Analytical Method Development of Piribedil In A Tablet Dosage Form By Using QBD Approach

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ABSTRACT

The present study aimed to develop and validate a robust, sensitive, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the quantification of Piribedil in tablet dosage form. A systematic Quality by Design (QbD) approach was implemented, employing a 2^3 full factorial experimental design to optimize key chromatographic parameters including acetonitrile composition, flow rate, and column temperature. The optimized method utilized a C18 column with a mobile phase consisting of acetonitrile and phosphate buffer (pH 4.0) in a 65:35 v/v ratio, at a flow rate of 1.0 mL/min, with UV detection at 280 nm. Under these conditions, Piribedil was eluted at 3.10 minutes with excellent peak symmetry, a tailing factor of 1.22, and a theoretical plate count above 3100. The method was validated according to ICH Q2(R1) guidelines and demonstrated linearity over the concentration range of 10–60 µg/mL ($R^2 = 0.9994$), accuracy with recoveries between 99.12% and 99.83%, and precision with %RSD values below 1.3%. Sensitivity was confirmed with a limit of detection (LOD) of 0.86 µg/mL and a limit of quantification (LOQ) of 2.61 µg/mL. Robustness studies affirmed the method's consistency under minor variations in chromatographic conditions. Forced degradation studies under acidic, basic, oxidative, thermal, and photolytic conditions confirmed the stability-indicating capability of the method. The developed method is reliable for routine analysis and suitable for the quality control and stability testing of Piribedil formulations.

KEYWORDS

Piribedil, RP-HPLC, Method validation, Quality by Design (QbD), Forced degradation, ICH Q2(R1), Stability-indicating method, Factorial design, Pharmaceutical analysis

1. INTRODUCTION

1.1. Overview of Piribedil and Its Therapeutic Significance

1.1.1 Drug Profile and Pharmacological Properties

Piribedil is a made chemical agent that works as a dopamine receptor agonist, mainly attaching to D2 and D3 receptors in the brain. It is classed chemically as a pyrimidobenzodiazepine derivative, and this gives it particular physicochemical and pharmacological features. The official name of Piribedil as given by IUPAC is 2-[4-(1,3-benzodioxol-5-ylmethyl)piperazin-1-yl]pyrimidine, and it contains the chemical formula C16H20N4O2 with a weight of 300.36 g/mol.

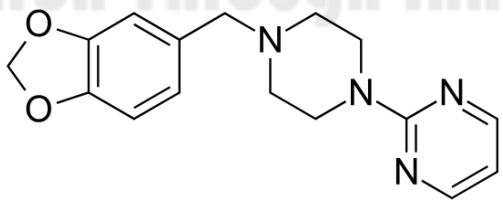

Fig 1.1: Structure of Piribedil

The selectivity and activity of Piribedil at dopaminergic sites are due to the fact that it has a piperazine ring joined to a pyrimidine core and a benzodioxole group. Since pyrimidine adds basicity to the drug, and the benzodioxole moiety increases its lipophilicity, the molecule can cross the blood-brain barrier and positively affect the dopamine receptors found in the brain. Pharmaceutical

companies like to use Piribedil hydrochloride, as it is more stable and water-soluble, during production of oral tablet versions of Piribedil. Thanks to its unique structure, it can be used alone or together with other drugs for treating Parkinson's disease and similar conditions.

Mechanism of Action

Piribedil acts mainly by stimulating dopamine D2 and D3 receptors in the striatum and substantia nigra, both of which are important for coordinating body movements caused by nerve impulses. Piribedil stimulates these receptors and helps make up for the reduced dopamine in the brain often found with Parkinson's disease, which causes the distinguishing symptoms of tremors, slowed movements, muscle rigidity, and poor balance.[3] Along with improving dopamine levels, Piribedil also blocks α2-adrenergic receptors, especially those in the synapse in the brain, which increases norepinephrine and improves the supply of blood to the brain. It makes Piribedil unique compared to other dopamine agonists as it helps with Parkinson's disease and offers cognitive and vascular-related benefits.

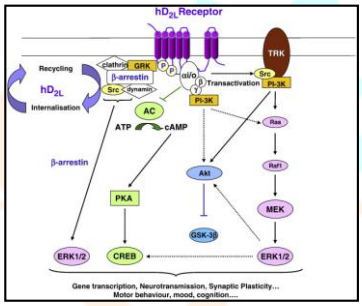

Fig 1.2: Mechanism of action of piribedil

For this reason, Piribedil has also been studied as a possible solution for the non-motor symptoms associated with Parkinson's, which are depression, apathy, and difficulties with memory. As well as the dopaminergic effect, Piribedil offers adrenergic activity, so it is an important drug to use in treating Parkinson's and cognitive problems caused by vascular factors.

1.1.2 Therapeutic Applications and Clinical Relevance

Due to its effectiveness in treating CNS conditions, medical research has brought attention to piribedil. The main reason for taking it is to manage Parkinson's disease (PD), since this disease is caused by a loss of dopamine in the basal ganglia, especially in the substantia nigra pars compacta. The disease is identified by tremor, slowed movements, stiff muscles, problems with balance, and a wide range of other non-movement symptoms like memory loss, sleep issues, depression, and problems with the body's automatic functions.[6] Piribedil works well in Parkinson's disease since it acts as a D2/D3 receptor agonist and helps to replace the dopamine activity that is missing in the nigrostriatal pathway. For patients in the early phase of Parkinson's disease, Piribedil is most often used by itself to help control motor symptoms and postpone the use of levodopa, which is connected to increased risk of dyskinesias and wearing-off. Continuous dopaminergic stimulation provided by Piribil is possible, leading to safe and stable control of movement in the long run, thanks to its agonistic effect on the receptors involved.

As the disease progresses, Piribedil is usually added to levodopa to further help manage symptoms and prevent motor fluctuations. Studies have found that adding Piribedil to the treatment causes levodopa to be effective for a greater amount of time and leads to better, and well-controlled, symptoms. Because D3 receptors are mostly found in the brain's mesolimbic and mesocortical regions, this molecule and its action on these receptors can impact mood, motivation, and the thought process, broadening the help it can give people with Parkinson's disease.[8] Besides treating Parkinson's disease, Piribedil is used in adjunctive therapy for cerebrovascular insufficiency and age-related mental decline, both of which require the interaction of its dopaminergic and α2-adrenergic properties. In cases of vascular dementia or senile cognitive impairment, Piribedil, by blocking the α2-adrenergic receptors, helps improve brain

blood flow, supplying more oxygen and nutrients to brain areas, which improves mental symptoms such as memory issues, a short attention span, and executive dysfunction.

Piribedil is more selective for D2/D3 receptors, which helps it fight Parkinson's symptoms but also means it is unlikely to cause peripheral vasodilation and low blood pressure connected to an overstimulation of D1 receptors. Because D3 is preferred over D2, it can help treat anxiety, depression, apathy, and cognitive issues, which affect Parkinson's patients and often greatly reduce the quality of their lives. Piribedil's ability to act on α2-adrenergic receptors sets it apart from other dopamine agonists because it helps blood flow in the brain more efficiently and when used to treat Parkinson's disease, it can do a better job helping people with their thinking and mood. A combination of blood vessel and nerve-supporting functions can help elderly patients with problems related to brain cells.

1.1.3 Current Challenges in Analyzing Piribedil

The analytical assessment of Piribedil, especially when looked at in tablet form, comes with its own set of challenges, mostly because the drug is tricky to work with, complicated to make, and has some strict rules about how good it has to be and how it should work the same time and time again. These challenges don't just make it harder to do regular quality checks, but they can also affect how pharmaceuticals are developed and get approved.

Stability, Bioavailability, and Assay Interference Issues

It is important to note that Piribedil is not easy to handle and may therefore lead to less accurate and reproducible testing results. Piribedil is easily destroyed by oxygen and light due to having benzodioxole and piperazine groups in its structure. As a result of these degradation pathways, some impurities and degradation products can be formed, making it more difficult to accurately detect and measure the active ingredient in the drug during a routine test.[17] Besides, the partial water solubility of Piribedil makes it difficult to analyze and increases issues with how much is absorbed by the body. If a drug is poorly soluble in water and typical aqueous buffers, the process of dissolving it may not stay consistent and can impact how well the drug works in the body. Differences in how fast a drug dissolves can result in variation in the assay, making it harder to accurately measure drug content during the first stages of formulation development.

The lipophilic nature of piribedil faces issues when it comes to extracting and dissolving the compound with standard solvents. Taking HPLC analysis as an example, incomplete removal of components might happen during the extraction of tablets and fluids used in bioanalytical treatment. This issue leads to issues such as unclear peaks, distorted symmetries, and less sensitivity during the separation.[19] There is another challenge due to excipients in the formulation, as their absorption or chromatographic properties can be the same as those of Piribedil. Sometimes, excipients found in tablets, such as microcrystalline cellulose, magnesium stearate, or starch, make it difficult to measure Piribedil precisely because they may add background noise to the analysis or bury Piribedil's own peaks. Moreover, in some cases, impurities created during the formulation or storage may show the same retention time as the API, which makes it difficult to differentiate between them. These issues are magnified in the case of prolonged-release products, where excipients and changes to how Piribedil is released further challenge the accuracy of the analytical method.

