DISSOLUTION METHOD VALIDATION OF TIEMONIUM METHYLSULFATE TABLET 50 MG

A thesis presented to the Department of Pharmacy at Daffodil International University, in partial fulfillment of the requirements for the degree of Master of Pharmacy (M. Pharm).

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APPROVAL

The thesis titled "Dissolution Method Validation of Tiemonium Methyl Sulfate Tablet 50 mg" has been submitted to the Department of Pharmacy at Daffodil International University. It has been deemed satisfactory in partial fulfillment of the requirements for the Master of Pharmacy degree, and its contents have received approval and authorization.

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CERTIFICATION

I, as an Assistant Professor in the Department of Pharmacy, Faculty of Allied Health Sciences at Daffodil International University, hereby certify that I supervised this thesis. It substantially fulfills the requisites for the Master of Pharmacy (M. Pharm) degree. Additionally, I affirm that the implementations presented in this thesis are original and have not been previously submitted to any degree program within this university.

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DECLARATION

I hereby affirm that I have independently undertaken and completed this project under the guidance of the Faculty of Allied Health Sciences at Daffodil International University, meeting the criteria for the Master of Pharmacy (M. Pharm) degree. I declare that this project is the result of my individual efforts. Furthermore, I emphasize that the execution of this project is distinctive and has not been previously presented in any degree program within this university.

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Foremost, I extend my gratitude to the Divine for granting me the capability to successfully complete this thesis and pursue studies in this field.

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Author

Sakib Hossain Sabbir

DEDICATION

Dedicated to the One and Only Almighty “ALLAH” who is the creator of everything.

Abstract

This study represents a comprehensive analytical method validation study focusing on the Dissolution method for Tiemonium Methyl Sulfate 50 mg tablets. The investigation encompasses crucial validation parameters, including Method Precision, Intermediate Precision, Solution Stability, System Suitability, Specificity, Filter Evaluation, Linearity, and Accuracy. Through rigorous testing, all parameters were found to fall within acceptable ranges, demonstrating the robustness and reliability of the Dissolution method. Notably, the extended Solution Stability, reaching up to 18 hours, emphasizes the method's practical utility and consistency. These favorable outcomes collectively affirm the suitability of the Dissolution method for routine analysis of Tiemonium Methyl Sulfate 50 mg tablets in pharmaceutical quality control. This research contributes valuable insights to the field, offering a validated and effective analytical approach for ensuring the accuracy and precision of dissolution testing in pharmaceutical formulations.

Keywords: *Analytical Method Validation, Dissolution Method, Tiemonium Methyl Sulfate, Pharmaceutical Analysis, Quality Control, Solution Stability, Method Precision, System Suitability, Linearity, Accuracy, Specificity.*

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Chapter 1. Introduction

1.1 General Introduction

In pharmaceuticals and other sectors, analytical method validation is a thorough and systematic procedure that evaluates and proves the accuracy, reliability, and consistency of analytical methods used to test, measure, and analyze diverse substances and products. This validation is crucial to ensure the safety and effectiveness of pharmaceutical goods and meet regulatory criteria. In order to guarantee the quality and safety of pharmaceuticals and other products across many industries, analytical method validation is an essential procedure. Manufacturers can give consumers trust in the precision and dependability of their analytical processes by adhering to established regulatory rules and standards, which will eventually improve public health and product quality. To meet quality and safety criteria, protect the public's health, and maintain compliance with regulatory bodies, it is imperative to adhere to regulatory rules and standards. [1][2][3]

1.2 Analytical Method Validation as per Recognized Guidelines Worldwide

1.2.1 ICH Guidelines

The International Council for Harmonisation (ICH) provides the following definition for analytical method validation in its guideline Q2(R1), titled "Validation of Analytical Procedures: Text and Methodology":

"Analytical method validation is the process of demonstrating that analytical procedures are suitable for their intended use."

The essential goal of analytical method validation is to establish and document the suitability for the purpose of analytical methods employed in the healthcare sector, and this brief definition captures this objective. The validation procedure guarantees that these methods can measure crucial quality aspects with accuracy and reliability, generating confidence in the caliber, safety, and efficacy of pharmaceutical products. [2]

1.2.2 FDA Guidelines

The U.S. Food and Drug Administration (FDA) defines analytical method validation as a systematic and recorded process that shows an analytical procedure is appropriate for its intended use in the context of pharmaceutical quality assurance and regulatory compliance. The FDA's guidance documents, such as "Analytical Procedures and Methods Validation for Drugs and Biologics" (May 2015), outline several critical method attributes, including specificity, accuracy, precision, linearity, and robustness. As part of this comprehensive verification process, these attributes are evaluated to ensure the method consistently and dependably provides accurate data for decision-making in pharmaceutical development, production, and quality control. [1]

1.2.3 EMA Guidelines

The European Medicines Agency (EMA) defines analytical method validation as a systematic and recorded process that shows an analytical procedure is appropriate for its intended use within the context of pharmaceutical quality assurance and

regulatory compliance. In order to make sure that the method consistently and dependably provides accurate data for decision-making in the development, production, and quality control of pharmaceuticals, a thorough validation process that takes into account the assessment of critical attributes, including specificity, accuracy, precision, linearity, and robustness, in accordance with EMA guidelines, is carried out. The specific standards and guiding principles for analytical method validation within the European Union regulatory framework are outlined in EMA references like the "Guideline on Validation of Analytical Procedures: Text and Methodology" and other pertinent materials.

