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"APPLICATION OF NOVEL QBD APPROACH IN ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ANALYSIS OF COENZYME Q10 IN SINGLE AND COMBINED DOSAGE FORMS"

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Abstract

Objective: A HPLC Method development and Validation by using QbD Tools/Approach for Coenzyme Q10 and its Combination.
Purpose: In Coenzyme Q10, Q refers to the quinine chemical group and 10 refer to the number of isoprenyl chemical subunit in its tail. In general ubiquinones, the number can be anywhere from 6 to 10. It is very important part of the Electron Transport Chain and participates in aerobic cellular respiration, which produces energy in the form of ATP. A study reported that 95% of the Human body's energy is produced this way. Therefore, those organs with the higher energy requirements - such as the liver, heart and kidney- have the highest CoQ10 concentrations. Coenzyme Q10 (CoQ10) is a fat soluble, vitamin-like naturally occurring substance.
The purpose of this research is to evaluate the recently published evidence on the development and validation of HPLC methods for Coenzyme Q10 using the QbD approach. While a few QbD-based stability-indicating RP-HPLC methods for Coenzyme Q10 have been reported, there are no QbD-based spectroscopic methods or QbD-based methods for Coenzyme Q10 in combination with other drugs. Therefore, it is worth considering the development of a QbD-based analytical method for Coenzyme Q10, both alone and in combination with clomiphene citrate, and applying this method to analyze its research or marketed formulations. Furthermore, a PSAR study indicates that no QbD-based analytical method has been patented for Coenzyme Q10, either alone or in combination with other drugs.

Keywords: - Coenzyme Q10 (CoQ10), RP-HPLC, Analytical QbD, Patent Search Analysis Research (PSAR)

1. INTRODUCTION [1-12]

Coenzyme Q10 (CoQ10), or ubiquinone, is a naturally occurring compound that plays a pivotal role in the mitochondrial respiratory chain, facilitating ATP production while exhibiting antioxidant properties. Due to its various therapeutic applications—from cardiovascular health to skin care—there is a rising need for reliable and efficient analytical methodologies to quantify CoQ10 in different formulation types, including tablets, capsules, and topical preparations. Traditional analytical methods often fall short in terms of robustness and regulatory compliance. Hence, the application of Quality by Design (QbD) principles has emerged as a compelling approach for developing and validating analytical methods.
Coenzyme Q10, or CoQ10, is a naturally occurring, fat-soluble vitamin-like compound. The "Q" in CoQ10 stands for the quinone chemical group, while the "10" represents the number of isoprenyl chemical subunits in its tail. For ubiquinone in general, this number can range from 6 to 10. CoQ10 is a crucial component of the Electron Transport Chain and plays a role in aerobic cellular respiration, which generates ATP, the body's primary energy source. According to research, this process is responsible for producing 95% of the energy in the human body. Consequently, the highest concentrations of CoQ10 are found in organs with greater energy demands, such as the liver, heart, and kidneys.
Clomiphene (also known as clomiphene citrate) is an FDA-approved selective estrogen receptor modulator (SERM) used to treat anovulatory or oligo-ovulatory infertility and to promote ovulation in individuals who wish to become pregnant. Using clomiphene to induce pregnancy can achieve a 6-month live birth rate of 20% to 40%. Clomiphene can be used alone or in combination with an adjuvant, such as acupuncture or dry needling therapy.
Patients most likely to benefit from clomiphene citrate include those with polycystic ovarian syndrome (PCOS), post-oral contraceptive amenorrhea, amenorrhea-galactorrhoea syndrome, psychogenic amenorrhea, and certain cases of secondary amenorrhea. However, individuals prescribed clomiphene must not have hepatic impairment, vaginal haemorrhage, or ovarian cysts.

1.1 Chemical Structure Of Coenzyme Q10 And Clomiphene Citrate:

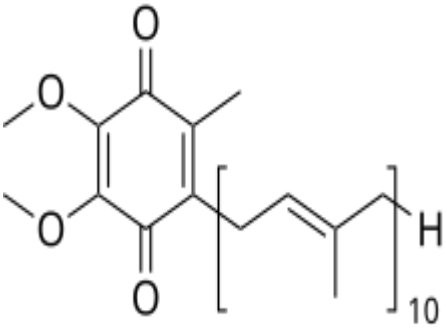

Figure 1 Coenzyme Q10

https://en.wikipedia.org/wiki/Coenzyme_Q10

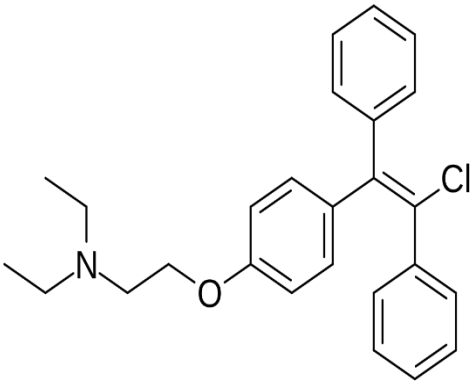

Figure 2 Clomiphene Citrate

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2. QUALITY BY DESIGN (QbD) APPROACH [4-22,37,38,39,43,52]