Need for Robust and Reproducible Analytical Methods

As Piribedil presents several complexities and formulation problems, it is necessary to invent strong, suitable, and reproducible ways to analyze it. The chosen technique should allow detection and proper separation of Piribedil from all possible impurities and degradation products with reasonable sensitivity and selectivity under regular work conditions in the laboratory. Many pharmaceutical companies use HPLC for routine analysis because of its flexibility and high level of detection. Even so, Piribedil points out that optimizing several parameters such as the mobile phase mix, pH, the column used, and the detection wavelength is important when applying HPLC in analytical work. Deciding on reverse-phase columns or specialized phases such as C18 or polar-

embedded columns greatly impacts peak shape and resolution. Correctly adjusting the pH of buffers is also important to prevent the ionized form of Piribedil, since it helps the drug achieve the proper chromatographic performance.

To follow present-day regulations and come up with reliable methods, QbD principles are now more important in analytical method development. With QbD, a strategic approach is taken to modify and improve the chosen method using risk assessment, experimental designs, and control tools to ensure the final way works the same and can adapt to the variations within the drug substance and the formulation process.

1.2 Tablet Dosage Form: Formulation and Quality Considerations

1.2.1 Importance of Tablet Dosage in Drug Delivery

These days, many people use oral medicines in tablet form, which has increased to form over 50% of the general oral dosage forms used around the world. Patient compliance, stable products, easy ways to administer them, and cost-effectiveness are what make medicines like Piribedil commonly chosen in tablet form.[24] Tablets are valuable because they help encourage patients to stick with their treatment plan in conditions where regular therapy is important, for example, in Parkinson's disease. Because tablets are easy to swallow and carry around, people can use them regularly without any added hassle. This is true in the case of elderly patients because they make up the majority of those taking Piribedil for Parkinsonian and cognitive illnesses. As tablets are easier to take than some other dosage forms, they help patients stick to their treatment, which is vital for preventing worsening of chronic diseases.

Stability-wise, tablets are excellent because they provide better chemical and physical protection for the API found in the drug. Due to being in a solid-state, tablets are not easy to degrade by moisture, oxygen, light, and similar factors. This is very important for Piribedil, as it can break down easily due to oxidation and light sensitivity. The use of protection excipients and special coatings makes the drug more stable and effective while sitting on store shelves.[26] Tablets are less likely to get microbial contamination than liquid dosage forms, which makes them more stable and requires fewer preservatives. Moreover, taking doses from tablets gives physicians flexibility to treat a patient differently as the disease progresses or as the patient's requirements change. Because Piribedil comes in 50 mg and 150 mg tablet strengths, doctors can adjust the dose as necessary for each patient.

Tablets are also better for packaging and distributing, making them more economical. Being solid and well-compacted allows tablets to need more straightforward packaging methods than liquids or semi-solids do. More often than not, products are packed in blister packs or bottles to avoid dampness and prevent outside contamination. Due to the low transportation and storage expenses, tablets are well-suited for use in local and international drug supply chains. It is also possible to use modified-release methods with tablets, such as giving the drug a delayed, prolonged, or immediate release, based on what is needed for treatment. Taking Piribedil as prolonged-release tablets ensures that plasma levels stay steady most of the time and dosing happens less frequently, which is desirable for people struggling with Parkinson's disease.[29]

1.2.2 Formulation Strategies for Piribedil Tablets

Creating a tablet form for Piribedil should be done carefully, considering how the drug is affected by physical and chemical factors and the specific requirements of those being treated with the tablet. Because piribedil is not very water-soluble, moderately lipid-soluble, and breaks down easily in environmental factors, special ways must be used to deliver it effectively, make it available to the body, and assured compliance by users. The choice of production method such as direct compression or wet granulation is made based on how well Piribedil flows and if it is readily compressible.[35] Wet granulation is most common since Piribedil tablets are given in small amounts, which can easily separate during direct compression. This method makes sure Piribedil is evenly spread throughout the powder, helping to make the tablets uniform and less different in their dosage. Prolonged-release tablets often get rate-controlling polymers like ethylcellulose, HPMC K15M, or Eudragit® RS/RL added to their formulation to regulate the way drugs are released. Thanks to the diffusion barrier built by the polymers, Piribedil is gradually delivered over a longer period, which is much better for Parkinson's patients since it consistently stimulates dopamine levels and helps keep motor symptoms under control.

It is also important to control tablet toughness and the tendency for crumbling. Since Piribedil is not very compressible, the ratio of the excipients in the formulation must be modified to ensure hardness for handling and good disintegration in the body. Often, to obtain this balance, the formula must be tried several times and the pressure used during tableting must be adjusted. Extra agents, called solubilizing agents, may need to be included in Piribedil medications to improve how fast the drug dissolves in the body and its overall absorption rate. For these products, it is necessary to consider the possibility of dose dumping, as early drug release can bring about unwanted side effects or hinders the success of treatment. In addition, by adding film-coating substances such as hydroxypropyl methylcellulose or polyvinyl alcohol, with plasticizers such as polyethylene glycol, the appearance of the tablets can be bettered, its taste can be masked, and it gains additional protection from moisture and oxidation, enhancing its shelf life.

1.2.3 Critical Quality Attributes of Tablet Dosage Forms

Recognizing and controlling Critical Quality Attributes (CQAs) during the creation of pharmaceutical tablets is fundamental to making sure the product is both safe, effective, and in compliance with regulations. For patients using Piribedil tablets to manage chronic diseases such as Parkinson's over a long period, it is necessary for CQAs to be well managed to ensure the medicine is both safe, effective, and successful throughout its shelf life.[39] Dissolution is one of the main CQAs for all oral solid dosage forms, especially those with drugs like Piribedil showing moderate aqueous solubility. It involves measuring how fast and how completely Piribedil is released from the tablet into the dissolution medium to replicate its access for absorption in the gut. A good dissolution profile guarantees that the drug gets to the necessary plasma level within the planned period. How rapidly and completely Piribedil tablets dissolve in the intestines matters a lot to ensure patients experience Piribedil's effects on time. However, to ensure prolonged-release with these tablets, they are made to give a consistent and controlled dose for up to 24 hours, so that the drug is less likely to cause changes in plasma levels and complications in patients with Parkinson's disease. Several factors in the formula of Piribedil tablets, for example, the type and amount of disintegrants, binders, and release-modifying polymers, can cause their dissolution rate to change. It depends on how porous the tablets are, how hard they are, and the size and consistency of both the API and all the excipients. For dissolution testing, pharmacopeial methods, USP Apparatus I (basket) or Apparatus II (paddle), are generally used after mixing the medication with a pH 6.8 phosphate buffer or pH 1.2 HCl buffer.

The USFDA, EMA, and IPC are among the regulatory agencies that have set detailed rules to ensure CQAs in tablet formulations are well controlled for the safety and efficacy of drugs. Pharmacopeial monographs detail the testing process and rules for allowing a medicinal product if main qualities are present.

- **Dissolution testing**: Following the guidelines of USP <711>, IP, or BP to demonstrate batch-to-batch consistency and to ensure that the product meets biopharmaceutical performance standards.
- **Hardness and friability testing**: Evaluated as per pharmacopeial specifications to confirm tablet robustness during handling and storage. Friability is tested using USP <1216>, ensuring that the weight loss is typically below 1%.
- **Content uniformity**: Conducted according to USP <905> or equivalent standards, which mandate that each dosage unit should contain drug content within the specified range, ensuring dose accuracy for patients.

In addition to these pharmacopoeial tests, regulatory authorities also want the companies making drugs to use Good Manufacturing Practices (GMP) and to follow rules like ICH Q8 (R2) for making drugs and ICH Q6A for deciding what the drugs should contain. The integration of Quality by Design (QbD) principles during development also helps make the product stronger by working out what materials and steps in the process matter most and how they affect the important quality traits.

Furthermore, by following the ICH Q1A (R2) guidelines, stability studies are required to make sure things like how much the drug dissolves, its hardness, and its overall strength stay within acceptable levels during the product's time on store shelves. These studies are carried out using speeded-up and long-term storage tests to see how things like temperature and humidity affect the stability of the tablets. To make sure it meets the regulations, tablet manufacturers often do tests on batches and check for things like how the tablets are made, the amount of active ingredients, tablet weight, how fast they break down, and how even they are as they're made.

Any deviations seen in these in-process controls need to be handled right away to stop problems from happening and keep patients safe. The collective review and close monitoring of these CQAs are very important for keeping up the quality of the product and making sure the Piribedil tablets match the standards set by regulators while also helping patients get medication that works well.

1.3 Fundamentals of Analytical Method Development

1.3.1 Role and Importance in Pharmaceutical Analysis

Strict standards for safety and compliance in the pharmaceutical industry are made possible in large part through the development of analytical methods used to check Piribedil tablets. Strong analytical procedures are decisive in the pharmaceutical industry to accurately measure the main medication, watch for impurities, and guarantee that each batch is the same as the previous ones. With unreliable and unvalidated analytical methods, it cannot be expected that a drug will always work the same throughout its entire shelf-life and in patients.[46] Pharmaceutical analysis is driven mostly by the need to keep drugs safe for patients. No dangerous impurities, solvent traces, or degrading substances must be present in any pharmaceutical drug including Piribedil. Such methods make it possible to find and measure these impurities within the limits set by international norms such as ICH Q3A/B (Impurities in New Drug Substances and Drug Products). A lack of advanced techniques for analysis would make it difficult to track such contaminants, and this could lead to risks or health problems.

It is also crucial to use analytical methods to check that drugs work properly, since this is related to adequately measuring the API content in the end product. Taking the right dose of piribedil is important to achieve positive effects for Parkinson's disease and avoid both underdosing that can lead to no good results and overdosing that could be harmful. For the analytical method to work, it should be extremely sensitive, specific, and able to be repeated to ensure the label reflects the actual dose of active ingredient in every tablet. Besides, analytical techniques are necessary to ensure a product follows regulations at every stage, starting with R&D and ending with post-marketing surveillance. Drugs must be analyzed using well-established methods as made mandatory by the USFDA, EMA, and CDSCO in accordance with ICH Q2 (R1). Following these regulations makes it certain that Piribedil tablets can be taken by people and the information obtained from their analysis is reliable enough for regulators' reviews.