1.3 Purpose of Analytical Method Validation in Pharmaceutical Industries

1.3.1 Achieving Product Safety and Quality: Analytical method validation verifies the accuracy and consistency of quality control methods. This ensures that pharmaceutical products meet quality and safety criteria. [4]

1.3.2 Ensuring Product Quality and Safety: Analytical method validation verifies the accuracy and reliability of methods used for quality control. This ensures that pharmaceutical products meet quality and safety standards. [4]

1.3.3 Regulatory Compliance: Validation is a regulatory requirement to demonstrate that analytical methods are suitable for their intended use. [6]

1.3.4 Stability and Shelf-Life Determination: By identifying degradation

products, validated methodologies aid in the evaluation of product stability and enable the calculation of shelf-life. [7]

1.3.5 Batch Release: Validated methods are essential for the release of each batch of pharmaceutical products to ensure they meet specifications. [8]

1.3.6 Optimizing Manufacturing Processes: Validation is integral to optimizing manufacturing processes by monitoring critical parameters and ensuring consistent product quality. [9]

1.3.7 Safety and Efficacy Assessment: Validation helps determine the safety and effectiveness of pharmaceutical goods by precisely measuring active components and identifying contaminants. [10]

1.3.8 Data Reliability for Research and Development: Validated methods provide reliable data for research and development activities, including formulation development and process optimization. [10]

1.4 Methods to be Validated

1.4.1 Assay Methods: Assay methods are essential for quantifying the active pharmaceutical ingredient (API) in a drug product. Ensuring accurate quantification is critical for dose consistency and therapeutic efficacy.[12] In the pharmaceutical sector, validation of assay methods is necessary to guarantee precise and reliable quantification of the active pharmaceutical ingredient (API) in therapeutic products. In order to ensure proper dosage calculation, regulatory compliance, and patient safety, validation is crucial for accuracy, precision, and dependability. It is a crucial part of pharmaceutical quality assurance

and control since it supports batch release, stability testing, process optimization, quality control, and regulatory filings.

1.4.2 Impurity Testing Methods: Validation of impurity testing procedures is necessary in the pharmaceutical sector to ensure precise identification and measurement of impurities, including degradation products and impurities related to processes. Validation guarantees the accuracy and dependability of these techniques, which is essential for adhering to regulations and averting any possible dangers to patient safety. Precise impurity profiling is essential for evaluating pharmaceutical products' overall quality, safety, and effectiveness. It also helps define acceptable limits and make sure impurities stay below designated thresholds, protecting public health and adhering to strict regulations**.** Impurity testing methods detect and quantify impurities, including process-related impurities and degradation products, which can impact product safety and efficacy. [13]

1.4.3 Dissolution Methods: Dissolution methods assess how a drug product releases the active ingredient over time, directly affecting bioavailability and therapeutic performance. [14] Validation of dissolution methods in the pharmaceutical sector is imperative to confirm their precision, accuracy, and dependability when assessing the release of an active ingredient from a drug product over time. This validation is essential in verifying the drug's bioavailability and therapeutic effectiveness, as the speed of active

ingredient release directly influences its absorption and performance. The accuracy of dissolution testing is a fundamental element of quality control, stability assessments, and regulatory submissions, all of which play a vital role in upholding public health, ensuring product consistency, and aligning with strict regulatory standards, ultimately enhancing the overall safety and efficacy of pharmaceutical products.

1.4.4 Content Uniformity Methods: Content uniformity methods ensure the even distribution of the API within a dosage form, which is critical to delivering consistent doses and therapeutic effect. Validation of content uniformity methods in the pharmaceutical sector is imperative to verify that every individual unit of a drug product contains the prescribed quantity of the active pharmaceutical ingredient (API).[15] It plays a pivotal role in quality control, ensuring regulatory compliance, and ultimately upholding patient safety by mitigating the potential hazards linked to under- or overdosing, while simultaneously reinforcing the overall effectiveness and trustworthiness of pharmaceutical products.

1.4.5 Microbiological Methods: Validation of microbiological methods in the pharmaceutical industry is essential to confirm their precision, accuracy, and dependability in the detection of microbial contamination and the assurance of sterility. This validation is of utmost importance to meet regulatory demands and pharmacopeial benchmarks, exemplified by the

United States Pharmacopeia (USP) Chapter <61> addressing the Microbiological Examination of Nonsterile Products and Chapter <71> focusing on Sterility Tests. The precise execution of microbiological testing is an indispensable element in protecting patient safety, preempting the perils linked to microbial contamination in pharmaceutical products. Moreover, validation plays a pivotal role in quality control, ensuring that the products align with prescribed standards and are suitable for their intended purposes, ultimately bolstering public health and regulatory adherence. [16][17][18]

1.5 Parameters of Analytical method validation

1.5.1 System Suitability

System suitability represents a pivotal element in method validation, serving to confirm the suitability of an analytical method for its designated use. This assessment encompasses a predefined set of criteria and tests that collectively evaluate the performance of the complete analytical system, encompassing instruments, reagents, and the proficiency of analysts, with the primary objective of ensuring consistent delivery of precise and dependable results. The execution of system suitability testing is a means to affirm that the entire system operates within predefined limits, thereby assuring the method's precision, specificity, and overall resilience. Its significance lies in preserving the integrity and dependability of data generated during pharmaceutical analysis, aligning with established regulatory guidelines. [19][20]

1.5.2 Specificity

A comprehensive evaluation of an analytical method's capacity to precisely and selectively detect the target analyte in the presence of probable interferents, contaminants, or closely related compounds is known as specificity, which is a crucial method validation criterion in the pharmaceutical industry.[19] It is done to make sure the method accurately and precisely measures and uniquely identifies the target compound in real-world sample matrices. As it protects the integrity and trustworthiness of analytical findings by preventing cross-contamination or misinterpretation due to co-eluting chemicals, specificity is of utmost importance. [4][21]

1.5.3 Filter Evaluation

In pharmaceutical analysis, filter evaluation is a key parameter for method validation. It entails a thorough evaluation of the filtering procedure used to prepare samples to make sure it successfully eliminates particles and impurities while maintaining analyte integrity.[4] As poor filtration might introduce mistakes, impair data quality, and result in inaccurate quantification, this evaluation is carried out to ensure the dependability and accuracy of analytical results. The role that filter evaluation plays in sustaining data integrity and the reliability of analytical procedures, harmonizing with good laboratory practices (GLP) and regulatory standards, emphasizes the significance of this process. [11]

1.5.4 Precision

Precision, a fundamental method validation parameter in pharmaceutical analysis, is the measure of a method's ability to consistently generate reproducible results under varying conditions. It is performed to ensure that the analytical method is dependable and can repeatedly produce closely clustered data points.[4] Precision is of paramount importance because it assures that the method can distinguish small differences in analyte concentration, detect impurities or variations in a sample, and provide reliable, consistent results. In pharmaceutical research and manufacturing, precise analytical methods are crucial to make informed decisions, meet regulatory requirements, and ensure the quality, safety, and efficacy of pharmaceutical products. [2]