The pharmaceutical sector is constantly looking for ways to improve and ensure the efficacy, quality, and safety of its products. However, in recent years, the industry has faced significant challenges, such as medication recalls, manufacturing failure costs, scale-up concerns, and regulatory complexity. Traditionally, end-product testing was primarily used to ensure product quality and performance, with limited understanding of the underlying processes or key process factors. As a result, regulatory agencies are now focusing on implementing Quality by Design (QbD), a science-based methodology that improves process understanding by reducing process variation and enabling process-control techniques. QbD is designed to build quality into the process from the start, rather than just testing the final product. This proactive approach helps mitigate risks and improve overall product reliability.

2.1 Overview Of Quality By Design (Qbd): QbD is an innovative approach that considers quality as a fundamental characteristic of products right from the development stage. It encompasses principles such as:

a. **Quality Target Product Profile (QTPP):** A clear definition of the desired product qualities, including parameters like potency, purity, and stability.

b. **Critical Quality Attributes (CQA):** The qualitative and quantitative properties of a product that must be controlled to ensure its verified quality.

c. **Critical Process Parameters (CPP):** Factors that may affect the CQA, which must be monitored and controlled during method development.

d. **Design Space:** A multidimensional space that defines the range of conditions under which a process can be expected to deliver the desired quality.

By applying these principles, the development and validation of analytical methods can become more systematic, predictable, and aligned with regulatory standards.