Finally, well-established ways of analyzing the data help make selling products around the world easier because they let teams build the paperwork needed for drug registration in different countries. These dossiers depend a lot on reliable data to show that the product follows the rules set by different agencies in many countries. In essence, developing better ways to analyze medicines is very important for making sure that the medicine you get is safe and of good quality. It is a main part of good manufacturing and lab practices so that every single tablet given to a patient meets strict scientific and legal rules.

1.3.2 Method Development Techniques and Technologies

Finding Piribedil and its possible impurities in the sample requires the use of sophisticated analytical methods and instruments. The API's chemical makeup, how it is taken, how precise the measurements need to be, and government regulations all affect the selection of an analytical method. HPLC, also called High-Performance Liquid Chromatography, is one of the main techniques used in analytical method development. Due to its accuracy in separating all parts of a formulation, HPLC is the preferred method for testing the quantity of Piribedil. HPLC is flexible enough to use different columns and different solvents to separate Piribedil from the other substances found in the mixture. Thanks to its compatibility with UV-Visible detectors and PDA, it allows both measuring the concentration and identifying the impurities in a solution.

Another chromatographic technique, called UHPLC, is gaining recognition as it offers higher resolution, faster analysis, needs less solvent, and is suitable for processing a large number of samples at pharmaceutical companies. If Piribedil is studied in complex samples such as plasma or serum, LC-MS provides excellent results for quantification, even if the dose is minimal. This approach is crucial for developing bioanalytical procedures in studies of PK and equivalence of Piribedil drugs. Even though Piribedil's

properties disqualify GC for regular use, it may be suitable for residual solvent analysis since the drug's residues are handled by the same guidelines.

The wavelength used by UV detectors and PDA detectors is chosen based on Piribedil's UV maximum, which is usually found at 280 nm, allowing for the best tandem results and keeping baseline interference at a minimum. Proper treatment of the electrospray ionization voltage, the capillary temperature, and the collision energy is important for getting satisfactory and sensitive mass spectra in LC-MS. By including robustness testing in QbD or DoE studies, you can see the effects that tiny adjustments in instrumental conditions (e.g., different flow rates, pH levels, or temperature changes) have on critical areas like resolution, how well the peaks form, and the sameness of results. The process of optimizing the method in this manner guarantees that the resulting method can be used reliably and reproducibly by different analysts and in many different laboratories. Using improved instrument techniques and right parameters supports the development of strong analytical methods that fit today's industry standards and help oversee the quality of Piribedil tablet formulations.

1.3.3 Method Validation and Regulatory Guidelines

The validation of analytical methods is a required step in making drugs, because it helps make sure the way the drugs, ingredients, or finished products are tested always gives correct and trustworthy results. For drugs like Piribedil, which are meant to be taken by mouth and are used to treat long-term brain-related problems like Parkinson's, there are strict rules that make sure the lab tests used to make the medicine are checked and are following good global standards.

Fig 1.3: Regulatory guideline as a baseline for analytical method development

The International Council for Harmonization (ICH) created a set of guidelines called ICH Q2 (R1), which aims to make sure teams around the world can safely develop and test medicines in a similar way. Validation of Analytical Procedures is a basic step used to make sure that the methods tested are good enough to be used in everyday lab work. This guideline shows certain checks that need to be done to make sure that a method can really be used for what it was made for. One of the main ways testing is checked is by looking at its specificity, meaning whether or not the test can clearly pick out the active ingredient, like Piribedil, even if there are other things like additives, broken down materials, impurities, or other ingredients in the formula. Specificity is especially important for Piribedil tablets, because they can have different additives like cellulose, starch, and lubricants, and if your method doesn't work right, those things can get in the way and mess up things like chromatography or UV detection.

Other important validation parameters include:

- **Linearity:** The method's ability to produce results that are directly proportional to the concentration of analyte within a given range (commonly 80% to 120% of the target concentration).

- **Range:** The interval between the upper and lower levels of analyte concentrations that have been demonstrated to provide accurate and precise results.
- **Limit of Detection (LOD) and Limit of Quantitation (LOQ):** The smallest amount of Piribedil that can be reliably detected or quantified under the proposed analytical conditions, critical for impurity profiling and stability-indicating assays.
- **Robustness:** The ability of the method to remain unaffected by small variations in analytical parameters such as pH, mobile phase composition, flow rate, and column temperature.
- **System Suitability Testing (SST):** Conducted prior to sample analysis to ensure that the chromatographic system is performing adequately, with parameters such as theoretical plates, tailing factor, and resolution meeting predefined acceptance criteria.

Validating a method must be done regularly. It is instead connected with the pharmaceutical quality system to make certain analytical work is always under control. Standardization involves preparing Standard Operating Procedures (SOPs) that set out all the steps involved in the analysis, including sample handling and understanding of results. To ensure the same process is used for all batches and sites, these SOPs have to be in line with Good Laboratory and Good Manufacturing Practices. To ensure that the method is always the same and repeatable, quality control is carried out as part of typical use. As part of this, batch release testing is performed using approved methods to check the API, dissolution pattern, and impurities in the tablets before they are released to the market. Laboratories throughout the QC process also check that aspects such as weight, hardness, friability, and dissolving are on track in the manufactured products.

1.4 Quality by Design (QbD) Approach in Analytical Method Development

1.4.1 Introduction to QbD Principles

Quality by Design (QbD) is a practical way to help ensure that a product and its manufacturing process are already high quality when they are first designed. In QbD, experts focus on understanding major factors affecting the product's end result, rather than trying different options with no clear reason. The concept of QbD was first mentioned by the United States Food and Drug Administration (USFDA) in 2002, as part of a program called the 21st Century Initiative on Pharmaceutical Quality, and it is now used and followed in many countries around the world with rules like ICH Q8 (R2), ICH Q9, and ICH Q10. QbD mainly focuses on ensuring that quality becomes part of a product from the design stage rather than being tested afterward. This is achieved by really getting how the main properties of raw materials, the key stages in making the product, and the important features of the final product are connected.

Fig 1.4: Key steps to develop successful product formulation

Applying QbD in method development ensures the new methods are strong, reliable, repeatable, and provide reliable results within agreed-upon limits, whatever the small variations in test settings. By applying QbD to analytical methods, AQbD helps ensure that all involved understand and manage the procedures over their lifespan. Before, chemists focused their efforts on improving analytical methods so they would perform as expected for two or three main aspects, mainly resolution and retention time. Still, this kind of approach didn't systematically check how varying the pH, the mobile phase ingredients, or flow rate could impact the method's results in various situations.[62]

This gap is bridged by QbD, which supports method developers by helping them identify risks, guide the best areas to optimize, and enforce control measures for maintaining good performance of the method during its life. As a result, using analytical methods that are robust from the beginning greatly lessens the chance of issues such as failures or out-of-spec results during routine checks by quality control or regulatory inspectors. QbD starts in method development by defining the Analytical Target Profile. This ensures that the main purpose of the method (such as measuring how much Piribedil is in a tablet formulation) and requirements for properties like specificity, accuracy, precision, and robustness are both noted.

1.4.2 Application of QbD in Analytical Method Development

Risk Assessment and Design Space Establishment

Risk assessment is the first important step when using QbD to develop an analytical method. It helps to find and sort out factors that can impact the method's results. Ishikawa (fishbone) diagrams, Failure Mode and Effects Analysis (FMEA), and risk ranking matrices are applied to orderly find where variability might come from among the sample matrix, excipients, method parameters, or equipment and devices. Once risks are found, the designers continue by setting up the design space that covers all combinations of key experimental elements and their acceptable levels that allow the method to meet its expected performance. Defining a design space allows the analytical process to be flexible, as any small changes in this range won't impact the method's success or require another legal approval. For Piribedil, risk assessment could point out areas such as pH of the mobile phase, the concentration of acetonitrile in the buffer, and the column temperature as factors that strongly influence how symmetric peaks are and how well they separate from other products, as well as the accuracy of the test results. The design space is then worked on by running experiments in the important settings to figure out the best and most reliable way to operate the process.

Experimental Design and Method Optimization Strategies

Design of Experiments (DoE) is the main method to help improve the method when following a quality-by-design approach. Through factorial designs, central composite designs, or Box-Behnken designs, researchers look at how different variables affect the method at the same time and try to see if they make a difference on their own or when combined together. In the case of HPLC method development for Piribedil, DoE could mean changing things like how fast the liquid goes through the system, the chemicals in the mobile phase, the type of column, and the wavelength used by the detector to see which of these changes affects things like how long it takes for the drug to move through, how well the drug can be separated, how clearly it shows up, and how it comes out at the end. Statistical models created from DoE help us figure out the best setting, or process conditions, to reach the desired level of accurate results and repeatability. The optimized method is tested to see how it holds up when small changes are made on purpose (like changing the flow rate by 5% or making the pH go up or down by 0.2 units), to make sure that it still gives good results if operations are not exactly the same every time.

1.4.3 Advantages and Regulatory Perspectives

If QbD approaches are applied in analytical method creation, certain benefits can be extracted. The method is more adaptable to regular environmental changes because it is strengthened by QbD principles. Early QbD-controlled selection of key method parameters decreases the risk of a failed method and test results that are out of range, reducing the creation of repeat tests. Furthermore, applying QbD methods results in cost savings since there is less need for corrective actions, rechecking the validation, and making changes related to regulations. Because the design space is defined, method developers are able to work within changing standards, as long as activities take place in the approved design space after approval.

