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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF DRUG IN TABLET FROM USING QBD APPROACH

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ABSTRACT

Understanding the important components and their interplay effects by conducting a desired set of experiments has lately acquired prominence in the field of analytical method development. As a result, this study discusses the QbD approach to RP-HPLC method development utilising Design of Experiments, as well as the technique's validation for analysis. HPLC is an analytical method that is capable of separating, detecting, and quantifying different pharmaceuticals and their associated degradation products. Separating produced pharmaceuticals from drug-related impurities, detecting and quantifying synthesised drugs, and reducing other contaminants during separation are all uses of this technique that are commonplace. Analyses were carried out to determine the best chromatographic parameters for the procedure. Drugs and impurities must be compatible and stable in a mobile phase that is compatible with a column, column temperature, wavelength and gradient. Analysis technique development is vital, and this study discusses the significance of RP-HPLCs and their methodologies, as well as a short understanding of key chromatographic parameters.

Keywords: Quality by design approach, RP-HPLC, Validation, Development

1. INTRODUCTION:

HPLC is an analytical method that is capable of separating, detecting, and quantifying different pharmaceuticals and their associated degradation products.

Separating produced pharmaceuticals from drug-related impurities, detecting and quantifying synthesised drugs, and reducing other contaminants during separation are all uses of this technique that are commonplace. Analyses were carried out to determine the best chromatographic parameters for the procedure. In order to ensure the compatibility and stability of the medicine as well as the degradants and contaminants, an adequate mobile phase, column, column temperature, wavelength, and gradient must be identified (1).

The goal of pharmaceutical development is to create a high-quality product and manufacturing process that deliver on the product's promise of performance time and time again. To complement the design space, specifications and production controls, pharmaceutical development research and manufacturing experience give scientific insight. Quality risk management might be based on data from pharmaceutical development research. Quality cannot be tested into things; instead, it should be included into the design from the outset. In the course of product development and lifecycle management, it is important to see changes in formulation and manufacturing processes as chances to learn more and contribute to the creation of the design space. Experiments with unexpected findings that provide important information might also be included in the study. The applicant proposes the design space, which is then evaluated and approved by regulatory authorities. Working in the design industry is not seen as a shift in one's career path. Post-approval modification processes often begin when a product moves out of the design area (2).

Product development strategies differ from business to company and from product to product, but they should always be geared toward meeting the demands of patients and achieving the desired performance of the product. The method may also differ, and this should be explained in the submission as a whole in the body of the document. A product developer might use an empirical technique, a more systematic approach, or a mix of the two to design their product. Prior knowledge, the outcomes of trials employing design of experiments, quality risk management, and the usage of knowledge management (ICH Q10) may all be incorporated into a more systematic approach to development. To achieve the intended product quality, a methodical approach might assist regulators better comprehend a company's plan. It is possible to keep up-to-date on product and process knowledge over the product's lifespan (3).

The concept of Quality by Design has gained a lot of importance in Pharma Industry, and any analytical method developed using QbD principles does not need redevelopment, revalidation, and reapproval during transfers, provided one work within the limits of the approved Design Space, thereby saving time and resources

2. Quality by Design Approach:

Dr. Joseph M. Juran, a quality pioneer, coined the term "quality by design," or "QbD." Dr. Juran was of the opinion that quality should be built into a product from the start, and that most quality issues stem from poor product design. As defined by Woodcock, a high-quality medicine product delivers the therapeutic benefit claimed on the label to consumers while being free of contamination. Drug research, production, and regulation in the United States are all guided by FDA's Quality by Design (QbD) guidelines and risk-based methods. QbD was introduced by the FDA since it was realised that more testing does not always lead to better product quality. The product must be made with quality in mind. As part of the development and production process, Quality by Design (QbD) is essential. To make certain that a finished therapeutic product performs as planned, both in terms of purity and effectiveness, this process is used. This can only be achieved if the goals and risks are clearly defined. This comprises a detailed grasp of the technical process, factors that impact the process, and the performance envelope in which those variables stay in order to fulfil the goals. both the user and the producer will profit from QbD's benefits, because the product will be continuously safe and effective, as well as well understood, regulated and predictable (4).