2.3 Representation Of QbD Approach:

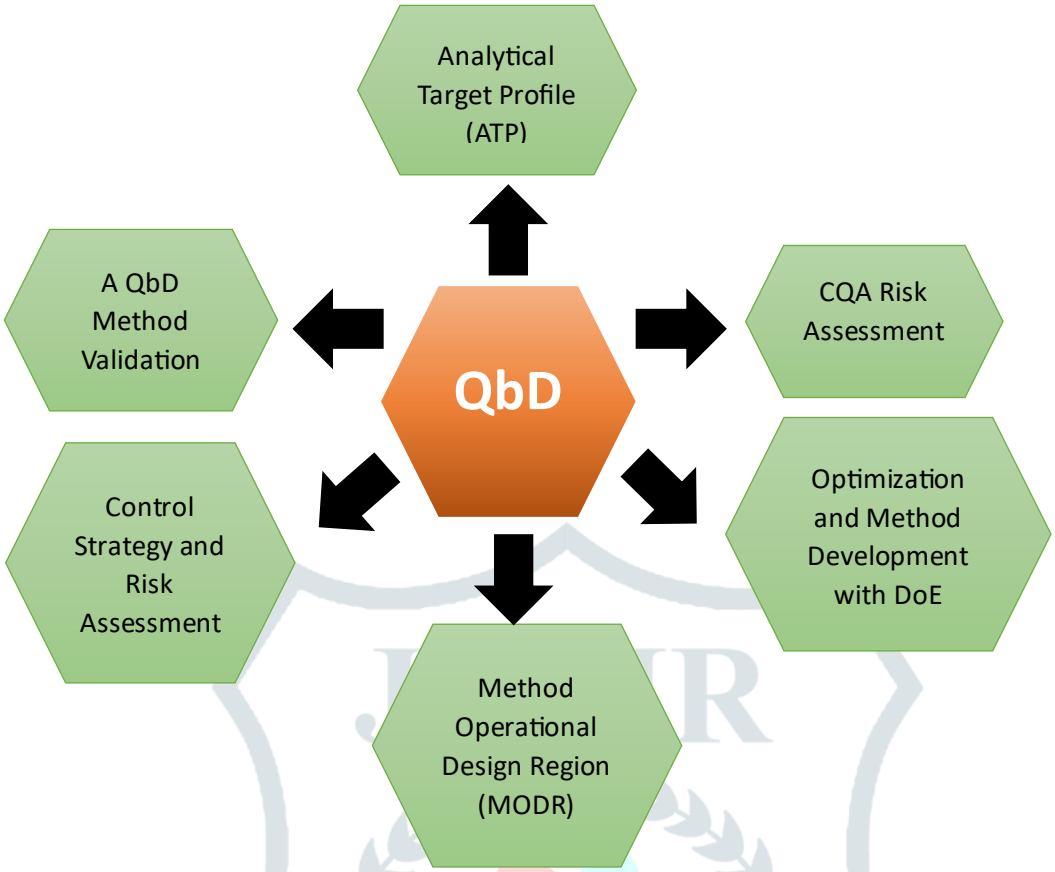
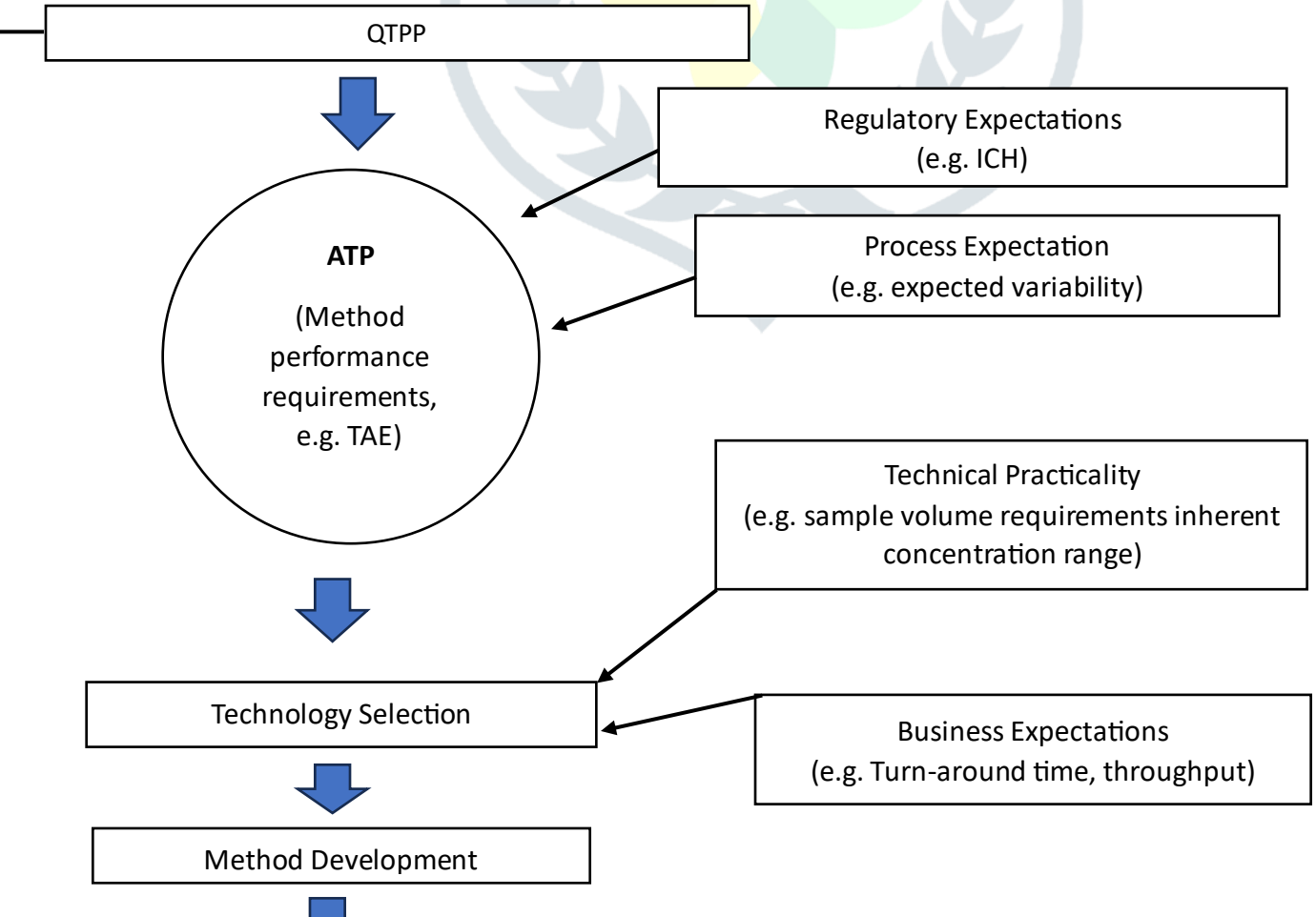


Figure 3: Pictorial Representation of QbD Approach.