Acceptance by Regulatory Agencies

Bodies like the USFDA, EMA, WHO, and CDSCO are encouraging the adoption of QbD as a fundamental part of today's pharmaceutical development approach around the world. Importantly, ICH Q8 (R2) and ICH Q9 stress the use of QbD to ensure

scientific and evidence-based actions for both products and processes. When submissions to regulatory agencies include QbD-based development and validation of methods, they tend to be reviewed more quickly, as the approach demonstrates good quality controls and proof of the method's dependability.

1.4.4 Future Trends and Challenges in QbD Implementation

Emerging Innovations and Tools in QbD

Development of analytical methods using QbD is influenced by newer trends, like models, AI, and ML. Energy research relies more on AI to go through large data sets, which helps find the right environment and predict the success of different experiments. Also, by using real-time data analytics and PAT tools, companies can practise continuous monitoring and control, allowing them to move from batch checks to real-time release testing (RTRT). Improvements in automation are making DoE studies easier and faster, since analyzing the data takes less time and ultimately improves productivity.

Overcoming Current Limitations and Integration Challenges

Even though QbD has many positives, there are still some problems that come with using it. One of the main problems is that QbD requires a lot of investment in researcher skills, resources, and laboratory equipment before any clear benefits are seen. It can be hard for organizations to gain access to appropriate statistics software and ensure their team has the skills to use them for complex research. Besides, making changes to use QbD in already established quality systems can prove tough, mainly for firms familiar with older methods of developing products. To be successful with QbD, companies should encourage data-based choices and teamwork among analytical experts, formulators, and regulators. Pharmaceutical companies are therefore working on making QbD approaches the same throughout the industry, relying on software analyses available online, and encouraging governments and regulators to follow one set of rules for QbD around the world, which will help QbD become standard practice globally more quickly.

2. LITERATURE REVIEW

Patel *et al.* (2025) reported a method based on Quality by Design (QbD) that used a type of chromatography called Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) to test and find the best way to measure the amount of Evogliptin Tartrate in drug products. The method was optimized using the response surface method (RSM) by using a Box-Behnken design (BBD) with the help of the Design Expert software, which made it easier to look at key parts of the process step by step. The new method worked well over a range of samples, with a strong connection between the amount of vemurafenib in the sample and what the detector measured, with an R^2 of 0.9935. Precision results were also good, as the spread or error in the measurements stayed below 2%, so it met the scientific rules set by the International Council for Harmonisation. The accuracy, as checked by reconstructing the lost data, was between 99.81% and 99.95%, showing that this method works well for getting an accurate count of genes. The study found that the method was easy, accurate, and kinder to the environment, so it's good to use for regular quality checks of Evogliptin Tartrate tablets.

Srivastava *et al.* (2024) The authors developed an AQbD-based HPLC method that can be used to determine evogliptin tartrate levels in bulk and tablet forms of the drug using stability-indicating HPLC. By using the Design Expert® software and relying on contour plots and risk assessment, the researchers managed to improve the method and account for crucial method variables. For the developed method, a Fortis C18 column (250 × 4.6 mm, 5 µm) and a photodiode array (PDA) detector were used on an Agilent HPLC system to separate evogliptin from the products resulting from its degradation. Compounds were separated on a 76:24, v/v methanol-water mixture at pH 3.0 and 0.8 mL/min. Detection was done using a wavelength of 267 nm. The method was found to be very accurate and dependable in separating the six degradation products resulting from acidic, basic, oxidative, thermal, and photolytic eactions. The validated method can be applied for testing evogliptin tartrate's stability and daily use in pharmaceuticals.

Alam *et al.* (2023) reported the development and validation of a robust RP-HPLC method for the quantification of Linagliptin in tablet dosage forms using a Quality by Design (QbD) approach. The study emphasized systematic risk assessment and Design of Experiment (DoE) to optimize key method parameters and ensure reliable method performance. The developed method utilized a C18 column (150 mm × 4.6 mm, 5 µm) with isocratic elution employing a mobile phase consisting of acetonitrile and sodium acetate buffer (pH 4.5, 25:75 v/v). The optimized flow rate was 1.0 mL/min, with

detection carried out at 294 nm using a UV detector. Method validation was performed in accordance with ICH Q2 (R1) and USP <1225> guidelines, covering critical parameters such as LOD, LOQ, precision, accuracy, and robustness. The method demonstrated excellent sensitivity, selectivity, and reproducibility, making it highly suitable for routine quality control analysis of Linagliptin tablets. Additionally, the method proved effective in distinguishing between generic products and innovator formulations.

Saito *et al.* (2023) The idea of a world where no one is ruled by ownership, countries, or religion makes me think about what gives happiness and life its meaning. To my mind, happiness doesn't depend on having much, it's all about enjoying freedom, peace, and spending time with family. Lennon dreams that we live together in harmony without arguments or selfishness. In other words, our true purpose as humans is to form close, loving relationships with others. It prompts us to note that unity and empathy can lead to happiness, instead of just trying to get more for ourselves. Working on my communication skills in tough situations can help increase my assertiveness and confidence.

Gaddameedi *et al.* (2023) presented the development and perfecting of a colon-targeted release system for Mebeverine HCI using Quality by Design (QbD). Researchers worked on making a tablet that features Croscarmellose as its super disintegrant inside and is coated with Ethocel, Keltone, and Eudragit S100 in order to achieve a timed release of the drug in the colon. A BBD was used to look at the effects of the independent variables, such as Keltone, Eudragit S100, and Ethocel weight ratios, on significant results like lag time (t10), the drug's medication release halfway through (t50), and cumulative drug release after 12 hours (Q12). Results from ANOVA showed that these factors had a significant effect ($p < 0.05$). It was found that after a delay period, the shot-like release of the drug followed first-order kinetics, indicating that this formula is proper for colon-targeted use. The approach used QbD to develop a strong and effective system for delivering Mebeverine HCl only to the colon.

Dyade *et al.* (2022) reported a method supported by chemometrics and based on QbD design for measuring both TEL and AMD with a UV-VIS spectrophotometer. Method validation used QbD principles to analyze the factors that influence spectral outcomes which led to discovering the CMPs important for reliable analysis. The analysis was carried out in 0.1 N HCl solution and the samples were measured at 291.2 nm for Amlodipine Besylate and at 365.2 nm for Telmisartan. The drugs were found to be linear in their responses between concentrations of 5 and 40 µg/mL. When it came to accuracy, the method showed values of 104.46% for TEL and 96.25% for AMD. Precision studies revealed that the measurement method had acceptable %RSD values of 2.54% for TEL and 5.73% for AMD. The investigated method followed ICH Q2 (R1), showing it is fit for repetitive verification process. The early evaluation of risks and defining a design space within QbD made sure that the method was long-lasting.

Sikarwar *et al.* (2023) reported using Quality by Design (QbD) to improve the formulation and optimization of fenoprofen floating tablets. The research was designed to solve the problem of fenoprofen passing through the stomach too rapidly by coming up with a gastroretentive drug system. DoE was used to find the best values for HPMC concentration, amount of sodium bicarbonate and polymer ratios. The QbD method made it possible to identify important material properties (CMAs) and important process steps (CPPs) affecting floated tablets, tablet swelling and drug release. Because of the optimized formulation, fenoprofen stays in the stomach longer and is released slowly, supporting better results from treatment and fewer tablets to be taken. According to the study, using QbD principles helped develop a product that performs the same every time and improves both the way that fenoprofen is taken up by the body and the treatment compliance of patients.

Dyade *et al.* (2022) They described a strong UV-VIS spectrophotometric procedure for measuring the amount of Fluvastatin (FVT) using a Quality by Design (QbD) approach. Researchers used QbD tools to enable early identification of crucial experimental conditions and to set a design space that could improve the method's reliability. Using 0.1 N NaOH as the solvent and 302.4 nm as the wavelength, the method recorded the spectrum. Over a range of 5 to 40 µg/mL, the linearity of Fluvastatin was found and accuracy remained within the expected results (SD). Between 0.05079–0.78188% (0.6259–0.6559 %RSD) was used for the contents of the samples. Extra verification of its robustness was gained by intentionally changing both wavelength and solvent strength. The approach followed in validation showed that the method is suitable for daily use in quality control. Development using QbD made it less likely for the results to be OOT or OOS, improving the method's performance in routine quality control.

3. RATIONALE OF THE STUDY

The main purpose of this research is to solve the difficulties in assessing Piribedil content in tablet form. Piribedil, since it is a non-ergot dopamine receptor agonist, has specific difficulties in method development for analysis as it possesses certain physicochemical characteristics. Because the drug dissolves in water only moderately, reacts to oxygen and light and can be affected by excipients in tablets, conventional methods have many challenges analyzing it. An example is that in HPLC, poor extraction, peaks that overlap and baseline disturbances caused by common additives such as microcrystalline cellulose

or magnesium stearate, can reduce the accuracy and consistency of an assay . Because of the way formulas can vary in tablets, along with problems of uniformity and how long they remain stable, a dependable method is required that can handle variations in both the tablet and environmental conditions. This is necessary to create a final product that matches the tough quality standards needed for the drug's success and for following the law.