1.5.4.1 System Precision

In pharmaceutical analysis, system precision, a crucial component of method validation, evaluates the reproducibility of results when the complete analytical system, including equipment, analysts, and circumstances, is taken into account. It is done to assess the method's overall consistency and dependability while taking into account all possible sources of variance.[5] System accuracy is crucial since it makes sure the whole thing can consistently produce reliable and accurate outcomes. This parameter is essential for upholding the quality, safety, and efficacy of pharmaceutical goods as well as data

integrity, supporting decision-making, and regulatory compliance. [2,4]

1.5.4.2 Method Precision

Method precision, a fundamental component of method validation in the realm of pharmaceutical analysis, serves as the cornerstone for assessing an analytical method's capacity to consistently and reproducibly yield precise results within well-defined parameters. Its execution is driven by the imperative need to ascertain that the method consistently and reliably quantifies analytes and impurities with unwavering accuracy. [4] Method precision assumes pivotal significance by virtue of its role in fortifying data quality and integrity, thereby facilitating informed decision-making, aligning with regulatory requisites, and upholding the paramount objectives of pharmaceutical product safety, effectiveness, and quality assurance. [5]

1.5.4.3 Intermediate Precision

Intermediate precision, a pivotal component of method validation in the realm of pharmaceutical analysis, evaluates the method's capacity to consistently yield precise and reproducible results in the face of diverse laboratory conditions, equipment, analysts, or temporal factors. This assessment is conducted to gauge the

method's robustness and its consistent ability to produce accurate and dependable results, even when exposed to variations arising from external factors. Intermediate precision assumes significant significance as it offers critical insights into the method's reliability and stability, thereby strengthening its capacity to meet stringent regulatory requisites and upholding the benchmarks for pharmaceutical product quality, safety, and efficacy. [22,23,24]

1.5.5 Linearity

Linearity, a fundamental facet in the validation of methods within pharmaceutical analysis, serves to assess an analytical method's capability to generate results that maintain a direct and predictable relationship with the concentration of the targeted analyte across a specified range. This evaluation is essential to confirm that the method adheres to a linear response in which the measured outcomes correspond accurately to changes in analyte concentration. The significance of linearity lies in its assurance that the method is well-suited for the precise quantification of analytes across a spectrum of concentrations, a crucial factor in determining accurate dosage levels and ensuring therapeutic effectiveness. Furthermore, this parameter underscores the method's compliance with stringent regulatory prerequisites and quality standards. [22,25,26]

1.5.6 Accuracy

Accuracy, a fundamental parameter in the context of method validation in pharmaceutical analysis, serves as the keystone for evaluating a method's capacity to yield results that closely approximate the true or intended values. This meticulous assessment is undertaken to substantiate the method's consistent and dependable aptitude for quantifying analytes with minimal systematic deviations, thus warranting that the reported outcomes align faithfully with the genuine concentrations.[22] The profound importance of accuracy cannot be overstated, given its direct and far-reaching implications on patient well-being and the therapeutic effectiveness of pharmaceutical products. By ensuring the precise presence of the designated active component, accuracy functions as a robust safeguard against the potential perils of under- or over-dosage. Additionally, it assumes a pivotal role in upholding regulatory mandates, fortifying product quality, and preemptively averting prospective health hazards. [24,25]

1.5.7 Solution Stability

Solution stability, a pivotal component of the method validation process in pharmaceutical analysis, involves the meticulous assessment of the stability of the solutions or reagents employed within the analytical methodology. This rigorous scrutiny is undertaken with the overarching objective of verifying the sustained integrity and unimpaired state of these solutions throughout the analytical procedures. [27,30] The significance of solution stability reverberates profoundly, given its direct and consequential influence on the precision and trustworthiness of the ensuing analytical outcomes. Any degradation or alteration in these solutions

can potentially introduce systematic deviations, thereby undermining the veracity and fidelity of the data generated, ultimately culminating in inaccuracies in quantification. Consequently, the assurance of solution stability emerges as a critical imperative, serving as the vanguard for the overall soundness of the entire analytical process, regulatory adherence, and the fortification of the safety and therapeutic efficacy of pharmaceutical products. [28,29,31]

1.6 Drug Profile

1.6.1 Tiemonium Methyl Sulfate

Tiemonium Methyl Sulfate, (4-[3-Hydroxy-3-phenyl-3-(2-tienyl) propyl]-4-metylmorpholinium methyl sulfate (IUPAC)), classified as a quaternary ammonium compound, serves as a valuable therapeutic agent for the management of smooth muscle spasms within the gastrointestinal and urinary systems. It boasts clinical significance in the treatment of an array of medical conditions, including irritable bowel syndrome (IBS), functional dyspepsia, and a spectrum of gastrointestinal disorders typified by distressing symptoms like abdominal pain, cramps, and irregular bowel patterns. The mechanism of action revolves around its capacity to impede the influence of acetylcholine on muscarinic receptors located in smooth muscle cells, a pivotal feature that underlies its effectiveness in alleviating the discomfort and distress associated with spasmodic conditions, thus substantially enhancing the overall well-being of those afflicted.