2.4 Flow Chart Representation of Process for Analytical Quality by Design :



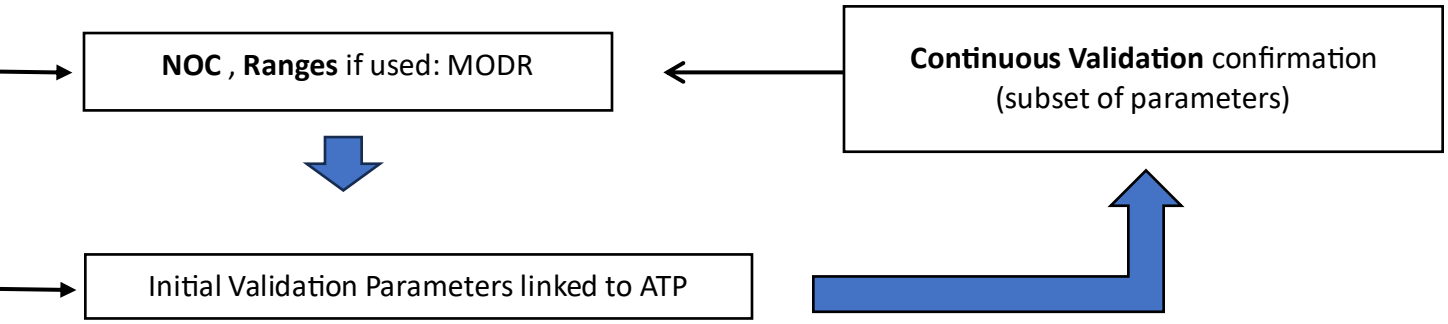


Figure 4: Flow Chart Representation of Process for Analytical Quality by Design

2.5 APPLICATION OF QBD IN ANALYTICAL METHOD DEVELOPMENT FOR COQ10 IN SINGLE AND/OR COMBINATIONS:

2.5.1 Defining The Quality Target Product Profile (QTPP)

In developing an analytical method for CoQ10, the QTPP must be defined. Key attributes include:

a. **Sensitivity:** The method's ability to detect low concentrations of CoQ10.
b. **Specificity:** The method's capability to measure CoQ10 accurately in the presence of other compounds, especially in combined formulations.
c. **Robustness:** The method's performance under varied operational conditions to ensure consistent results.

2.5.2 Identification Of Critical Quality Attributes (CQA)

The CQAs for CoQ10 analytical methods might include:

i. **Linearity:** The method's ability to provide results that are directly proportional to the concentration of CoQ10 within a specified range.
ii. **Accuracy:** The closeness of the test results to the true value.
iii. **Precision:** The degree of agreement among individual test results when the method is applied repeatedly to multiple aliquots of a homogeneous sample.

2.5.3 Risk Assessment And Design Of Experiments (DOE)

Employing tools like Failure Mode and Effects Analysis (FMEA) can help identify risks associated with various analytical parameters, such as temperature, solvent composition, and instrument calibration. Using a Design of Experiments (DoE) approach facilitates the systematic exploration of the method parameters to find an optimal analytical method for CoQ10.

2.5.4 Implementation Of Design Space

The determined design space for CoQ10 analytical methods includes variable ranges for temperature, time, pH, and mobile phase composition. This can lead to the establishment of a robust method capable of withstanding variations in analytical conditions while consistently delivering valid results.

3. HIGH PERFORMANCE LIQUID CHROMATOGHRAPHY (HPLC) [8-12,19,20,23-43,52]

3.1 Introduction Of HPLC

For analytical chemistry, one of the most effective tools is High Performance Liquid Chromatography (HPLC). Any substance that can dissolve in a liquid can be identified, separated, and quantified using this method. For both quantitative and qualitative drug product analysis, as well as for assessing and determining the stability of drug products, the HPLC is the most precise technique available.

One of the most crucial instruments in analytical chemistry nowadays is High Performance Liquid Chromatography (HPLC), which was developed from traditional column chromatography. The methods for identifying, separating, and quantifying the chemical components of both natural and manmade materials are the focus of analytical chemistry.

The qualitative and quantitative composition of materials is studied using analytical chemistry. Understanding the sample material requires both of these elements. Quantitative and qualitative are the two subfields of analytical chemistry. The presence or absence of specific components provides us with information about the sample's nature through a qualitative examination. Numerical information regarding the proportion of one or more of these components is provided by a quantitative analysis. Different analytical techniques are frequently employed for pharmaceutical formulation and bulk drug sample analysis.

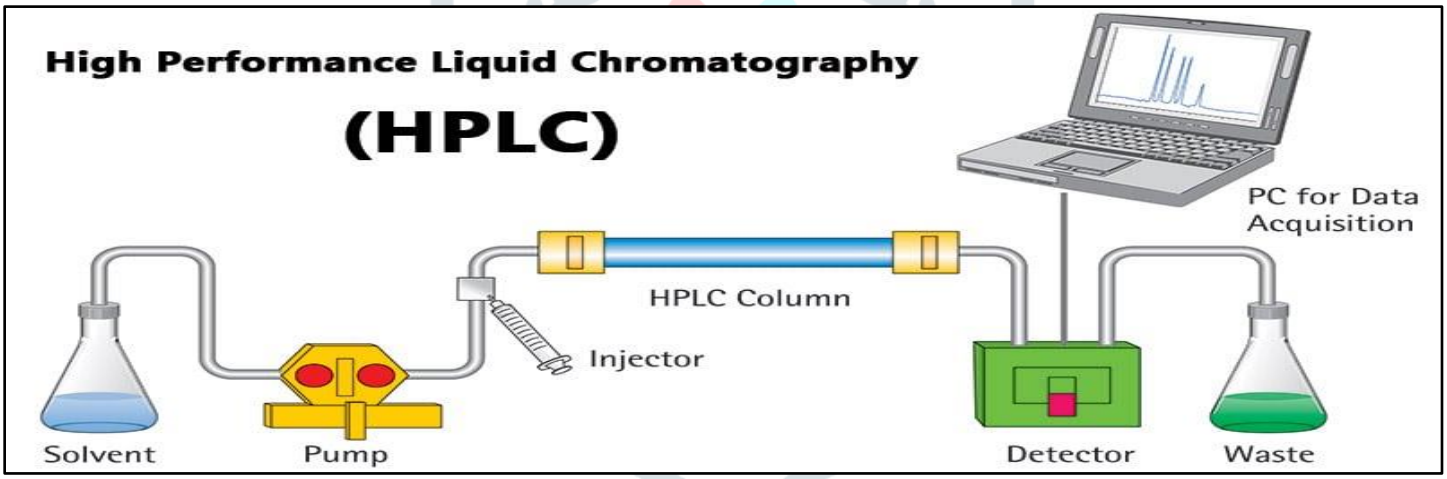
High performance liquid chromatography (HPLC) is a key and essential analytical technology used in all phases of medication research, manufacture, and discovery in the contemporary pharmaceutical business. One of the greatest techniques for determining the peak purity of novel chemical entities, tracking changes in reactions during scale-up or synthesis processes, assessing novel formulations, performing quality control, and ensuring the final therapeutic product is HPLC. The primary medication, any reaction impurities, all available synthetic intermediates, and any degradants are to be separated and quantified using the HPLC method.