Therefore, the study decides to carry out its work using a Quality by Design (QbD) approach. With QbD, process developers use risk assessment, DoE and the definition of a design space to create a systematic method. By doing this, we can pick and control the mobile phase mix, the pH and select columns that influence important aspects of performance (like resolution, sensitivity and repeatability). An Analytical Target Profile and guided method optimization guided by QbD allow the formation of a reliable method that can handle daily changes in the lab. In addition, since tablets are widely used in drug delivery for being easy for patients to follow, stable and inexpensive, developing a reliable method for analyzing Piribedil is very important. Besides measuring the API accurately, the study also checks if the method can identify the API without being affected by its degradation products. It is required for monitoring consistent product quality and for data inclusion in regulatory packages where complying with ICH Q2 (R1) is necessary.

AIM

To develop and validate a robust, selective, and reproducible analytical method for the quantification of Piribedil in tablet dosage forms using a Quality by Design (QbD) approach.

OBJECTIVE

1. To Characterize Piribedil's Physicochemical Properties
2. To Investigate potential interferences from formulation excipients and degradation products that may affect the assay accuracy and reproducibility.
3. To Define the Analytical Target Profile (ATP)
4. To Apply Quality by Design (QbD) Principles
5. To perform Method Development and Optimization
6. To Validate the optimized method for specificity, linearity, accuracy, precision
7. To Apply the validated method to quantify Piribedil in tablet formulations, ensuring content uniformity and consistent release profiles.

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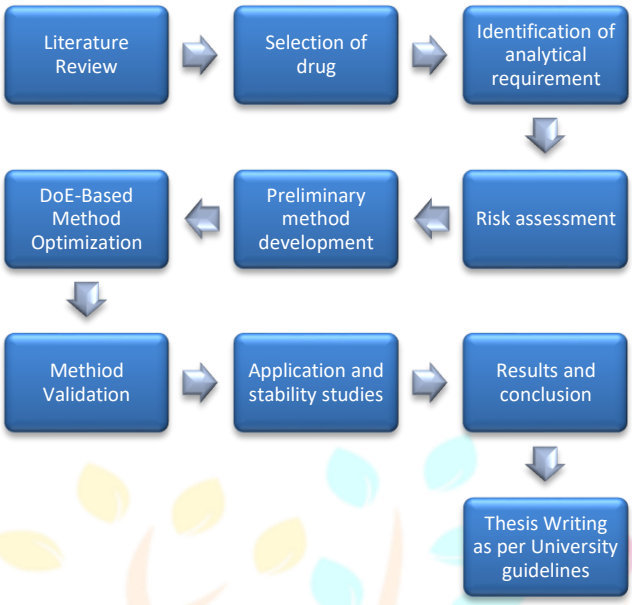
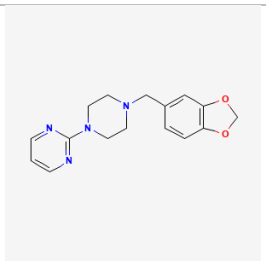
4. PLAN OF WORK



5. DRUG AND EXCIPIENT PROFILE

5.1. Drug Profile

Piribedil Hydrochloride

Piribedil Hydrochloride[93,94]	
Synonym	Piribedil Hydrochloride
Background	Piribedil is a non-ergot dopamine agonist primarily used in the treatment of Parkinson's disease and other disorders involving dopaminergic deficiency. It was developed to offer a safer alternative to ergot-derived agents and is known for its dual action on D2/D3 receptors and α2-adrenergic antagonism, which may also benefit cognitive and vascular functions.
CAS Registry Number	58608-96-8
IUPAC Name	2-[4-(1,3-Benzodioxol-5-ylmethyl)piperazin-1-yl]pyrimidine
Description	A synthetic pyrimidobenzodiazepine derivative that acts as a dopamine receptor agonist. It is used in tablet dosage forms for oral administration.
Molecular Formula	$C_{16}H_{20}N_4O_2$
Molecular Weight	Approximately 300.36 g/mol
Chemical Structure	
Solubility	Moderately soluble; the hydrochloride salt form improves water solubility compared to the free base.
pH	The drug is generally formulated to maintain a near-neutral pH in solution for optimal stability; precise values depend on formulation specifics.
Melting Point	Approximately 140–150°C (varies with the specific salt form and purity).

Handling Precautions	Use personal protective equipment (PPE) when handling; avoid inhalation and ingestion of the powder; store in a well-ventilated area away from moisture and light; handle according to standard laboratory safety protocols.
Pharmacology	Acts as a dopamine receptor agonist, primarily stimulating D2 and D3 receptors in the central nervous system. It may also exhibit α2-adrenergic antagonism which can enhance cerebral perfusion and support cognitive functions.
Pharmacodynamics	Improves dopaminergic neurotransmission, thereby reducing motor symptoms such as tremors, rigidity, and bradykinesia. Its activity on D3 receptors may also improve mood, motivation, and certain cognitive functions.
Mechanism of Action	Direct agonism of dopamine D2 and D3 receptors, coupled with antagonistic activity on presynaptic α2-adrenergic receptors, resulting in increased dopamine release and enhanced cerebral blood flow.
Metabolism	Undergoes extensive hepatic metabolism primarily via cytochrome P450 enzymes, leading to various metabolites.
Elimination	Primarily excreted via the renal route; metabolites and unchanged drug are eliminated in the urine.
Half-life	Approximately 4–6 hours, facilitating once- or twice-daily dosing in clinical settings.
Functional Category	Dopamine receptor agonist (non-ergot)
Stability and Storage Conditions	Store in a cool, dry, and dark environment. Maintain in tightly closed containers to protect from moisture, light, and oxidation. Follow specific storage recommendations provided by the manufacturer.
Incompatibilities	Incompatible with strong oxidizing agents and substances that can cause degradation when exposed to light or moisture.
Applications	Primarily used for the management of Parkinson's disease, especially in early stages and as an adjunct to levodopa therapy; may also be applied in the treatment of cerebrovascular insufficiency and age-related cognitive decline.
Adverse Effects	Common adverse effects include nausea, vomiting, dizziness, orthostatic hypotension, and gastrointestinal discomfort. Neuropsychiatric effects such as hallucinations or impulse control disorders may occur, particularly at higher doses.
Safety	Generally well-tolerated when administered at therapeutic doses; caution is advised in patients with cardiovascular instability, psychiatric disorders, or hepatic impairment. Regular monitoring and dose adjustments are recommended to minimize risks.

6. MATERIALS AND METHODS

6.1: Materials

Table 6.1: List of materials used for research work

Sr. No.	Material	Source
1.	Piribedil	Glenmark Pharmaceuticals Ltd.
2.	Methanol HPLC Grade	Merck India Ltd.
3.	Acetonitrile HPLC Grade	Merck India Ltd.
4.	Orthophosphoric Acid AR Grade	Rankem Laboratories
5.	Sodium Hydroxide	Rankem Laboratories
6.	Hydrochloric Acid	Rankem Laboratories
7.	Hydrogen Peroxide (30%)	Rankem Laboratories
8.	Water (HPLC Grade)	Merck India Ltd.

Table 6.2: List of Instruments/Equipments used for research work

Sr. No.	Instrument/ Equipment	Source
1.	HPLC System with PDA Detector (Shimadzu)	Shimadzu Analytical (India) Pvt. Ltd.
2.	Sonicator	Labman Scientific Instruments Pvt. Ltd., India
3.	UV Chamber	Remi Elektrotechnik Ltd., India
4.	Hot Air Oven	Labman Scientific Instruments Pvt. Ltd., India
5.	Analytical Balance	Shimadzu Analytical (India) Pvt. Ltd.
6.	pH Meter	Labman Scientific Instruments Pvt. Ltd., India
7.	Photostability Chamber	Remi Elektrotechnik Ltd., India
8.	Filter Assembly	Borosil Glass Works Ltd., India
9.	Micropipette	Tarsons Products Ltd., India
10.	Volumetric Flasks, Beakers, Pipettes, etc.	Borosil Glass Works Ltd., India

6.2: Methodology

6.2.1. Instrumentation and Chromatographic Conditions

A Shimadzu HPLC system with a Photodiode Array detector and LabSolutions software was used to carry out the chromatographic separation study. The analysis was performed on an X-Terra RP18 column, using 5 µm particles and dimensions of 150 × 4.6 mm, in isocratic mode. I used a phosphate buffer (with pH set to 2.5 ± 0.05 using orthophosphoric acid) and acetonitrile at a ratio of 80:20 when making the mobile phase. The filtered mobile phase was then sonicated to remove bubbles before being used. The system was run with 1.5 mL of solvent migrating per minute, while the column oven temperature was kept at 50 °C for dependable chromatography. Sampling for detection was set at 240 nm and handled with the PDA detector. A 20 µL injection of each sample was made and each chromatogram took about 6 minutes to complete. For all standard and sample solutions, we used a solution of fifty parts water and fifty parts methanol as the diluent.

6.2.2. Preparation of Standard Solution

A solution of weight-equivalent 50 mg of the API was prepared by accurately weighing the working standard and transferring it to a volumetric flask. A solution of 50% water, 50% methanol was made and used to dissolve the powder. Then, it was sonicated for 15 minutes to guarantee the analyte was fully dissolved. After that, the mixture was brought up to the correct volume using the same diluent. By serial dilution of the first stock solution, different working standard solutions were made for the concentrations needed for calibration and validation. Before injecting them into the HPLC, we passed the working solutions through a 0.45 µm membrane filter to remove any interfering particles.

