Compliance with Regulatory standards: According to ICH recommendations, QBD complies with regulatory criteriafor the creation and verification of analytical techniques.

Fig.No. 12: Benefits of QbD in HPTLC

Analytical Quality of Design (AQBD):

The ICH defines QbD, which is founded on sound science and quality risk management, as"a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control." It suggests that in order to accomplish the stated objectives, performance attributes of the product and process must be developed scientifically. AQbD produces a stable, well-understood, and long-lasting technology that consistently delivers the desired performance throughout its lifespan, much like process QbD. A Method Operational Design (MODR) refers to a multi-dimensional framework that incorporates various method factors and parameters, ensuring optimal performance. This approach is designed to analyze and define the necessary conditions for achieving desired results in a given process or system., is created using the thorough information acquired from this methodology. System appropriateness is one of the relevant method controls that are constructed using it. [29] provides a high-level overview of AQbD procedures.

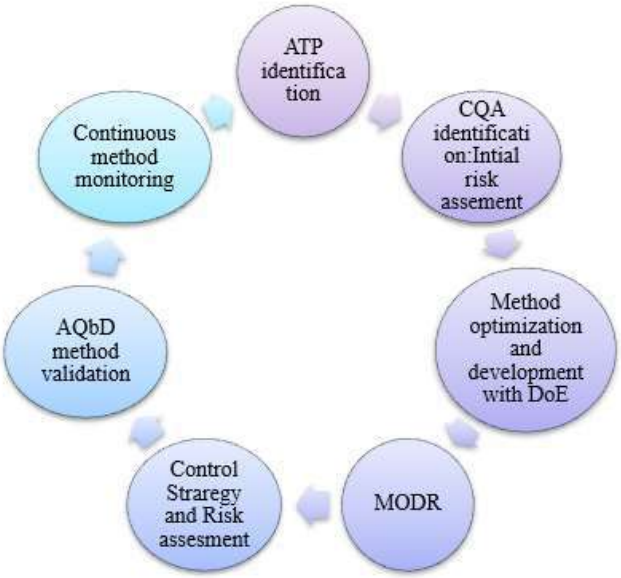


Fig. No.13: AQbD tools and life cycle

Elements QbD:

Adopting Quality by Design (QbD) leads to a resilient and effective approach that aligns with International Council for Harmonisation (ICH) guidelines, which is why it is increasingly being embraced by pharmaceutical industries. This approach facilitates the ongoing improvement of methods. [30-32] One application of QbD in the pharmaceutical industry is in High-Performance Liquid Chromatography (HPLC), which is widely used for stability testing, method development, and impurity detection in pharmaceutical products.

1. Karl Fisher tritation to determine moisture content.
2. Bio-Pharmaceutical Process
3. Studies on Dissolution
4. Hyphenated methods such as LC-MS
5. Cutting-edge methods like UHPLC capillary electrophoresis and mass spectroscopy
6. Examination of Genotoxic contaminants.

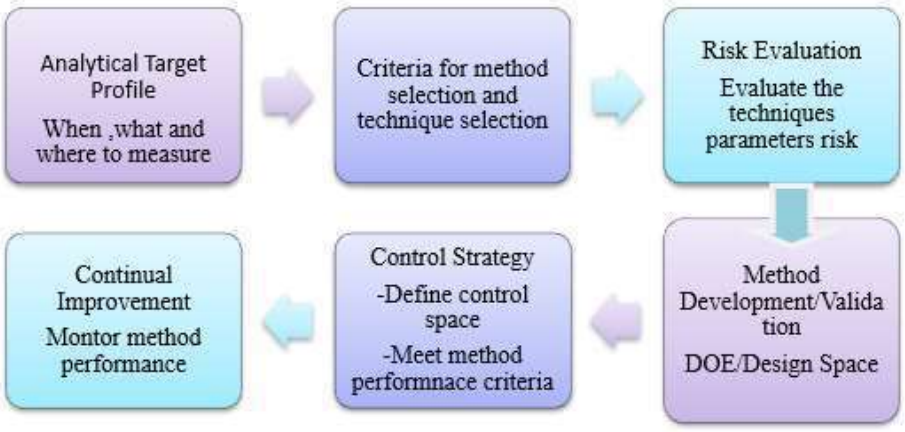


Fig.No.14: Aspects of QbD to analytical Method Development

1. Analytical Target Profile (ATP):

An analytical target profile that is linear to QTPP is the first step in QbD. The analytical target profile outlines the goal of the process of developing analytical techniques by connecting the technique's outcomes to achieving QTPP. The data and scientific reasoning from the analytical procedure are used to define the analytical target profile. The ATP outlines what the technique must measure (approval criteria) and what degree of measurement is necessary (functional level attributes such precision, accuracy,