3.2 HPLC Principle:

Adsorption is the primary principle used in HPLC. A liquid (mobile phase) is pushed through a porous material column (stationary phase) under high pressure after the sample solution is put into the column. Based on the variations in migration rates through the column caused by distinct sample partitions between the stationary and mobile phases, the sample is separated.
A combination of components moves through an HPLC column in accordance with their relative affinities for the stationary phase. Elution occurs at different times depending on the partition behavior of various components. A component with a higher affinity for the adsorbent moves more slowly. The component that moves more quickly has a lower attraction for the stationary phase.

The technique of HPLC has following features:

a. High resolution
b. Rapid analysis
c. Controlled flow rate of mobile phase
d. Small diameter, Stainless steel, Glass column
e. High sensitivity
f. High accuracy
g. Easy to purify the sample
h. Relatively high inlet pressure and controlled flow of mobile phase
i. Ease of automation
j. Good repeatability
k. Simultaneous Analysis



3.3 HPLC Method Development:

Methods are created for new products when there aren't any approved techniques. For current products, alternative approaches include cutting manufacturing time in exchange for increased resilience and precision. Comparative laboratory data, including advantages and disadvantages, is made available when a different method is suggested to replace an established practice. The primary active ingredient, any reactive impurities, any synthetic intermediates that may be available, and any degradants are all attempted to be extracted and quantified using the HPLC method.

Figure 5: Diagrammatical representation of HPLC
Source: https://images.app.goo.gl/6f98iSF7LEbHrkYE9