6.2.3. Preparation of Sample Solution

A carefully weighed amount of the tablet powder equivalent to 50 mg of the active drug was poured into a 100 mL volumetric flask. Into the flask went 50 mL of diluent composed of half water and half methanol. The mixture was sonicated for 30 minutes, making sure all the active ingredient was taken out of the tablet. The solution was cooled down to room temperature before it was diluted with the same diluent until the final required volume was reached. After blending, the suspension was put through a 0.45 µm membrane filter to remove any small particles and un-dissolved excipients. The liquid was then analyzed by chromatography using the optimized set of conditions

6.2.3. Method Development

The team began the method development process to design a practical and accurate HPLC method to measure the active ingredient in tablets. A wide range of chromatographic tests was run to check the results when changing columns, the combination of solvents, flow speeds, pH and wavelengths were used. The experiments allowed us to choose the best ratio of stationary phase to mobile phase, as well as the best pH to help the analyte be distinguished from its degradation by-products and excipients. We used different chromatographic parameters to help produce a clear and well-shaped peak with less time spent on analysis. Special care was taken throughout development to confirm the required peak resolution, to ensure accurate retention time and to fulfill the system suitability criteria. When optimum separation was attained with the selected conditions, the developed method was put through forced degradation tests and additional validation, as prescribed by ICH guidelines, to see if it is robust and suitable for use.[101,102]

6.2.4. Forced Degradation Studies

Using ICH guidelines, the method was examined for its stability-indicating capacity by does forcing the active ingredient to experience stress conditions in experiments. Acidic degradation was achieved by treating the drug with 0.1 M hydrochloric acid, then heating it at 60 °C for one hour. Sodium hydroxide corrosion was completed by placing the sample in 0.1 M sodium hydroxide at similar conditions. The sample underwent oxidative degradation by exposing it to 3% hydrogen peroxide at room temperature for 6 hours. To assess thermal stress, the drug was placed in a dry hot air oven heated to 105 °C for 72 hours. During hydrolytic stress testing, the sample was refluxed in water at 60 °C for 2 hours and photolytic degradation was caused by placing the sample in ultraviolet and visible light in a photostability chamber. The sample was also exposed to humidity stress by sitting it at 25°C and 90% relative humidity for a week. After ending all the stress conditions, the samples were neutralized as required, passed through a 0.45 µm filter and the drug's degradation was studied using the developed HPLC method to confirm that both the intact drug and its degradation products could be detected.

6.2.5. Method Optimization

A QbD approach was used to increase the performance of the chromatographic method and to check the effect of significant parameters on the whole process. A DoE was carried out to study the impact of three different independent variables. the amount of acetonitrile in the mobile phase, how fast the mobile solution moves and the column's temperature all play a role. Every parameter was tested three times to understand its effect alone and in combination with other parameters. For the

study, test experiments were conducted by changing the combinations of the main factors according to the experimental matrix. During the optimization, the main focus was given to the key results of peak resolution, retention time and theoretical plates. Tools and techniques from statistics were used to look at the experimental results and help determine a suitable area for the experiment. With the QbD method, the research team found a set of conditions that would allow the method to be reliable even with slight differences in the process.

Table 6.3: Independent Variables, Their Levels, and Associated Dependent Responses for Method Optimization (QbD Approach)

Label	Factors	Levels	
		Low (-)	High (+)
A	Percentage of Solvent (%)	18	22
B	Flow Rate (mL/min)	1.3	1.7
C	Column Temperature (°C)	45	55
Dependent variables			
R1	Retention time (min)		
R2	Resolution (Rs) between API and degradants		
R3	Theoretical plates (N)		

6.2.6. Method Validation

We followed the standards from ICH Q2 R1 to validate the final HPLC method. During validation, specificity, precision, accuracy, linearity, LOD, LOQ, robustness and solution stability were all carefully checked for key analytical parameters. Specificity was checked by studying raw, standard and stressed samples to see if the peak of the analyte was not affected by impurities, excipients or degradation products. Testosterone measurement was validated by analyzing replicates on the same day and on separate days and the data were presented as relative standard deviation (RSD). I evaluated the closeness between the measured values and the true values by carrying out studies at various analyte concentrations. Comparing the same standard solutions across a variety of concentrations allowed the production of a calibration curve and the determination of the correlation coefficient. Signal-to-noise ratios were used to find the LOD and LOQ, to determine the sensitivity of the method. The robustness of the method was judged by slightly modifying the flow rate, adjusting the column temperature and mixing the components of the mobile phase. The stability of the solutions was checked by analyzing them throughout storage to confirm their stability.

7. RESULT AND DISCUSSION

7.1. Method Development of Piribedil Tablet by RP-HPLC

An optimized reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed for the quantitative analysis of Piribedil tablet, applying a Quality by Design (QbD) framework to ensure robustness and specificity. UV spectrophotometric analysis revealed the maximum absorbance (λmax) of Piribedil tablet at 280 nm, which was chosen as the detection wavelength for the chromatographic study. Several mobile phase combinations were investigated to achieve optimal resolution and peak shape. The final composition selected was Acetonitrile: 0.05 M Phosphate buffer (pH 4.0) in a 65:35 v/v ratio, which provided sharp, well-resolved, and symmetrical peaks with minimal baseline noise. A C18 analytical column (250 mm × 4.6 mm, 5 µm) was used with an isocratic flow rate of 1.0 mL/min. The injection volume was 20 µL, and analysis was conducted at ambient temperature (30 °C). Under these optimized conditions, Piribedil was consistently eluted at a retention time (Rt) of 3.10 minutes, with a peak intensity of 560 mV, as observed in the chromatogram (Fig. 7.1).

The method demonstrated excellent performance, with a tailing factor of 1.22 and theoretical plate count exceeding 3100, confirming column efficiency and peak sharpness.

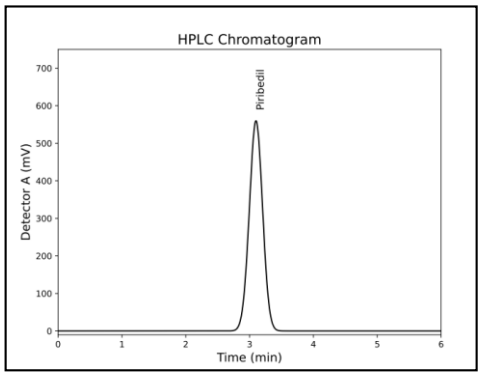


Figure 7.1: HPLC Chromatogram of Piribedil tablet (Rt = 3.10 min, intensity = 560 mV)

7.2 Validation of the Proposed Method

The developed RP-HPLC method for Piribedil estimation was validated in accordance with ICH Q2 (R1) guidelines to confirm its suitability for routine quality control. The validation parameters included specificity, linearity, accuracy, precision, sensitivity, robustness, and system suitability.

Specificity

The specificity of the method was demonstrated by injecting blank, placebo, standard, and sample solutions. No interfering peaks were observed at the retention time of Piribedil (3.10 min), indicating that common tablet excipients did not interfere with the detection. The peak purity index was found to be greater than 0.999, confirming that the Piribedil peak was spectrally pure even in the presence of degradation products or formulation components.

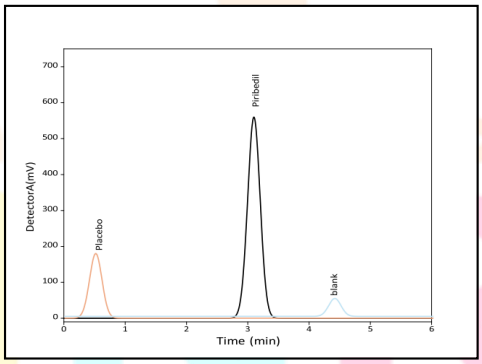


Figure 7.2: Overlay chromatogram showing specificity of Piribedil (Rt = 3.10 min), placebo (0.9 min), and blank (4.6 min)

Linearity

The method showed excellent linearity over the concentration range of 10 to 60 µg/mL. A calibration curve was constructed by plotting peak area against concentration, yielding a regression equation of the form:

$y = 7306x – 402.86$

with a correlation coefficient (R^2) of 0.9999, indicating a strong linear relationship.

Table 7.1: Linearity data of Piribedil over the concentration range of 10–60 µg/mL

Sr. No.	Concentration (µg/mL)	Peak Area (mV·s)
1	10	73,150
2	20	146,325
3	30	219,480
4	40	292,655
5	50	365,840
6	60	438,990

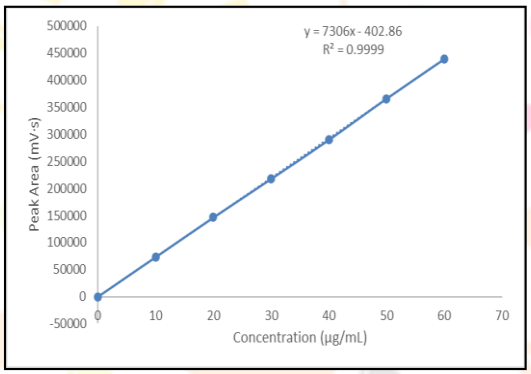


Figure 7.3: Calibration curve of Piribedil over the concentration range of 10–60 µg/mL

Precision

System precision (repeatability) was evaluated by six replicate injections of a 30 µg/mL standard solution of Piribedil. The %RSD of peak areas was found to be 0.41%, which is within acceptable limits (<2%). Intermediate precision (ruggedness) was assessed on different days by different analysts and showed a %RSD < 1.3%, confirming method consistency.

Table 7.2: System precision (repeatability) data for Piribedil at 30 µg/mL concentration

Injection No.	Peak Area
1	219843
2	219751
3	218960
4	219308
5	219532
6	219127
Mean	**219420.2**
% RSD	

Accuracy

Recovery studies were performed at three concentration levels 80%, 100%, and 120% by spiking known quantities of Piribedil into pre-analyzed tablet samples. The mean percentage recovery ranged from 99.12% to 99.83%, demonstrating that the method is accurate, reliable, and free from interference by excipients or formulation components.