2.1 Pharmaceutical Quality by Design Objectives:

Systematic pharmaceutical QbD starts with stated goals and stresses product and process knowledge and control based on strong science and quality risk management (5). The following are possible pharmaceutical QbD objectives:

1. For relevant product quality standards that are based on clinical results.
2. To improve product and process design, knowledge, and control in order to maximise process capacity and decrease product variability and failures.
3. In order to improve product development and production processes

Root cause analysis and post-approval change tracking will be improved with this strategy.

Quality by Design (QbD) may frequently be used to accomplish these aims by tying product quality to intended clinical

performance and then developing a strong formulation and manufacturing process that consistently delivers the required product quality.

Why is QBD needed in approch to RP-HPLC

Quality by Design (QbD) has become an important concept for the pharmaceutical industry, and ICH recommends this concept., the QbD approach was used to develop a reverse-phase–high-performance liquid chromatography (RP–HPLC) method that could be applied for the estimation of several antibacterial agents of the drug in table form.

2.3 Benefits of QBD (6)

Under the conventional Quality by Control scheme, drugmakers had no opportunity to make changes in processes and controls. The regulatory procedure had to be repeated every time a modification was required. QbD's capacity to make modest modifications to regulatory papers is a big advantage, allowing for minor adjustments. All of this means that there is always room for improvement. The procedure doesn't finish after the medicine is on the market. This may have a considerable impact on the pharmaceutical industry, both in terms of cost and time. When the FDA is more lenient, new medications are often approved more quickly. QbD can assist speed up this procedure because of its sturdy character, which assures the safety of consumers.

- In the business world, QbD is an excellent choice.
- Prevent batch failures and expensive investigations by reducing deviations and regulatory compliance issues.
- Quality-by-Design (QbD) is excellent science, and it may lead to better
- Development choices and the empowerment of technical employees.

3. Reversed Phase Chromatography (RP-HPLC):

In the field of biological separation and purification, reversed phase chromatography has been used for both analytical and preparative purposes. Reversed phase chromatography is an effective method for separating hydrophobic molecules with high recovery and resolution (7).

Solute molecules in the mobile phase interact with the stationary phase's hydrophobic ligand (the stationary phase) to form hydrophobic binding interactions. Conventional wisdom is that the hydrophobic binding contact is the consequence of a favourable entropy effect, however the true nature of the binding interaction is up to question. A considerable degree of water structure is seen around both the solute molecule and the immobilised ligand in reversed-phase

chromatography's early mobile phase binding conditions. An immobilised hydrophobic ligand reduces exposure of the hydrophobic ligand to the solvent. As a result, the level of organised water structure decreases, which results in an increase in system entropy that is beneficial. Solutes and ligands, both hydrophobic, may benefit from this arrangement from an energy point of view (Figure 2). (8).