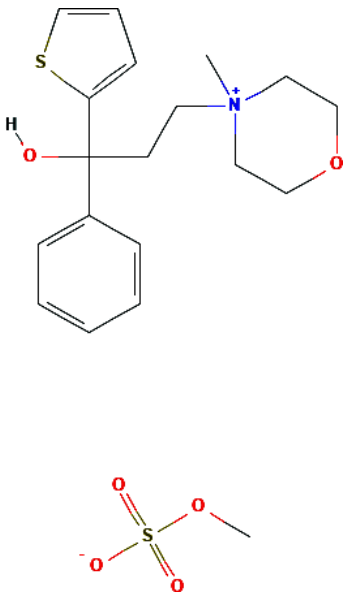


Figure 1: Chemical Structure of Tiemonium Methyl Sulfate

1.6.2 Chemical and Physical Properties

Chemical Name	4-[3-Hydroxy-3-phenyl-3-(2-tienyl)propyl]-4-metylmorpholinium methyl sulfate
Molecular Formula	$C_{19}H_{27}NO_6S_2$
Molecular Weight	429.6 g/mol
Melting Point	167.74°C
Boiling Point	337.9°C
Solubility	Sparingly Soluble in water and Readily soluble in organic solvent
Physical Status	White to off white crystalline powder
Elimination Half Life	About 8 Hours
ROA	Oral, Parenteral, Rectal

Excretion	Renal

Table 01: Chemical and Physical Properties of Tiemonium Methyl Sulfate

1.6.3 Pharmacological Properties

Tiemonium Methyl Sulfate serves as a fundamental antispasmodic medication with a core focus on mitigating smooth muscle spasms encountered within the gastrointestinal and urinary systems. Its clinical utility encompasses the following domains:

1.6.3.1 Irritable Bowel Syndrome (IBS): Tiemonium Methyl Sulfate emerges as an indispensable therapeutic choice for individuals grappling with the challenges of IBS, a prevalent functional gastrointestinal disorder typified by a spectrum of discomforts including abdominal pain, cramps, bloating, and unpredictable bowel patterns. Its efficacy lies in the alleviation of these distressing symptoms, achieved by tempering the excessive smooth muscle contractions in the gastrointestinal tract.

1.6.3.2 Functional Dyspepsia: This medication extends its application to the management of symptoms associated with functional dyspepsia, a recurring source of upper abdominal discomfort, indigestion, and bloating. Through its ability to induce relaxation in the smooth muscles of the digestive tract, Tiemonium Methyl Sulfate serves as a means to alleviate these discomforts effectively.

1.6.3.3 Gastrointestinal Spasms: Tiemonium Methyl Sulfate finds purpose in addressing both acute and chronic spasms occurring in the stomach and intestines, frequently attributed to conditions such as gastritis, gastroenteritis, or inflammatory bowel diseases. By mitigating these spasmodic contractions, the medication plays a pivotal role in the mitigation of pain and discomfort.

1.6.3.4 Biliary Disorders: The application extends to the relief of spasms in the biliary tract, encompassing the gallbladder, a particularly pertinent intervention in cases of cholecystitis where spasms contribute significantly to the experience of pain and discomfort.

1.6.3.5 Urinary Tract Spasms: The efficacy of this medication extends to the management of spasms within the urinary tract, a condition that manifests in urinary tract infections (UTIs) or colic associated with urinary stones. By mitigating these spasms, it contributes substantially to the alleviation of pain and discomfort.

1.6.3.6 Other Medical Conditions: In addition to the outlined applications, Tiemonium Methyl Sulfate retains the potential for deployment in addressing additional medical conditions characterized by the presence of smooth muscle spasms. The selection of this usage is

guided by the discretion of healthcare professionals, who carefully assess its appropriateness on a case-by-case basis.

1.6.4 Side Effects

1.6.4.1 Dry Mouth (Xerostomia): This is a prevalent side effect, as Tiemonium Methyl Sulfate can reduce salivary secretion, leading to a sensation of dryness in the mouth.

1.6.4.2 Blurred Vision: Some individuals may encounter transient visual disturbances, such as blurred vision or difficulty focusing, while using the medication.

1.6.4.3 Constipation: Tiemonium Methyl Sulfate can slow down bowel motility in certain cases, potentially resulting in constipation.

1.6.4.4 Urinary Retention: In rare instances, the medication may lead to difficulty in urination or urinary retention, where emptying the bladder becomes challenging.

1.6.4.5 Dizziness: Some individuals may experience dizziness or lightheadedness, which can affect their balance and coordination.

1.6.4.6 Nausea: Nausea and mild gastrointestinal discomfort can occur in some cases.

1.6.4.7 Allergic Reactions: Though uncommon, allergic reactions are possible. Immediate medical attention is necessary if any signs of an allergic reaction appear, such as skin rash, itching, swelling, severe dizziness, or difficulty breathing.

1.6.5 Contraindication

Tiemonium methylsulphate is contraindicated in cases of glaucoma or when there is acute eye pain accompanied by vision disturbances. Additionally, this medication should not be administered to individuals with conditions affecting the prostate or urinary bladder.

1.6.6 Available brands in Bangladesh market

- Algin (Reneata Limited)
- Emonium (Beximco Pharmaceuticals Ltd.)
- Norvis (Square Pharmaceuticals Ltd.)
- Visral (Opsonin Pharma Ltd.)
- Xelcom (Radiant Pharmaceuticals Ltd.)

1.7 Test Sample Details

- **Name** **:** Tiemonium Methyl Sulfate 50 mg Tablet

- **Name of API** **:** Tiemonium Methyl Sulfate
- **Methodology** **:** Non-Compendial
- **Method Reference** **:** INN
- **Specification Reference** **:** INN
- **Label Claim** **:** Each Tablet contains Tiemonium Methyl Sulfate 50

mg.

1.8 Formulation of Tiemonium Methyl Sulfate Tablet

S/N	Name of Material	Quantity/Tablet (mg)
1	Tiemonium Methyl Sulfate INN	50.000
2	Microcrystalline Cellulose (Avicel pH 102)	22.066
3	Lactose Monohydrate, Spray Dried	53.462
4	Pregelatinized Maize Starch (Starch 1500)	14.200
5	Magnesium Stearate	1.420
6	Purified Talc	0.852
7	Opadry II 85F18422 White	4.260

Table 02: Formulation of Tiemonium Methyl Sulfate 50 mg Tablet

1.9 Formulation of Placebo

S/N	Name of Material	Quantity/Tablet (mg)
1	Microcrystalline Cellulose (Avicel pH 102)	22.066
2	Lactose Monohydrate, Spray Dried	53.462