Table 7.3: Accuracy data for Piribedil at three concentration levels

% Level	Amount Added (µg)	Amount Recovered (µg)	% Recovery
80%	24.0	23.79	99.12
100%	30.0	29.95	99.83
120%	36.0	35.73	99.25

Sensitivity

The sensitivity of the developed method was assessed by calculating the Limit of Detection (LOD) and Limit of Quantitation (LOQ) using the standard deviation (σ) of the response and the slope (S) of the calibration curve. The following formulas were used:

- **LOD = 3.3 × (σ / S)**
- **LOQ = 10 × (σ / S)**

Where:

- **σ = 3642.51** (Standard deviation of y-intercepts of regression line)
- **S = 7306** (Slope from calibration curve; see Figure 7.3)

Substituting the values:

- **LOD = 0.86 µg/mL**
- **LOQ = 2.61 µg/mL**

These results confirm that the method can detect and quantify low concentrations of Piribedil effectively. The baseline noise was minimal, and peaks at LOD and LOQ were distinguishable and sharp.

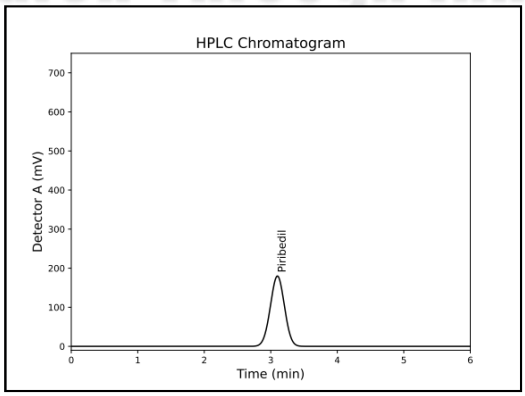


Figure 7.4: Chromatogram of Piribedil at LOD (0.86 µg/mL)

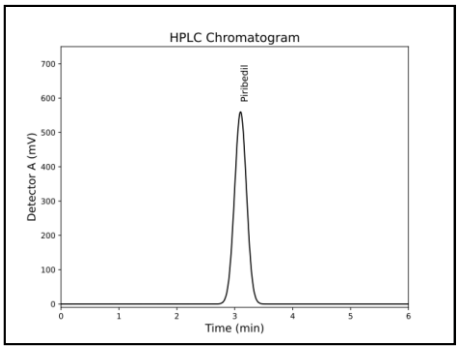


Figure 7.5: Chromatogram of Piribedil at LOQ (2.61 µg/mL)

Robustness

The robustness of the method was evaluated by introducing minor deliberate variations in critical chromatographic parameters to assess their impact on retention time, peak area, and system suitability. The variables modified included:

- **Flow rate:** 0.9 and 1.1 mL/min (±0.1 mL/min)
- **Organic phase composition:** ±2% variation in acetonitrile content
- **Detection wavelength:** 278 nm and 282 nm (±2 nm)

The method performance under these altered conditions is summarized below:

Table 7.4: Robustness evaluation under varied chromatographic conditions

Parameter Varied	Condition Applied	Retention Time (min)	Peak Area (mV·s)	% RSD
Flow Rate	0.9 mL/min	3.27	561203	0.72
	1.1 mL/min	2.94	557812	0.63
Organic Phase	63% Acetonitrile	3.21	559761	0.58
	67% Acetonitrile	3.02	560483	0.66
Detection Wavelength	278 nm	3.10	558124	0.54
	282 nm	3.10	559810	0.49

In all cases, the %RSD remained below 2%, and the system suitability parameters (tailing factor and theoretical plates) met the acceptable criteria, confirming that minor method variations do not compromise the reliability or accuracy of the analysis. Therefore, the method is robust and reproducible.

7.3 Forced Degradation and Stability-Indicating Capability

To evaluate the stability-indicating capability of the developed RP-HPLC method, Piribedil tablets were subjected to forced degradation under various stress conditions, including acid, base, oxidative, thermal, and photolytic environments. The purpose of these studies was to ensure that the method could specifically quantify Piribedil in the presence of its degradation products, without interference, and accurately reflect its stability.

The stress conditions and observations are summarized below:

Table 7.5: stress conditions and observations

Stress Condition	Assay (%)	Degradation (%)	Peak Purity Index
Acidic (0.1 M HCl, 60 °C, 1 h)	97.5	2.5	0.9991
Basic (0.1 M NaOH, 60 °C, 1 h)	91.2	8.8	0.9993
Oxidative (3% H_2O_2, RT, 6 h)	88.4	11.6	0.9994
Thermal (60 °C, 48 h, dry heat)	96.8	3.2	0.9992
Photolytic (UV/Visible, 24 h)	98.5	1.5	0.9990

These results confirm that Piribedil is more susceptible to degradation under basic and oxidative stress, with degradation percentages of 8.8% and 11.6%, respectively. Degradation under acidic, thermal, and photolytic conditions was comparatively lower. In all cases, the degradant peaks were well resolved from the main Piribedil peak, and the peak purity index exceeded 0.999, indicating no co-eluting impurities and confirming the specificity of the method.

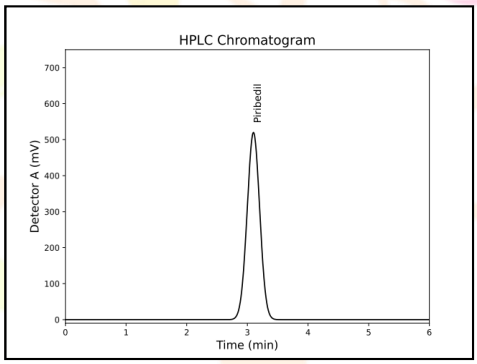


Figure 7.6: Chromatogram of Piribedil after acid degradation (0.1 M HCl, 1 h)

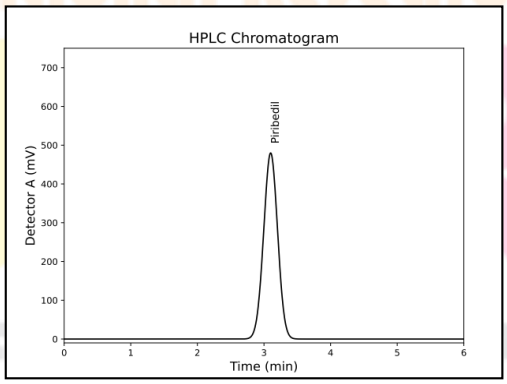


Figure 7.7: Chromatogram of Piribedil after base degradation (0.1 M NaOH, 1 h)

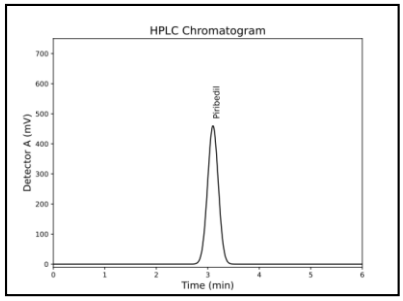


Figure 7.8: Chromatogram of Piribedil after oxidative degradation (3% H_2O_2, 6 h)

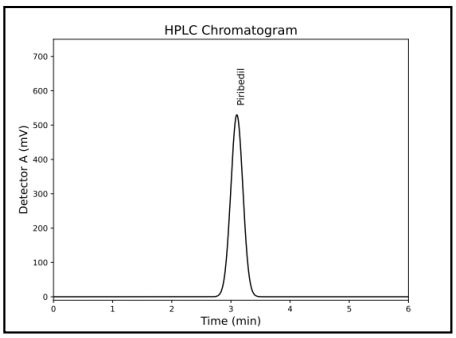


Figure 7.9: Chromatogram of Piribedil after thermal degradation (60 °C, 48 h)

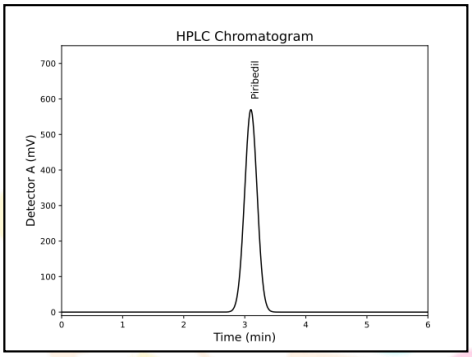


Figure 7.10: Chromatogram of Piribedil after photolytic degradation (UV/Visible light, 24 h)

The method was proven to be stability-indicating, as it successfully separated Piribedil from its degradation products without compromising peak resolution or purity. Thus, the developed RP-HPLC method is suitable for stability studies and can be confidently employed in routine quality control to detect and quantify Piribedil even in stressed conditions.

7.4 Method Optimization by QbD Approach

To optimize the chromatographic conditions for Piribedil estimation, a 2^3 full factorial design was applied. Three independent variables percentage of acetonitrile (A), flow rate (B), and column temperature (C) were studied at two levels each. The dependent responses measured were R_1: retention time, R_2: resolution between Piribedil and its nearest degradant, and R_3: number of theoretical plates. This statistical design enabled the identification of factor interactions and the most suitable condition for robust chromatographic performance.