3.3.1 The Following Steps are Involved in HPLC Method Development:

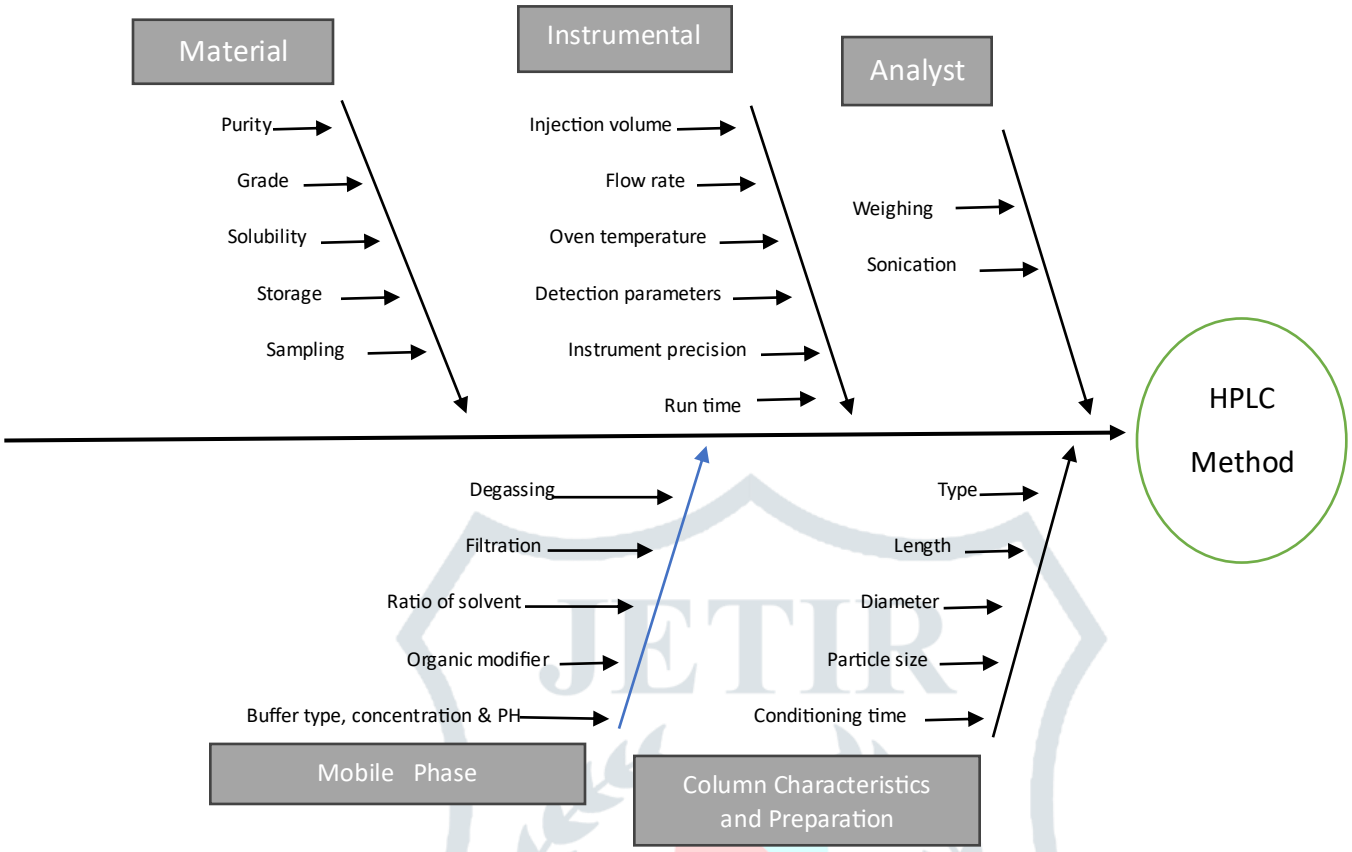


Figure 6: Steps involved in HPLC Method Development

3.3.2 The Following Steps are Involved in Optimization of HPLC Method Development:

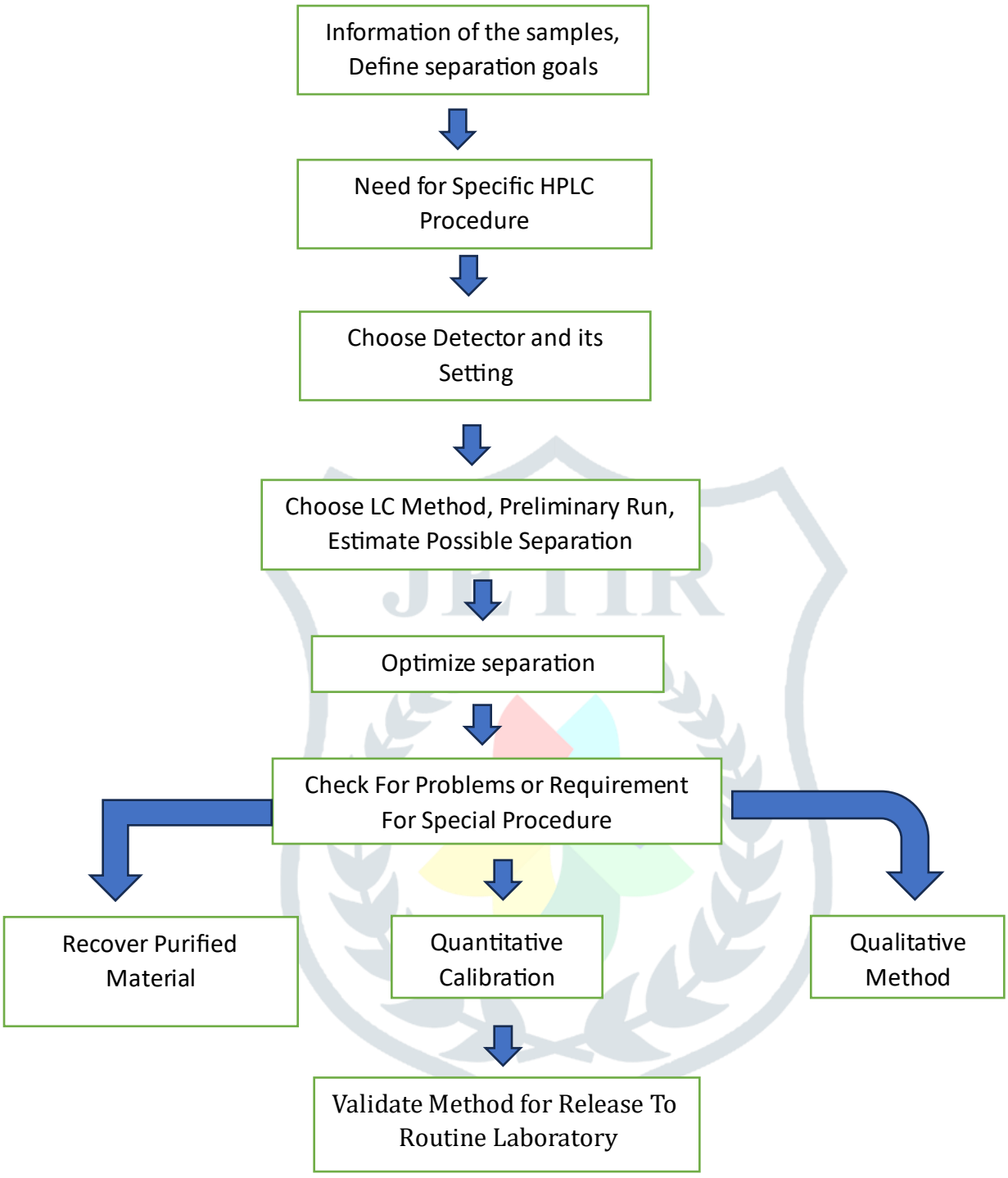


Figure 7: Steps involved in Optimization HPLC Method Development

4. ANALYTICAL METHOD DEVELOPMENT AND VALIDATION WITH THE HELP OF NOVEL QUALITY BY DESIGN [10,14,17,18,22,34-53]

The process of creating a particular analytical technique for pharmaceutical products from the in-process to the final product stage, together with the validation that must be completed before routine, investigation, and stability sample analyses may start, is known as analytical method development. The development and validation of analytical methods are crucial processes that are crucial to the discovery, creation, and production of pharmaceuticals. Pharmaceutical items' identity, potency, purity, and performance are guaranteed by these methods. Accurate, trustworthy, and coherent data can be obtained through the development and validation of

analytical techniques. Method development processes are costly, intricate, and time-consuming. An analysis method outlines the procedures and methods needed to do an analysis. This could entail employing tools, preparing standards, reagents, and samples, among other things.

Data that is accurate, dependable, and coherent is produced via the development and validation of an analytical process. Procedures for developing new methods are expensive, intricate, and time-consuming. An analysis method describes the procedures and methods required to carry out an analysis. This could entail running machinery and preparing standards, reagents, and samples.