3	Pregelatinized Maize Starch (Starch 1500)	14.200
4	Magnesium Stearate	1.420
5	Purified Talc	0.852

Table 03: Formulation of Placebo for Tiemonium Methyl Sulfate Tablet

Chapter 2. Objective of The Study

The primary objective of analytical method validation (AMV) is to demonstrate that an analytical method is suitable for its intended purpose and to ensure the reliability, accuracy, and consistency of the generated results. His comprehensive research aims to validate the analytical method for the dissolution of Tiemonium Methyl Sulfate 50 mg tablets, encompassing a thorough examination of key parameters crucial for method reliability and accuracy. The primary aim of this study is to comprehensively validate the analytical method for the dissolution of Tiemonium Methyl Sulfate 50 mg tablets. This includes evaluating key parameters such as system suitability, specificity, filter evaluation, precision, linearity, accuracy, and solution stability. The study seeks to establish the reliability, precision, and robustness of the Dissolution method, ensuring its suitability for routine pharmaceutical analysis. By rigorously examining these parameters, the objective is to provide a validated and standardized analytical approach for the accurate assessment of Tiemonium Methyl Sulfate release from its tablet formulation. This research contributes to enhancing the overall quality control processes in pharmaceutical manufacturing and ensures the consistency and accuracy of dissolution testing for Tiemonium Methyl Sulfate 50 mg tablets. The objectives are demonstrated below,

- To evaluate the suitability of the analytical system, ensuring it consistently provides reliable and precise results.

- To assess the method's ability to accurately measure Tiemonium Methyl Sulfate in the presence of potential interfering substances, confirming its selectivity.
- To scrutinize the effectiveness and appropriateness of the filter in separating components during the dissolution process, ensuring its impact on results is well- understood and controlled.
- To determine the method's sensitivity to detect and quantify low concentrations of the analyte, ensuring it meets the required limits of detection and quantification.
- To assess the stability of the analytical solutions, reagents, and standards over time to ensure the method's reliability during routine use.
- To assess the stability of the analytical solutions, reagents, and standards over time to ensure the method's reliability during routine use.
- To ensure accurate quantification of pharmaceutical ingredients in drug products.

Chapter 3. Literature Review

The amalgamation of regulatory guidelines from the U.S. Food and Drug Administration (FDA), International Conference on Harmonisation (ICH), and the European Medicines Agency (EMA) delineates a comprehensive framework for analytical method validation in the pharmaceutical industry. The FDA's "Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics" issued in May 2015 serves as a foundational document, addressing critical aspects of analytical method validation to ensure the robustness of drug development processes.

The ICH's "Validation of Analytical Procedures: Text and Methodology Q2(R1)," introduced in 2005, stands out as an internationally recognized guideline facilitating global harmonization. This tripartite guideline provides a standardized approach, fostering consistency in analytical method validation across different regulatory jurisdictions.

Similarly, the EMA's "ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology" released in November 2005 aligns with the ICH guidelines, emphasizing the importance of harmonized standards in analytical method validation within the European context.

These regulatory documents collectively underscore the critical role of analytical method validation in ensuring the reliability, accuracy, and consistency of drug analysis. They offer a detailed roadmap, encompassing parameters such as specificity, precision, linearity, accuracy, and system suitability. The emphasis on global harmonization reflects the industry's commitment to establishing uniform standards, thereby streamlining drug development processes and safeguarding patient safety worldwide.

Analytical method validation (AMV) is a critical component of pharmaceutical analysis, ensuring the reliability and accuracy of analytical procedures employed in drug development and manufacturing.

Watson's "Pharmaceutical Analysis: A Textbook for Pharmacy Students and Pharmaceutical Chemists" serves as a foundational resource, covering diverse analytical principles essential for pharmaceutical professionals. Ahuja and Dong's "Handbook of Pharmaceutical Analysis by HPLC" provides an in-depth exploration of high-performance liquid chromatography (HPLC), a pivotal analytical technique widely used in the pharmaceutical industry.

"Pena's Analytical Chemistry for Assessing Medication Adherence" and Davani's "Pharmaceutical Analysis for Small Molecules" address unique aspects of pharmaceutical analysis. Pena focuses on the role of analytical chemistry in evaluating medication adherence, while Davani delves into the intricacies of analyzing small molecules.

Huynh-Ba's "Pharmaceutical Stability Testing to Support Global Markets" and Gibson's "Pharmaceutical Preformulation and Formulation" contribute insights into stability testing and preformulation studies, crucial for ensuring the quality and stability of pharmaceutical products.

Niazi's "Handbook of Pharmaceutical Manufacturing Formulations" and Gad's "Pharmaceutical Manufacturing Handbook: Production and Processes" offer perspectives on the formulation and manufacturing aspects of pharmaceuticals. These works delve into the intricacies of creating pharmaceutical formulations and optimizing manufacturing processes.

The literature is further enriched by works focusing on quality control and regulatory aspects. Yu's "Pharmaceutical Quality by Design: Principles and Applications" emphasizes the principles of quality by design, a paradigm promoting systematic approaches to ensure product quality throughout the lifecycle. Barnette's "Pharmaceutical Quality Control Lab Guidebook: GMP" provides practical insights into establishing good manufacturing practices (GMP) in pharmaceutical quality control laboratories.

Chapter 4. Materials and Methods

4.1 Materials

Name	Function	Specification/Grade	Manufacturer/ Origin	Source
Water	Media and Diluent	Type-1	Wassserlab, Spain	C2C Pharma Ltd

Table 04: Materials Required

4.2 Instruments

Name	Manufacturer/ Origin	Model	Source
Dissolution Tester	Copley	DiS 800i	USA
	Labindia	DS 14000	India
Electronic Balance	Mettler Toledo	MS105	Switzerland
Ultrasonic Bath	Isolab	Ultrasonicleaner	Germany
UV Spectrophotometer	Shimadzu	UV-1900i	Japan

Table 05: Instruments used during testing

4.3 Apparatus

- Pipette
- Beaker
- Conical Flask
- Volumetric Flask

- Measuring Cylinder
- Whatman #1 Filter

4.4 Working Standard and CRS Status

Sl No.	Name of Material	Batch / Lot. No.	Manufacturer / Supplier
01	Tiemonium Methyl sulfate CRS	PA-TMM-02100	Pharma Affiliates
02	Tiemonium Methyl sulfate WS	TMS-IH 2203027	C2C Pharma Ltd

Table 06: Working Standard and Reference standard used during test.