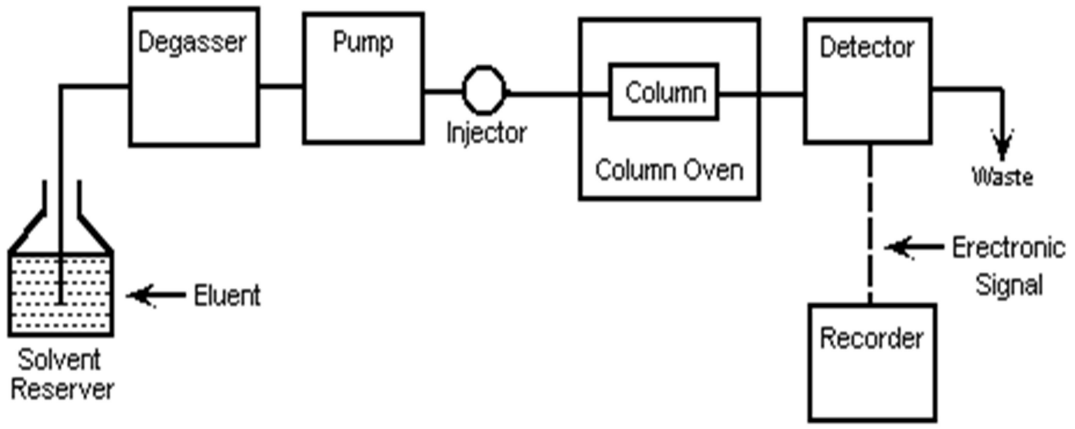

Fig 1: Components of HPLC System

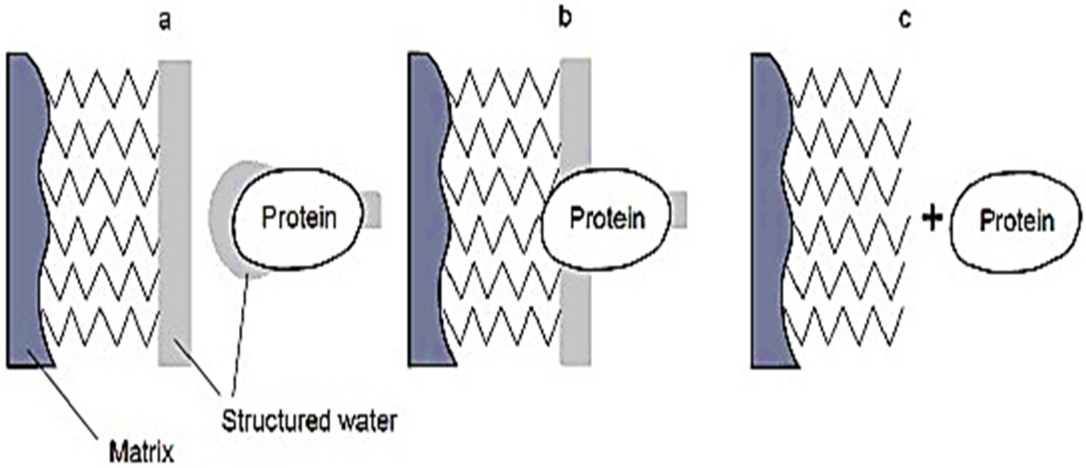

Figure 2: Interaction of A Solute with A Typical Reversed Phase Medium

For quantitative analysis, HPLC is one of the most helpful technologies available. RPC refers to the use of reverse phase chromatography, which is a kind of chromatography that employs polar mobile phases and non-polar stationary phases. For quantitative and qualitative analysis, HPLC is always injected with another analytical equipment. The system's mode of operation is isocratic, which means that a single solvent or mixture is pumped throughout the analysis in order to make a specific determination. In order to achieve gradient elution, it is possible to progressively alter

the solvent composition. Diffusion regulates the rate of dispersion between the stationary and mobile phases. Decreases in diffusion allow for quicker and more efficient purification (9).

RP-HPLC Method development as per qbd approach

i. **Define method intent**

QbD is a systemic, scientific, risk based, holistic and proactive approach that begins with predefined objectives and emphasizes product and process understanding and control

ii. **Perform experimental design**

It is an efficient and comprehensive experimental design based on systematic scouting of two key components of the RP-HPLC method (mobile phase and pH) is presented. It forms a chromatographic database that will assist with method understanding, optimization and selection. In addition, it can be used to evaluate and implement change of the method.

iii. **Evaluate experimental results and select final method conditions**

These were evaluated using the three tiered approach. At the first, the conditions were evaluated for peaks symmetry, retention time and peaks tailing. This resulted in different chromatographic conditions for API. The best suited experimental conditions shall be optimized using design expert software

iv. **Perform risk assessment with robustness and ruggedness evaluation**

the evaluation of method robustness and ruggedness to be carried out as the final step of method development is mainly for the method verification and finalization

- **Stability of analytical solution**

A stability analysis will be performed on both the normal and test solutions. A test sample of tablet will be utilised to determine the stability of the test solution. The stability test will be performed in a standard laboratory environment

The solution will be held in a brightly lit laboratory for 12 to 24 h before being analyzed. The discrepancy between the test solution's results at each

stability time point and the original will be calculated for the test solution stability analysis. The discrepancy between the effects of the stability time point and the original will be calculated in a standard solution stability analysis.