4.1 Outline the Process of Analytical Method Development and Validation:

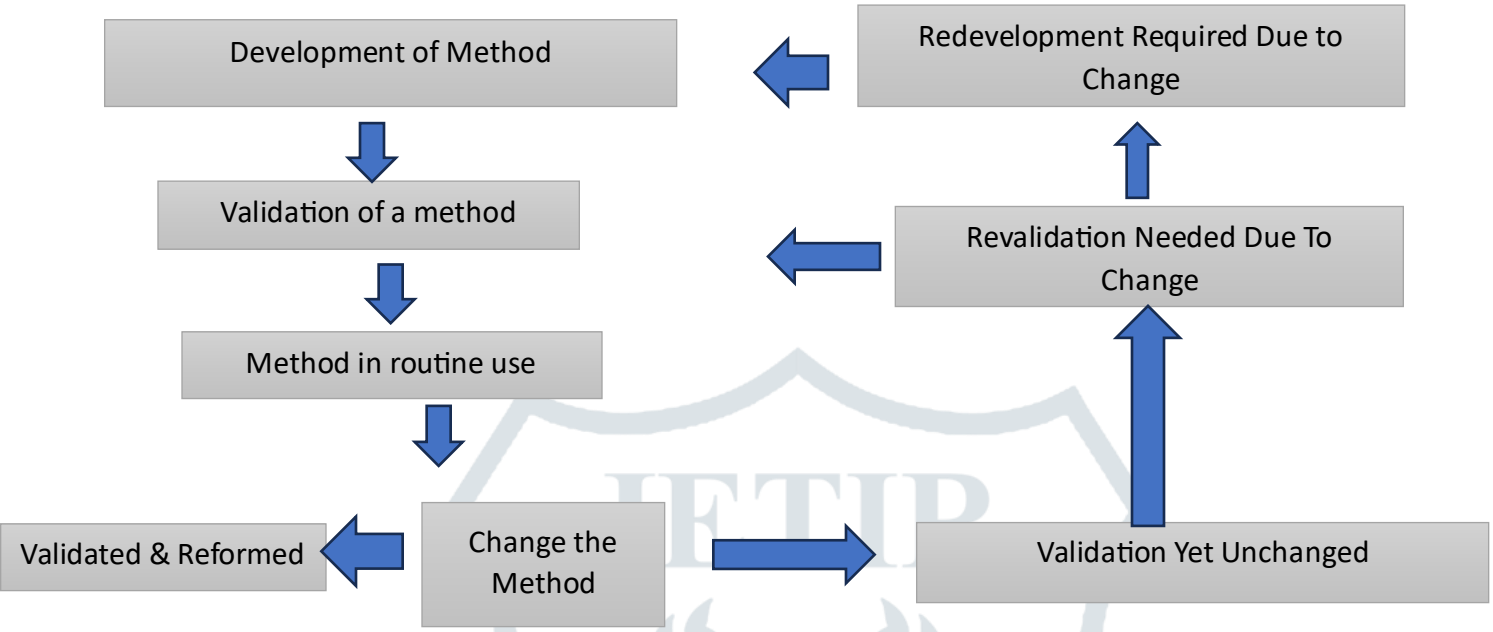


Figure 8: The Lifecycle of an Analytical Method Development

4.2 Physicochemical Properties of Drug:

Method development heavily relies on a drug molecule's physical characteristics. The first step in developing a method is to examine the drug molecule's physical characteristics, including its solubility, polarity, pKa, and pH. One of a compound's physical characteristics is polarity. The composition of the solvent and mobile phase can be ascertained with its help. Two atoms share an equal number of electrons in a non-polar covalent link. One atom is more drawn to electrons in a polar covalent bond than the other. Molecular solubility can be explained by a molecule's polarity.

Water and benzene are examples of polar and non-polar solvents that do not mix. Like dissolves like in general, meaning that substances with comparable polarity can dissolve in one another. The selection of diluents is based on the analyte's solubility. It is necessary for the analyte to dissolve in the diluents and not react with any of them. To guarantee that there is no peak distortion, particularly for early eluting components, the diluent should match the starting eluent composition of the test. A key factor in the development of HPLC methods is pH and pKa.

4.3 Quality by Design Approach In Analytical Method Development:

In a number of works, Dr. Joseph M. Juran initially introduced the idea of Quality by Design (QbD), which holds that quality can be planned. The ICH Q8 standards, which state that "quality should be built into the product by design, but quality cannot be tested in the product," addressed the idea of QbD. Quality is the acceptability of a medicine or drug substance for its intended uses. This phrase includes qualities like strength, identity, and purity. Quality by design is referred to as "a systematic approach to development that begins with a predefined objective and emphasizes product and process understanding and process control, based on sound science and quality risk management" according to ICH Q8 (R1).

The idea of incorporating quality into the analytical technique during its creation is the primary emphasis of the quality by design (QbD) principles' application to analytical method development. Thus, it is necessary to arrange the real method development process for an analytical quality by design (QbD) technique. The creation of QbD methods aims to achieve predetermined goals. HPLC serves as an example to illustrate the purpose of the development of the QbD method. The major component and any critical quality attributes (CQA) that could compromise the quality of the drug product are to be separated and quantified using the HPLC method for API. The specs should be specific, linear, accurate, precise, robust, and rugged.

4.4 Validation of Analytical Methods:

Validation is a critical step in ensuring that the analytical methods for CoQ10 in single and/or in combinations meet the required standards.

The following validation parameters, as per ICH guidelines, should be addressed:

i. **Linearity and Range**: Establishing that the method provides a linear response across the expected concentration range of CoQ10.
ii. **Accuracy and Precision**: Quantifying the reliability of the analytical method through repeated trials and comparison against known standards.
iii. **Specificity and Selectivity**: Evaluating the method's performance in the presence of excipients and potential degradation products.
iv. **Stability Indicating**: Ensuring that the method effectively indicates the stability of CoQ10 in varied formulations.
v. **Challenges in Implementation:** Despite its advantages, implementing a QbD approach presents several challenges.
vi. **Complexity of Analytical Methods:** The intricate nature of certain analytical techniques (e.g., HPLC, LC-MS) can complicate establishing CQAs and CPPs.
vii. **Regulatory Acceptance:** There is still a degree of variability in how regulatory agencies view and accept QbD-based submissions.
viii. **Resource Intensive:** Initial setup and training may demand significant time and resources.

4.5 Advantages of Adopting A QbD Approach for Analytical Method Development.:

1. Helps decrease the variation in analytical properties, strengthening the method's robustness.
2. Enhances the scientific comprehension of pharmaceutical processes and associated methods.
3. Provides the necessary design space to guide method development.
4. Allows for greater flexibility in analyzing APIs, impurities in dosage forms, stability samples, and biological metabolites.
5. Helps decrease the variation in analytical properties, strengthening the method's robustness.
6. Minimizes unnecessary deviations and expensive investigations.
7. Ensures a seamless transition for method transfers to the manufacturing stage.
8. Improves compliance with regulatory expectations and requirements.
9. Facilitates the invention of new techniques by enabling continuous improvements over the method's lifecycle.
10. Identifies and evaluates critical quality attributes and their impact on the product's end quality.

5. CONCLUSION

The application of a QbD approach in the analytical method development and validation of CoQ10 in single and/or in combinations can significantly enhance the quality, reliability, and efficiency of quantification in both single and combined dosage forms. Adopting these principles fosters a more systematic understanding of analytical processes, enabling more robust methodologies that comply with regulatory expectations. As the field of pharmaceutical analysis continues to evolve, integrating QbD principles will be pivotal in meeting the growing demand for high-quality CoQ10 formulations.

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