range and sensitivity). [28] The selection of target analyte (API and impurities), analytical methodology (HPLC, HPTLC, Gas chromatography, ion chromatography etc.), and method requirements are often included in ATP for analytical operations. [33]

2. Critical Quality Attributes (CQA):

The second phase of Quality by Design is CQA. CQA is defined by ICH Q8 as a physical, chemical, biological attribute that needs to fall within a permissible range or limit in order to guarantee the desired product quality. (8) Method parameters and characteristics are part of the CQA for analytical procedures. There may be differences in the analytical methods used for CQA. Oven and program temperature, injection temperature, gas flow rate, sample diluents and concentration are among the CQA requirements for the GC technique. Mobile phase buffer, pH, column selection, organic modifier and elution process are all included in CQA for HPLC procedures. TLC plates, mobile phase, injection volume and concentration, plate development time, and color detection reagent are all included in

3. Risk Management [36]:

The definition of Quality Risk Management (ICH Q9) is "a systematic process for assessing, controlling, communicating and reviewing quality risks throughout the life cycle." An integral part of the Analytical QbD process is risk assessments. Risk assessments make it easier to identify and rate elements that may have an impact on method performance and ATP complianceRisk assessments are usually conducted at the end of method development, with product modifications (e.g., route, formulation, or process), and prior to method transfer. They are often iterative throughout a method's lifespan. These RAs draw attention to possible discrepancies (such as reagent suppliers, testing cycle times, laboratory procedures, and surroundings). During the methodology selection and method development with product changes, example (root, formulation and viruses) and before method transfer, should be identified and considered [31]. ICH guideline Q9 mentions the following risk assessment methods:

•Failure Mode Effect Analysis (FMEA).
•Failure Mode, Effect, and Criticality Analysis (FMECA); Fault Tree Analysis (FTA).
• Hazard Analysis and Critical Control Points (HACCP).
•Hazard Operability Analysis (HAZOP).
• Preliminary Hazard Analysis (PHA).
•Risk ranking and filtering.