4.5 Methods

4.5.1 Dissolution Condition

- **Apparatus :** USP Type-II (Paddle)
- **Media :** Water
- **Time :** 60 Minutes
- **RPM** 75
- **Volume :** 900 ml
- **Temperature :** 37℃ ± 0.5℃
- **Wavelength :** 235 nm

4.5.2 Preparation of Standard

Weighed accurately 22.2 mg of Tiemonium Methyl sulfate working standard and transferred it into a 100 ml volumetric flask. About 70 ml of

dissolution media was added and sonicated for 10 minutes to dissolve. Kept on standby for ambient temperature and volume up to the mark with the dissolution media and mixed well.

10 ml of the solution was transferred into a 100 ml volumetric flask and volume up to the mark with the same solution and mixed well.

Following this procedure two Standard were prepared naming Standard-1 and Standard-2.

Concentration of Tiemonium Methyl sulfate: 0.0222 mg per ml

4.5.3 Preparation of Sample Solution

900 ml of dissolution medium was placed into each vessel (6) and the apparatus was assembled. The medium was allowed to equilibrate to a temperature of 37 ± 0.5°C. 1 tablet to each of six vessels was placed, taking care to exclude air bubbles from the surface of the dosage unit and operated the apparatus for specified conditions.

After 60 minutes 25 ml aliquot of 6 specimens (for 6 vessels) withdrew from a zone midway between the surface of the dissolution medium and the top of the rotating paddle, not less than 1 cm from vessel wall and filtered through Whatman#1 filter paper discarding the first 5 ml.

10 ml of the solution was transferred to a 25 ml volumetric flask. Volume up to the mark with dissolution media and mixed well.

Concentration of Tiemonium Methyl sulfate: 0.0222 mg per ml

4.5.4 Analysis Procedure

The absorbance of the Standard-1, Standard-2 and sample solution was measured using a 1 cm cell at 235 nm with dissolution media as the blank.

Sl. No	Solution Name	Number of Measurements
01	Blank	01
02	Standard-01	05
03	Standard-02	02
04	Dissolution Sample-01	01
05	Dissolution Sample-02	01
06	Dissolution Sample-03	01
07	Dissolution Sample-04	01
08	Dissolution Sample-05	01
09	Dissolution Sample-06	01
10	Standard-01 (End Standard)	01

Table 07: The Sequence of Measurements

- The reproducibility of standard solution-1 and standard solution-2 must be between 98.0% to 102.0%.
- Calculated the standard reproducibility of the absorbance of standard solution-1 and standard solution-2 by the following equation:

$$\text{Standard Reproducibility} = \frac{Absorbance\ of\ standard-02}{Absorbance\ of\ standard-01} \text{ x } \frac{Weight\ of\ standard\ 01}{Weight\ of\ standard\ 02} \text{x } 100$$

- If reproducibility complies with the limit, Dissolution sample solution-1 sample solution-2, sample solution-3 sample solution-4 sample solution-5 and sample solution-6 absorbance were recorded.
- At the end of the analysis absorbance of the standard solution-1 as end standard was recorded. The % RSD between the absorbance of the end standard and the absorbance of the initial standard must not be more than 2.0.

4.5.5 Dissolution Calculation

The content of Tiemonium Methyl Sulfate was calculated by following calculation,

$$= \frac{\text{Asam. X Wstd. X 10 X 900 x 25 X P X 100}}{\text{Astd. x 100 x 100 x LC x 10 x 100}} \%$$

= % of Tiemonium Methyl Sulfate

Where,

A_{sam} =Absorbance of Sample

A_{std} = Absorbance of

Standard W_{std} = Weight of

Standard

P = Potency of

standard LC = Label

Claim

4.5.6 Acceptance Criteria

Stage	Number Tested	Acceptance Criteria
S1	6	Each Unit is not less than Q+5%.
S2	6	Average of 12 units (S1 + S2) is equal to or greater than Q, and no unit is less than Q-15%
S3	12	Average of 24 units (S1 + S2 + S3) is equal to or greater than Q, and not more than 2 unit are less than Q-15%, and no unit is less than Q-25%.

Table 08: Acceptance Criteria for Dissolution Method

4.6 Validation Parameters

The validation procedure for the test method is the assessment of whether the procedure can be used for its intended purpose, under the actual condition of use for a specified drug product or drug substance.

Analytical method validation parameters selected for the dissolution test method of Tiemonium Methyl sulfate Tablet 50 mg are as follows:

Sl. No	Parameters
01	System Suitability
02	Specificity
03	Filter Evaluation
04	Precision

	▪ System Precision ▪ Method Precision ▪ Intermediate Precision
05	Linearity
06	Accuracy
07	Solution Stability

Table 09: Validation Parameters

4.7 Validation Design Matrix

4.7.1 System Suitability

- **Procedure:**

  Standard-01 and standard-02 are prepared as per the method of analysis. The absorbance of the solutions was measured as per below mentioned sequence using dissolution media as a blank.

Name of Solution	**Number of Measurements**
Standard-01	05
Standard-02	02

Table 10: Measurement Sequence for System Suitability

Acceptance Criteria:

- The %RSD of five replicate measurements must be not more than 2.0%.
- The recovery of standard solution-1 and standard solution-2 must be 98.0% to 102.0%

4.7.2 Demonstration of Specificity

- **Procedure**

  The blank solution, standard solution, and sample solution are prepared as per the method of analysis.

- **Preparation of CRS solution:**

  Accurately weighed 2.22 mg of Tiemonium Methyl sulfate CRS were transferred into a 100 ml volumetric flask. Approximately 70 ml of dissolution media was added, and the mixture was sonicated for 10 minutes to facilitate dissolution. The solution was allowed to stand at ambient temperature, and the volume was adjusted to the mark with dissolution media, ensuring thorough mixing.

  The concentration of Tiemonium Methyl sulfate achieved was 0.0222 mg per ml.