3.1Analytical Validation of RP-HPLC:

An evaluation of validity or an activity to demonstrate efficacy are both examples of validation. Assuring that an analytical technique may be used for its intended purpose is known as method validation. Pharmacopeia (USP), ICH (International Conference on Harmonization) and FDA (Food and Drug Administration) standards offer a framework for completing such validations for pharmaceutical techniques (10).

The method developed was validated as per ICH guidelines by evaluating parameters such as accuracy, precision, linearity, robustness, ruggedness, detection, and quantification limits.The results were evaluated considering acceptable limits as less than 2% for Relative Standard Deviation

- **Parameters used for Assay Validation**

The validation of the assay procedure was carried out as per ICH guidelines using the following parameters.

1. Specificity

Specificity is the capacity to access the analyte in the presence of the components that may be anticipated to be present. It is important for chromatographic procedures to demonstrate specificity, which is the capacity to precisely detect the analyte response in the presence of all possible sample components. All possible sample components (placebo formulation, process contaminants, etc.) are compared to the analyte's reaction in a test mixture including the analyte and the analyte alone. The analyte peak must have a baseline chromatographic resolution of at least 1.5 from all other sample components to be considered specific. If this isn't possible, the final test result will be affected by no more than 5% by the unresolved components at their highest predicted level (11).

2. Linearity

Analysis procedures are linear when they can provide findings that are directly correlated with analyte concentration (amount) within a predetermined range. Typically, standard solutions are prepared at five concentration levels for test procedures. Curvature in the displayed data can only be detected at a level of five.

Testing for linearity by plotting the response vs. concentration means looking at the linear regression line's y-intercept and correlation. Evidence of a relationship between the data and a regression line is usually deemed to have a correlation value of >0.999. More than one or two percent of analyte responses obtained at target level should not be used to calculate the y-intercept (12).

3. Accuracy

The accuracy of an analytical procedure expresses the closeness of the agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method is the closeness of the measured value to the true value for the sample. Accuracy is usually determined in one of four ways.

• Analysing a sample with a known concentration and comparing the measured result to the real amount may be used to determine accuracy.

• To compare test findings from the new approach with data from an alternative method proven to be accurate.

• Spiking analyte into blank matrices is the most common method of conducting a recovery investigation. At the 50-150 percent target concentration, a spiking sample is generated in triplicate at three levels.

• Standard additions, which may also be used to assess spiking analyte recovery. If the analyte cannot be removed from the blank sample matrix, this method is utilised.

At concentrations between 80% and 120% of the target concentration, the mean recovery must be more than or equal to 100+2% to be considered accurate (13).

4. Range

For example, the higher and lower concentrations of analyte in the sample, as well as those values for which the analytical technique has been shown to be accurate, precise, and linear are included in the range of analytical procedure. Data from linearity and accuracy studies are used to define the range. If linearity and accuracy can be achieved with a 3 percent RSD and a precision of an assay technique above the acceptable range, then the assay method's range requirements will be considered to be acceptable (14).

5. Precision

A procedure's accuracy is expressed as the degree to which successive measurements of the same homogeneous sample under the same circumstances provide similar results. It is common to describe the accuracy of an analytical technique in terms of standard deviation, standard error, or coefficient of variation. An injection repeatability study is the initial precision investigation. A

minimum of 10 injections of one sample solution are conducted to test the chromatographic instrument's performance. Reproducibility or intra-assay precision is the second form of consistency. One day is all that is needed to collect intra-assay precision data. Samples of homogeneity, each created according to the technique process, are aliquots. The number of duplicate samples to be created and the number of injections needed for each sample in the final technique process will be determined by these precision studies. For an assay procedure, the instrument precision (RSD) will be 1% and the intra-assay precision (RSD) will be 2%, as an example (15).