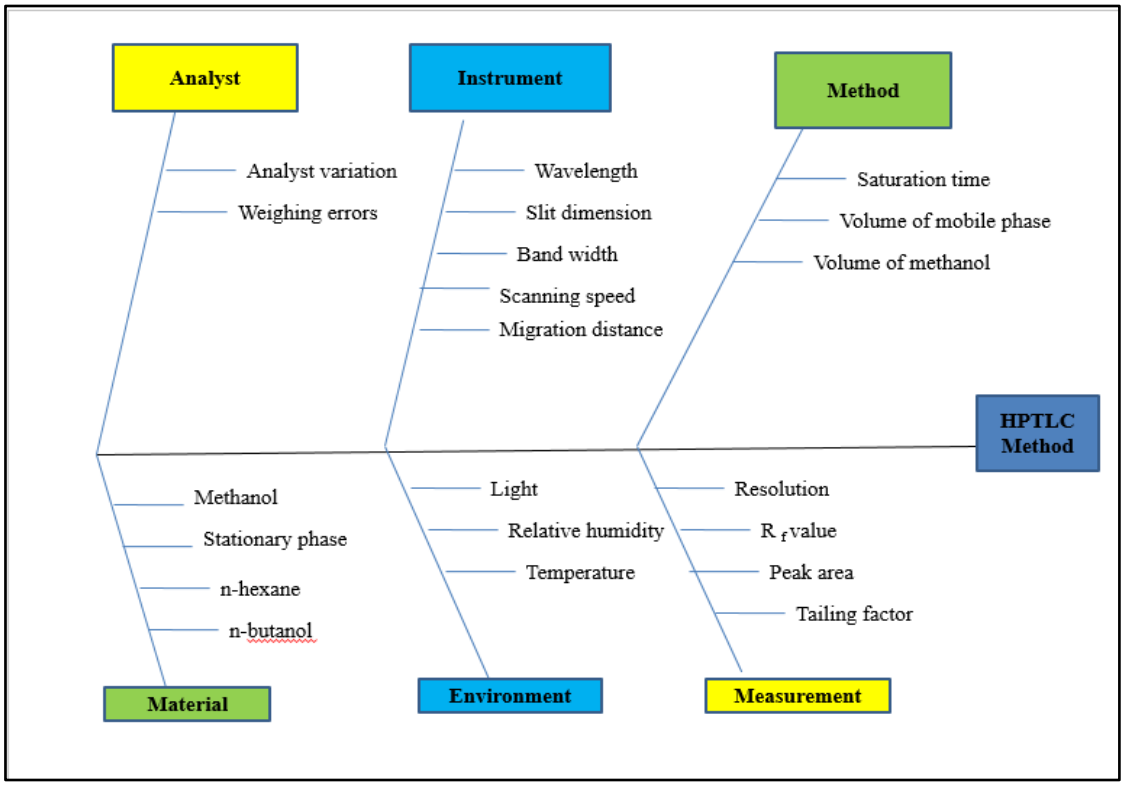


Fig.No. 15: Ishwika Fish Bone Diagram

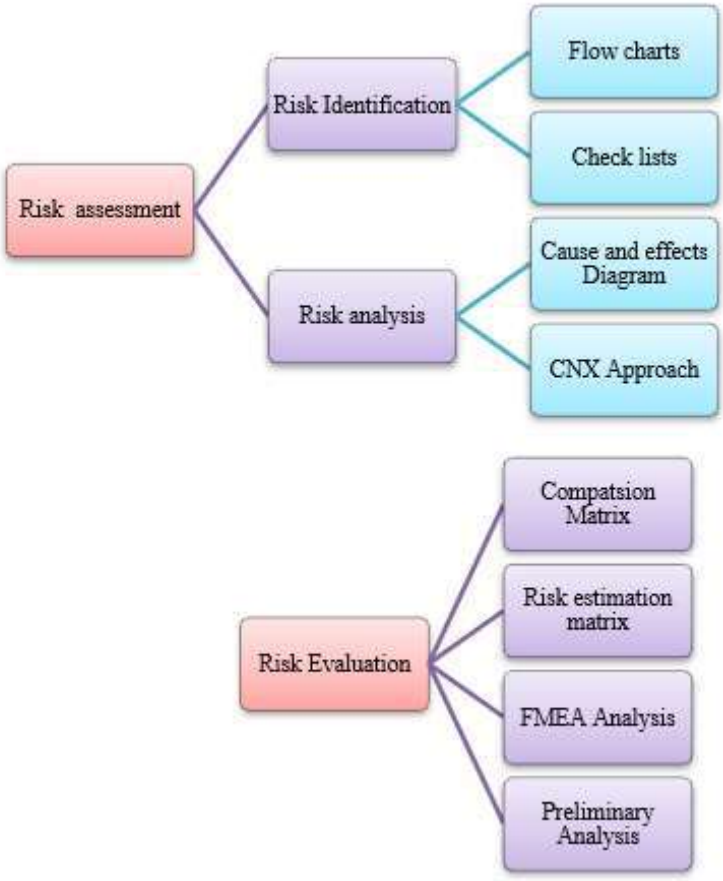


Fig.No.16: Steps Involved in Risk Management

4. Method operational design Space [37]:

Establishing the method operational region comes after method development and risk assessment. Operating zones are systematically constructed for everyday usage using MODR. MODR is a multivariate, risk-based, scientific approach to examining how various factors affect method performance. Critical method controls including system appropriateness, RRT, and RRF are also configured using it.

Steps involved in Method Development [38]:

Step 1 Defining method intent

The goals of developing an HPLC technique need to be clearly stated since pharmaceutical QbD is a structured, scientific, all-encompassing, threat-based, and pragmatic strategy that emphasizes product and process understanding control and begins with predefined goals. Finding and measuring the main chemical is the ultimate goal of the analytical procedure.