- **Preparation of Placebo solution:**

  Accurately weighed 96.26 mg of the placebo were transferred into a vessel of the apparatus, with careful exclusion of air bubbles from the surface of the dosage unit. The apparatus was immediately operated at the specified rate according to the dissolution test conditions. After 60 minutes, a 25 ml aliquot was withdrawn from a zone midway between the surface of the dissolution medium, maintaining a distance of not less than 1 cm from the

vessel wall. The withdrawn solution was then filtered through Whatman #1 filter paper, with the first 5 ml solution being discarded.

Transferred 10 ml of the solution into a 25 ml volumetric flask. The volume was adjusted to the mark with dissolution media, and the mixture was thoroughly mixed.

- **Preparation of API solution**

  Accurately weighed 50.0 mg of Tiemonium Methyl sulfate API were transferred into a vessel of the apparatus, with careful exclusion of air bubbles from the surface of the dosage unit. The apparatus was immediately operated at the specified rate as per the dissolution test conditions. After 60 minutes, a 25 ml aliquot was withdrawn from a zone midway between the surface of the dissolution medium, maintaining a distance of not less than 1 cm from the vessel wall. The withdrawn solution was then filtered through Whatman #1 filter paper, discarding the first 5 ml of the solution.

  Subsequently, 10 ml of the filtered solution were transferred into a 25 ml volumetric flask. The volume was adjusted to the mark with dissolution media, and the mixture was thoroughly mixed.

  Concentration of Tiemonium Methyl sulfate: 0.0222 mg per ml

- **Preparation of Spiked Sample Solution**

  Accurately weighed 50.0 mg of Tiemonium Methyl sulfate API and 96.26 mg of placebo were placed into a vessel of the apparatus, ensuring the

exclusion of air bubbles from the surface of the dosage unit. The apparatus was immediately operated at the specified rate as per the dissolution test conditions. After 60 minutes, a 25 ml aliquot was withdrawn from a zone midway between the surface of the dissolution medium, maintaining a distance of not less than 1 cm from the vessel wall. The withdrawn solution was then filtered through Whatman #1 filter paper, with the first 5 ml of the solution being discarded.

Subsequently, 10 ml of the filtered solution were transferred into a 25 ml volumetric flask. The volume was adjusted to the mark with dissolution media, and the mixture was thoroughly mixed.

Concentration of Tiemonium Methyl sulfate: 0.0222 mg per ml.

Sl No.	**Name of Solution**	**Number of Measurements**
01	Standard-1 Solution	05
02	Blank Solution	01
03	Placebo Solution	01
04	CRS Solution	01
05	API Solution	01
06	Spiked Sample Solution	01
07	Sample Solution	01
08	Standard-1 Solution (End Standard)	01

Table 11: Sequence of Measurement's for Determination of Specificity

- **Acceptance Criteria:**

  Blank solution: No significant absorbance should be found at 235 nm for the blank solution.

  Placebo solution: Interference should not exceed 2.0%.

  Identification: The spectrum of both standard solution and sample solution should correspond to each other.

4.7.3 Demonstration of Filter Evaluation

- **Procedure**

  Prepare the blank solution, standard solution-01, and standard solution-02 as per the method of analysis.

- **Preparation of Sample Solution for Filter Evaluation:**

  900 ml of dissolution medium was placed into each of the six vessels, and the apparatus was assembled. The medium was allowed to equilibrate to a temperature of 37 ± 0.5°C. Subsequently, 1 tablet was placed in each of the six vessels, with care taken to exclude air bubbles from the surface of the dosage unit. The apparatus was operated at the specified rate as given in the dissolution test conditions. After 60 minutes, a 60 ml aliquot was withdrawn from a zone midway between the surface of the dissolution medium and the

top of the rotating paddle, maintaining a distance of not less than 1 cm from the vessel wall.

- **Unfiltered Sample:**

  Direct sample solution (Centrifuge). Transfer 10 ml of the solution in 25 ml volumetric flask. Volume up to the mark with dissolution media and mix well.

- **Filtered Sample Through Whatman# 1 filter paper:**

  Filter 20 ml of the sample through Whatman# 1 filter paper. Transfer 10 ml of the solution in 25 ml volumetric flask. Volume up to the mark with dissolution media and mix well.

- **Filtered Sample through Whatman# 42 filter paper:**

  Filter 20 ml of the sample through Whatman# 42 filter paper. Transfer 10 ml of the solution in a 25 ml volumetric flask. Volume up to the mark with dissolution media and mix well.

Sl No.	**Name of Solution**	**Number of Measurements**
01	Standard-01 Solution	05
02	Standard-02 Solution	02
03	Sample Unfiltered Solution	01

04	Sample Whatman#1 Filter Paper	01
05	Sample Whatman#42 Filter Paper	01
06	Standard-01 Solution (End Standard)	01

Table 12: Sequence of Measurements for Determination of Specificity

- **Acceptance Criteria**

  The absolute difference between the filtered and unfiltered sample solution should not be more than ±2.0%.

4.7.4 Precision

4.7.4.1 System Precision

- **Procedure:** The blank solution and standard solution-1 were prepared according to the method of analysis. The absorbance of the following samples was measured using a 1-cm cell at a wavelength of 322 nm, with dissolution media used as a blank.

Solution Type	**Number of measurements**
Standard Solution-1	06

Table 13: Sequence of Measurements for Determination of System Precision

- **Acceptance Criteria:** RSD of the absorbance of the standard solution must not be more than 2.0%.

4.7.4.2 Method Precision / Repeatability Study

To demonstrate method precision (Repeatability), six dissolution Drug Product samples for 50 mg strength were prepared, respectively, as per the test method at 100% of the test concentration. The six samples were analyzed, and the average and relative standard deviation of the dissolution values were reported.

- **Procedure:** The blank solution, standard solution-1, standard solution-2, and sample solution were prepared as per the method of analysis.

  The absorbance of the solutions was measured in the following sequence:

Solution Type	**Number of measurements**
Standard Solution-1	05
Standard Solution-2	02
Sample Solution-1	01
Sample Solution-2	01
Sample Solution-3	01
Sample Solution-4	01
Sample Solution-5	01
Sample Solution-6	01
Standard Solution-1(End Standard)	01

Table 14: Sequence of Measurements for Determination of Method Precision

- **Acceptance Criteria:**

- Release of Tiemonium Methyl sulfate Tablet 50 mg in 60 minutes: NLT:75% (Q).
- RSD of release of Tiemonium Methyl sulfate must be NMT 5.0%.