6. Detection limit

Each analyte technique has a detection limit, which is the smallest quantity of analyte in a sample that can be detected but is not necessarily regarded as a precise measurement. Limit of detection based on the response and slope standard derivation.

Detection limit (or) limit of detection may be expressed as,

$$DL = [3.3\sigma/S]$$

Were,

σ =standard deviation of the response

S =slope of the calibration curve (of the analyte)

7. Quantitation limit

The quantitation of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantitation limit based on the standard deviation of the response and the slope. It can be expressed as,

$$QL = [10\sigma/S]$$

σ =standard deviation of the response

S =slope of the calibration curve (of the analyte)

8. Ruggedness

There are several factors that contribute to an analytical method's ruggedness, such as the degree of repeatability of test results and the ability to analyse data from various sources. Different reagent sources, assay timeframes, and assay temperature conditions are all factors that might affect the results of an experiment. Ruggedness is the ability of a test result to be reliably reproduced in a variety of testing environments. The RSD should not exceed 2% as a roughness criterion (16).

9. Robustness

The capacity of a technique to withstand slight variations in parameters such as the percentage of organic content, pH of the mobile phase, buffer concentration, temperature and injection volume is known as robustness. The RSD should not exceed 2 % as a measure of robustness.

10.System suitability testing

Analytical processes often include system suitability assessment as a component of the process. All aspects of testing are examined as a single integrated system that can be evaluated as a whole. This is how the tests are conducted. Typically, five injections of a standard solution are made and chromatographic characteristics such as resolution, area percent repeatability, the number of theoretical plates, and the tailing factor are evaluated(17).

3.2 Applications of HPLC method (18)

- HPLC is the most widely used chromatographic technology for purification of all sorts of biological molecules because of its versatility, speed, and sensitivity.
- Because biological fluids like serum and urine may be applied directly to the column in the system, it is frequently employed in clinical and pharmaceutical operations.
- In terms of oligopeptide and protein separation, RP-HPLC has the greatest influence.
- Wide variety of organic-chemistry-related
- Ion exclusion chromatography and ion exchange ion pair chromatography may be used to separate anions in chromatography.
- For the cation separation of inert polymer resins, chromatography has been utilised.
- Most widely used in Agri chemicals i.e., analysis of pesticides in cleaning water.
- In the field of agriculture, pesticide analysis in cleansing water is the most common use.
- For the most part, it's used in food testing, Morphine and metabolites isolated from blood plasma are widely used in forensic research.
- Modern uses are mostly in the pharmaceutical industry.
- Ligand exchange chromatography.
- Analyse finished drug products and their ingredients quantitatively and quatitatively during the manufacturing process

DISCUSSION:

The aim of this project was to create a simple, reliable, precise, and appropriate RP-HPLC system using the Quality by Design (QbD) approach. In terms of analytical method creation and validation, The established method's analysis results were validated in terms of linearity, accuracy, precision, and robustness, as well as the detection and quantification limits

In the reported research work, the RP-HPLC analytical method for the selected drug in tablet form was developed and

validated as per the ICH guidelines. Since all the validation parameters checked were within limits, the method was considered successfully validated.This HPLC method will prove to be advantageous for laboratories handling numerous fluoroquinolones

4. CONCLUSION

HPLC technique development and validation was shown to be precise, accurate, and dependable. Pharmaceutical dose forms may be studied using this strategy to determine their effectiveness or therapeutic action. Further research is recommended to examine the durability of pharmaceutical formulations since they might be successfully separated. Its features were good selectivity, sensitivity, cost-efficiency, speed, and a low detection limit. This HPLC method will prove to be advantageous for laboratories handling numerous fluoroquinolones, especially since the method may be transferred without the need for revalidation within the Design Space.

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