Step 2: Performing experimental design

Rapid and methodical method optimization can be effectively achieved through experimental design. It is believed that in order to carry out optimization and fully understand the procedure, a methodical experimental design is required. It creates a chromatographic database to aid in the comprehension, optimization, and selection of methods. Additionally, it can be used to assess and apply method changes, if necessary, in the future, such as when an impurity becomes irrelevant or the chromatographic column is no longer commercially available.

Step 3 Evaluation of experimental results and selection of final method conditions

The method's conditions must be evaluated using the three-tiered process. The conditions for peak symmetry, peak tailing and peak fronting should be accessed first. These conditions should thereafter be further assessed using stricter standards, like the tailing factor being smaller than 1.5, etc.

Step 4: Performing risk assessment with robustness and ruggedness evaluation

Once the method has been selected based on technical attributes, it is highly likely to be reliable and to continue working for the rest of the product's life. Verifying and finalizing the technique and assessing its robustness and ruggedness are the main goals of the fourth step of the method development process. Risk-based approach based on the QbD principles described in ICH Q8 and Q9 can be used to evaluate the robustness and ruggedness of a technique. A variety of situations, such as various laboratories, analytes, instruments, reagents, days, etc., or the consequence of little medication in the method parameters, may be utilized to establish the method's potential risk using fishbone diagrams, which are organized methodologies for risk assessment.

5.ControlStrategy [39]:

The Control design set is the same as the control strategy. The estimate is determined by the type of the analyst and their understanding of MODR. All of the statistical information gathered during the MODR may be used to create the approach control plan. The control strategy can change over the course of the method lifespan; it is not a one-time approach that is only applied during the method development phase. The technique control strategy for Qbd techniques is the same as for traditional methods, it should be noted.

6. Lifecycle Management [40]:

The life cycle approach differs from the usual approach to method development. According to More field, it comprises ongoing improvement of technique performance, and the design space provides flexibility for Because of the previously established design space, continuous improvement of the analytical method is possible without prior regulatory permission.

7. Experimental Designs [41]:

Before beginning experimental research, the experimental design is a statistical method for organizing trials such that the required data is gathered accurately and effectively. The experimental domain, also known as the region of interest, must be defined within a factor space before choosing a suitable experimental design.

Design of Experiments (DoE): -

The process of determining the optimal composition and operating parameters is known as optimization. The word "optimizes" literally means to make anything as near to as near to perfection as is practical. Many elements are involved in the design and development of pharmaceuticals. Factors, sometimes referred to as independent variables, are those that the maker can control and that might affect the characteristics of the analytical procedure and outcomes. Levels are the values of the factors. Response variables, often referred to as dependent variables, are traits that end products exhibit. Any change in independent factors is accompanied by a change in the dependent variables. [42]. The different types of DoE optimization methodologies are illustrated in Fig No. []. DoE has developed into a potent instrument that gracefully offers a lot of data with the fewest runs.

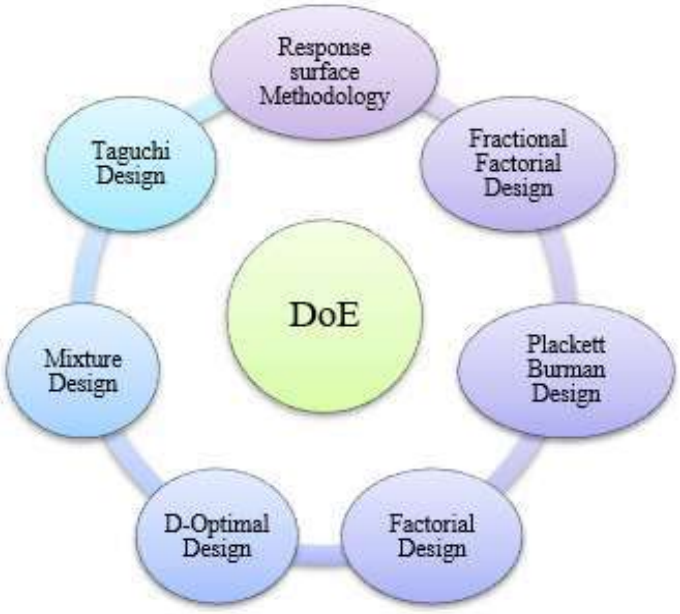


Fig.No.17: Design of experiments (DoE) Optimization Methodologies

A. Screening: -

Finding one or more CMPs to include in optimization trials and screening qualitative input variables are both part of the screening process.