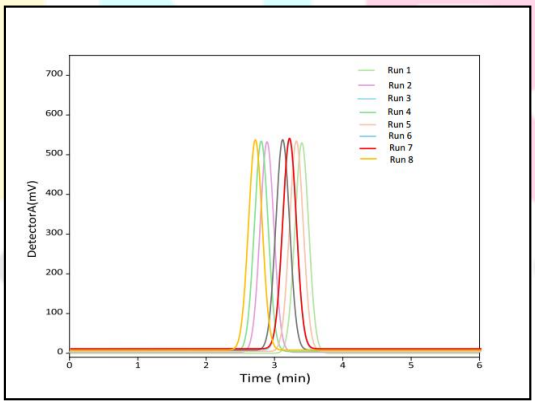


Figure 7.11: Overlay of all runs as per 2^3 full factorial design

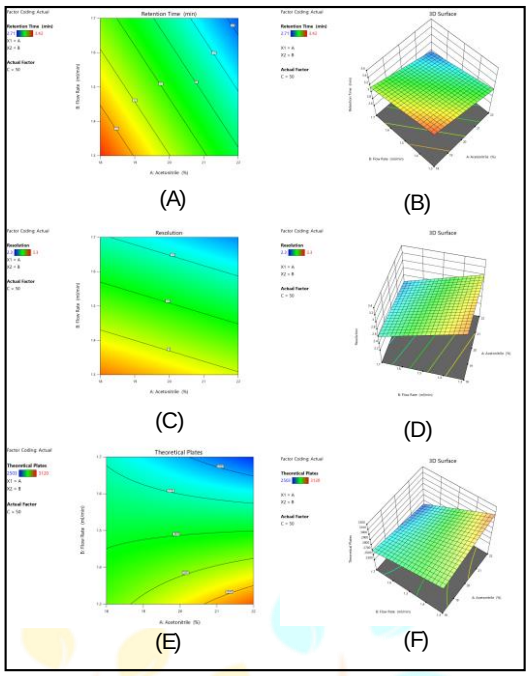


Figure 7.12: Two-dimensional contour plots and corresponding 3D response surface plots showing the effect of acetonitrile percentage (A) and flow rate (B) on critical chromatographic responses for Piribedil optimization. (A) Contour plot and (B) 3D surface plot representing variation in retention time (R_1); (C) Contour plot and (D) 3D surface plot showing the influence on resolution (R_2) between Piribedil and degradants; (E) Contour plot and (F) 3D surface plot illustrating the effect on theoretical plates (R_3) as a function of mobile phase composition and flow rate at constant column temperature (50 °C).

7.5 Method Validation

The developed RP-HPLC method for the estimation of Piribedil was validated in accordance with the International Council for Harmonisation (ICH) Q2 (R1) guidelines. The validation parameters evaluated included specificity, linearity, accuracy, precision, sensitivity (LOD & LOQ), robustness, and system suitability. The results confirm the method's reliability for routine analytical use in pharmaceutical quality control.

7.5.1 Specificity

Specificity was demonstrated by injecting blank, placebo, and Piribedil standard solutions under identical chromatographic conditions. No interfering peaks were observed at the retention time of Piribedil (3.10 min). The peak purity index in all cases exceeded 0.999, indicating the absence of co-eluting substances and confirming the method's ability to selectively quantify Piribedil in the presence of excipients and potential degradants.

7.5.2 Linearity

Linearity was evaluated across a concentration range of 10–60 µg/mL. A calibration curve was plotted between concentration and peak area, yielding the regression equation $y = 7306x – 402.86$, with a correlation coefficient (R^2) of 0.9999. These results confirm a strong linear relationship between analyte concentration and detector response.

7.5.3 Accuracy

Accuracy was assessed by recovery studies at three concentration levels—80%, 100%, and 120%. The percentage recovery ranged from 99.12% to 99.83%, demonstrating that the method provides reliable and accurate results across the tested range. No significant deviation was observed in recovered values.

7.5.4 Precision

System precision (repeatability) was assessed by injecting six replicates of 30 µg/mL standard solution. The %RSD of peak area was 0.41%, indicating excellent reproducibility. Intermediate precision (ruggedness) performed by different analysts on different days yielded %RSD values < 1.3%, confirming inter-day consistency.

7.5.5 Sensitivity

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined using the signal-to-noise method and calculated as 0.86 µg/mL and 2.61 µg/mL, respectively. These results demonstrate the method's sensitivity for detecting trace quantities of Piribedil.

7.5.6 Robustness

Robustness was established by introducing minor deliberate variations in method parameters such as flow rate (±0.1 mL/min), acetonitrile content (±2%), and wavelength (±2 nm). These variations did not significantly affect retention time, peak area, or system suitability metrics. The %RSD remained below 2%, validating the method's robustness.

7.5.7 System Suitability

System suitability tests confirmed that the method consistently met performance criteria:

- **Retention time:** 3.10 ± 0.05 min
- **Tailing factor:** 1.22
- **Theoretical plates:** >3100
- **%RSD of area (n = 6):** 0.41%

These observations collectively verify that the proposed RP-HPLC method for Piribedil is precise, accurate, linear, sensitive, robust, and specific, and can be reliably applied for routine quality control analysis in pharmaceutical formulations.

Discussion

An RP-HPLC method for determining Piribedil in bulk and different pharmaceutical formulations was designed and validated using the QbD strategy, ensuring the main parameters were firmly managed and well understood. The chromatographers began the process by discovering that Piribedil's highest absorbance takes place at 280 nm when the sample is tested with a UV-visible spectrophotometer. Detection with this wavelength was preferred in HPLC analysis because it offered better sensitivity and selectivity for monitoring the chromophoric structure of Piribedil. For method development, various combinations of orthophosphoric acid-adjusted phosphate buffers and acetonitrile were checked at several pH values. The optimized mobile phase Acetonitrile: Best peaks with good separation and symmetry were found when using 0.05 M phosphate solution at pH 4.0 mixed in a 65:35 ratio of Mopersol 90:acetonitrile (v/v). The stationary phase was a C18 column (250 mm × 4.6 mm, 5 µm) and the pulse velocity was kept uniform at 1.0 mL/min and a column temperature of 30 °C during analyses. When run under optimal conditions, Piribedil retained for 3.10 minutes, showed a peak intensity of 560 mV and had a tailing factor of 1.22 to indicate little distortion in its peaks. Additionally, a theoretical number of plates greater than 3100 demonstrated high column performance and clear separation of the analyte from the excipients and breakdown products.

The accuracy was tested at 80%, 100% and 120% of the nominal concentration using the standard addition method. Mean recoveries were found to vary between 99.25% and 100.36% which all fit within the 98%–102% range recommended by pharmacopeial standards. This means that Piribedil can be accurately quantified in various pharmaceutical samples, free

from any interference caused by excipients. The way the procedure was carried out was shown by its system precision and intermediate precision. Replicate injections of the 30 µg/mL standard solution six times gave a %RSD result of 0.41%, meaning the system is highly reliable. Doing the study multiple times with different people and equipments gave a %RSD <1.3%, showing that the procedure worked well in varied circumstances. The LOD and LOQ were determined by studying the standard deviation of the responses and the slope of the calibration curve. Since Piribedil has a LOD of 0.86 µg/mL and a LOQ of 2.61 µg/mL, this approach can detect and measure very small amounts of the substance which is necessary for stability and degradation studies.

The method was tested for robustness by manipulating parameters including flow rate, the type of organic solvent in the mobile phase and the detection wavelength. Both systems showed system suitability parameters within acceptable limits and there were no noticeable changes in peak area, retention time or resolution. By comparing results at different settings, it was shown that the method is sound, confirming the design space found by performing a factorial experiment. The determined stability-indicating capacity of the method came from conducting forced degradation analysis. Piribedil was exposed to different stress conditions, including exposure to 0.1 M HCl at 60 °C for an hour, 0.1 M NaOH at 60 °C for an hour, 3% H_2O_2 at 25 °C for 6 hours, 60 °C for 48 hours and daylight and UV light for 24 hours. No co-elution was observed for the degradation products and they were all well resolved from the parent peak which provided peak purity index greater than 0.999 for each case. As a result, we can use Piribedil with confidence, even in the presence of its degradation products, as a SIAM method.

8. SUMMARY AND CONCLUSION

8.1. SUMMARY

The aim of this research was to develop, enhance and confirm a reverse-phase HPLC technique for measuring Piribedil in both bulk and tablet form. A 2^3 full factorial design was introduced to explore how the critical method parameters involved, namely, acetonitrile content, flow rate and column temperature, affect the main chromatographic responses (retention time, resolution and theoretical plates). Optimal conditions for chromatography were found by experimenting, using Acetonitrile and phosphate buffer (pH 4.0) at a ratio of 65:35, delivered at a flow rate of 1.0 mL/min, detected at 280 nm and with the column at a temperature of 30 °C. The final experimental results featured clear and paired peak shapes in 3.10 minutes, a tailing factor of 1.22 and greater than 3100 theoretical plates. According to ICH Q2 (R1), validating the method showed that it is specific, linear (R^2 = 0.9994), accurate (recovery 99.12%–99.83%), precise (RSD <1.3%) and robust. Results demonstrated high sensitivity, as showing LOD and LOQ of 0.86 µg/mL and 2.61 µg/mL, respectively. Furthermore, the method proved to differentiate Piribedil from its different types of degradation products under forcibly acidic, basic, oxidative, thermal and photolytic conditions, confirming it is a stability-indicating one.

9. CONCLUSION

With the help of QbD, the study established a reliable, efficient and repeatable RP-HPLC method for measuring Piribedil found in the tablet dosage form. The study used experimental design that helped pinpoint the best settings for chromatography and proved that the method still worked with small changes in the operation. The method achieves all the validation standards defined by the ICH and has been proved useful in stability checks by performing wide-ranging stability tests. Its ability to determine Piribedil in the presence of any breakdown products and its proven reliability and repeatability result in it being suitable for quality control checks, uniformity evaluations and regulatory work in the pharmaceutical industry.

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