Screening employs a variety of tools, including factorial design, central composite design, placket-Burman design, Taguchi design, and D-optimal mixture design. [43].

B. Optimization: -

Several CMPs from the risk assessment and screening process are chosen for optimization. [44] The performance of the approach is affected by the replies from different CQA, and during optimization, the relationship between CQA and their output response is identified using scientific concepts. The Box-Behnker, Full factorial, Fractional factorial and Doehlert designs are the most often used experimental designs for the development of HPTLC techniques.

Various Designs Used forScreeningand Optimization of Method: -

Table No.2. Designs Selection Guide

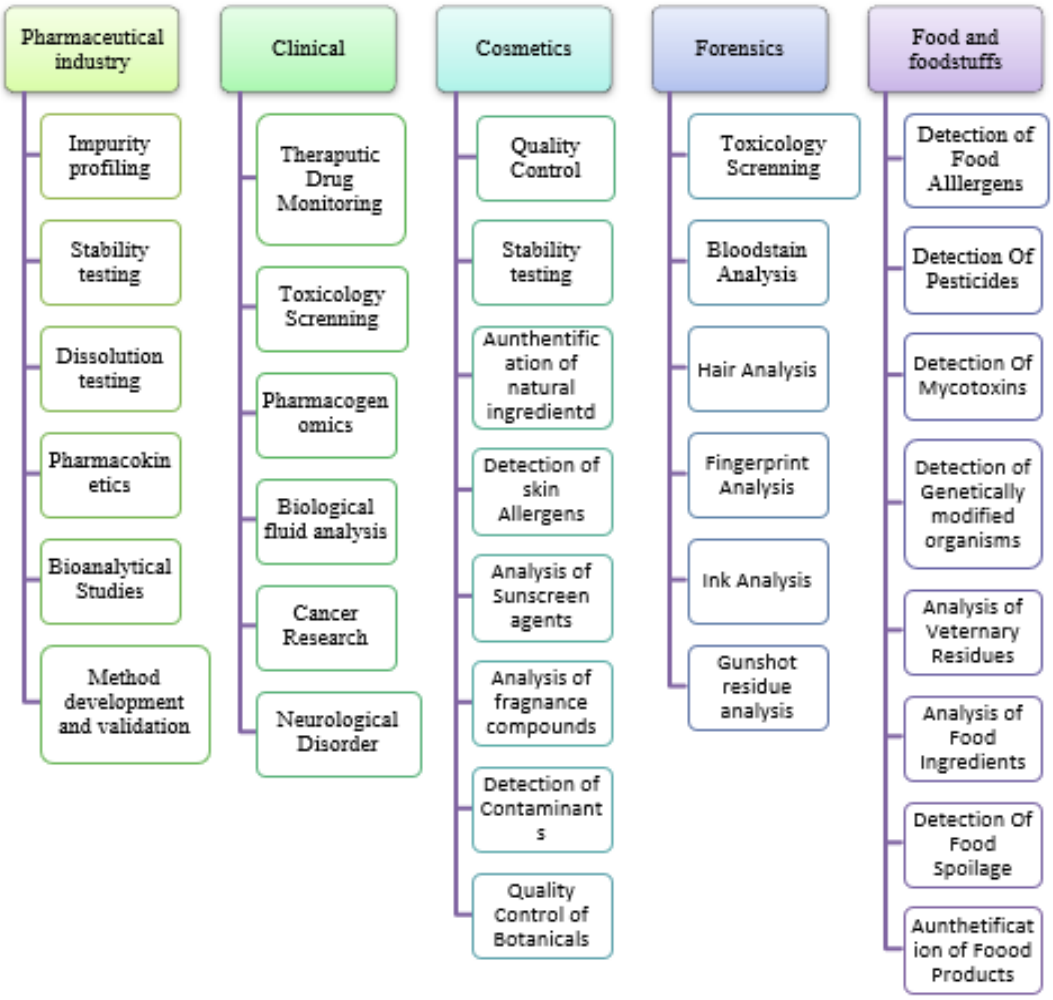
Objective	**Screening**			**Optimization**				
				Factorial	**Response surface**		**Mixture**	
No. of factors	7<F< 32	4<F<6	F<3	F= 2	2<F<6	3<F<5	2<F<6	3<F<5
Design to be selected	PBD	FFD	Full factorial	Full factorial	CCD	BBD	Simplex Lattice	Simplex Centroid
No. of levels	2	2	2	3	5	3	2/3	2/3
Application	Main effects	Major and minor	Main and interaction effects	Possible curvature in response	Opt of curvature	Optcenter of design	Factors are component of mixture and must total to be constant	
Levels	Additive (Linear)		Synergistic	Quadratic	Quadratic cubic		Schiff's Mixture	Schiff's Mixture

Validation and Post Method Considerations:

A crucial step that follows method development is method validation. In some tests, it validates that the analytical process is appropriate for the intended use. The method validation results may contribute to the consistency and dependability of analytical results. [45] Validation occurs following the establishment of

the QbD-based HPTLC technique development. Regulatory authorities, such as the USFDA and ICH Q2, provide guidance on method validation. Because the method has been thoroughly designed and reviewed, errors are unlikely to occur during the validation stage. [46] The following validation parameters are evaluated: system appropriateness, specificity, range, linearity, accuracy, precision, quantification limit, detection limit and robustness. [44]

Applications of HPTLC:



Application of QbD in HPTLC Approach:

Sr.No.	Drugs	Experimental conditions	Design used	Reference
1	Ceritinib	Pre-coated silica gel G60 F254 Mobile phase- Chloroform: Methanol: triethylamine (8.9:1.6:0.07 v/v/v) Rf:0.37	Box Behnken design, ANOVA	[47]
2	Berberine and Conessine	Type of Plate: Pre-coated silica gel 60 F 254 (5mm*0.45) Mobile phase: Ethylacetate: Methanol: Ammonia (6.5:1:0.3 v/v/v) Rf of Berberine: 0.22 Rf of Conessine: 0.85	Box Behnken design	[48]
3	Rottlerin	Type of plate: Pre-coated silica gel 60 F 254 Mobile phase: Toluene: Ehylacetate: Methanol: Water (5:4:2:0.2)	Box Behnken design	[49]
4	Fluoxetine	Type of Plate: Precoated silica gel 60 F 254 Mobile Phase: Acetone: Water (8.5:1.5 v/v) Rf: 0.72+0.07	Central Composite Design	[50]

5	Azilsartan and Clinidipine	Type of Plate: Pre-coated silica gel G60 F 254(10*10) Mobile Phase: Toluene: Ethylacetate: Methanol (6.5:1.5:2.0 v/v/v) Rf AZL:0.51+0.02 Rf value of CLN :0.71+0.02	Taguchi and Box-Behnken Design	[51]
6	Febuxostat	Type of plate: Pre-coated Silica gel aluminium Plate 60 F-254(20 cm*10 cm) Mobile Phase: Chloroform: Methanol: Formic Acid (6.7:2.9:0.1 v/v/v) Rf:0.728	Box-Behnken Design	[52]
7	Silymarin	Type of Plate: Pre-coated silica Gel Aluminum F254 (20CM *10cm) Mobile Phase: Chloroform: Ethyl acetate: acetone: Formic acid (40:30:20:10 v/v/v/v) Rf :0.15	Box-Behnken Design	[53]
8	Vildagliptin	Type of Plate: Pre-coated Silica gel aluminium plate 60 F-254(10*10 cm) Mobile Phase: Isopropyl alcohol: Methanol: Ammonia (6:4:0.2 v/v/v/v) Rf :0.5	Box-Behnken Design	[54]

CONCLUSION: -

Additionally, routine studies of clinical and pharmacological data, as well as investigations of traditional medicines and medicinal plants plants, greatly benefit from the HPTLC technique, analyses of foods and dietary supplements, analyses of environmental factors, analyses of cosmetics and toxicology, analyses of plants and herbs, and analyses of food and food supplements. AQbD in HPTLC is essential to understand different factors showing significant impact on method outcome. These are the TLC plate, volume of injection, selected mobile phase, The amount of time needed for plate development, Detection method. In conclusion, the HPTLC method should display robustness and facilitate use for a longer period, along with very low potential of failure. The approach's overall benefits include increased method proficiency, less variability, fewer trials, which lowers method costs, and decreased time consumption.

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