4.7.4.3 Intermediate Precision

The following variation was considered for the intermediate precision study.

- ✓ Analyst to Analyst.
- ✓ Day to day.

- **Procedure:**

The blank solution, standard solution-1, standard solution-2, and sample solution for 50 mg strength were prepared as per the method precision. The absorbance of the solutions was measured in the following sequence:

Solution Type	Number of measurements
Standard Solution-1	05
Standard Solution-2	02
Sample Solution-1	01
Sample Solution-2	01
Sample Solution-3	01
Sample Solution-4	01
Sample Solution-5	01
Sample Solution-6	01

Standard Solution-1(End Standard)	01

Table 15: Sequence of Measurements for Determination of Intermediate Precision

- **Acceptance Criteria:**
  - ✓ Release of Tiemonium Methyl sulfate Tablets 50 mg in 60 minutes: NLT: 75% (Q).
  - ✓ RSD of release of Tiemonium Methyl sulfate must be NMT 5.0%
  - ✓ Cumulative RSD of 12 determinations of method precision and intermediate precision should not be more than 2.0%.

4.7.5 Linearity

The Linearity study was carried out over a range of 50% to 150% of the 100% test concentration (0.0222 mg/ml of Tiemonium Methyl sulfate).

- **Preparation of Linearity Stock Solution:**

  About 22.2 mg of Tiemonium Methyl sulfate WS were weighed and transferred into a 100 ml volumetric flask. Approximately 70 ml of dissolution media were added, and the solution was sonicated to dissolve. It was allowed to stand at ambient temperature, and the volume was adjusted to the mark with dissolution media, followed by thorough mixing.

  Concentration of Tiemonium Methylsulfate: 0.222 mg per ml

- **Linearity solution-50%:**

5 ml of the linearity stock solution were transferred into a 100 ml volumetric flask. The volume was adjusted to the mark with dissolution media, and the solution was mixed well.

Concentration of Tiemonium Methyl sulfate: 0.0111 mg per ml

- **Linearity solution-80%:**

  4 ml of the linearity stock solution were transferred into a 50 ml volumetric flask. The volume was adjusted to the mark with dissolution media, and the solution was mixed well.

  Concentration of Tiemonium Methyl sulfate: 0.0178 mg per ml

- **Linearity solution-100%:**

  10 ml of the linearity stock solution were transferred into a 100 ml volumetric flask. The volume was adjusted to the mark with dissolution media, and the solution was mixed well.

  Concentration of Tiemonium Methyl sulfate: 0.0222 mg per ml

- **Linearity solution-120%:**

  6 ml of the linearity stock solution were transferred into a 50 ml volumetric flask. The volume was adjusted to the mark with dissolution media, and the solution was mixed well.

  Concentration of Tiemonium Methylsulfate: 0.0266 mg per ml

- **Linearity solution-150%:**

  15 ml of the linearity stock solution were transferred into a 100 ml volumetric flask. The volume was adjusted to the mark with dissolution media, and the solution was mixed well.

  Concentration of Tiemonium Methylsulfate: 0.0333 mg per m

  Prepared series of concentrations from the stock linearity solution as shown in the following table:

Sl. No.	Concentration (%)	Volume in ml taken from linearity stock solution	Total Volume (ml) with Dissolution Media	Concentration of Tiemonium Methyl Sulfate
01	50%	5	100	0.0111
02	80%	4	50	0.0178
03	100%	10	100	0.0222
04	120%	6	50	0.0266
05	150%	15	100	0.0333

Table 16: Preparation of Linearity Solutions

Sample Name	**Number of Measurements**
Standard Solution-1	05
50% Linearity Solution	03

80% Linearity Solution	03
100% Linearity Solution	03
120% Linearity Solution	03
150% Linearity Solution	03
Standard Solution-1 (End Standard)	01

Table 17: Sequence of Measurements for Determination of Linearity.

- **Data Evaluation:**

  A Linearity graph of average absorbance of each level against the concentration plotted & the correlation coefficient determined.

- **Acceptance Criteria:** The correlation coefficient must not be less than 0.995

4.7.6 Demonstration of Accuracy

Was carried out over a range of 80%, 100%, and 120% (3 concentrations in 3 replicates for each, totaling the entire analytical procedure) of the test concentration.

- **Procedure:**

  The blank solution and standard solutions 1 and 2 were prepared according to the method of analysis.

- **Accuracy solution 80%:**

Weighed and transferred about 40.0 mg of Tiemonium Methyl sulfate API and 77.01 mg of placebo into a vessel of the apparatus, taking care to exclude air bubbles from the surface of the dosage unit, and immediately operated the apparatus at the specified rate given in the dissolution test conditions.

After 60 minutes, withdrew a 25 ml aliquot from a zone midway between the surface of the Dissolution medium, not less than 1 cm from the vessel wall. Filtered the solution through Whatman# 1 filter paper, discarding the first 5 ml solution.

Transferred 10 ml of the solution into a 25 ml volumetric flask. Volume was adjusted up to the mark with dissolution media and mixed well. Samples were prepared in triplicate (n = 3).

Concentration of Tiemonium Methyl sulfate: 0.0178 mg per ml

- **Accuracy solution 100%:**

  Weighed and transferred about 50.0 mg of Tiemonium Methyl sulfate API and 96.26 mg of placebo into a vessel of the apparatus, taking care to exclude air bubbles from the surface of the dosage unit, and immediately operated the apparatus at the specified rate given in the dissolution test conditions.

  After 60 minutes, withdrew a 25 ml aliquot from a zone midway between the surface of the Dissolution medium, not less than 1 cm from the vessel

wall. Filtered the solution through Whatman# 1 filter paper, discarding the first 5 ml solution.

Transferred 10 ml of the solution into a 25 ml volumetric flask. Volume was adjusted up to the mark with dissolution media and mixed well. Prepared samples in triplicate (n = 3).

Concentration of Tiemonium Methyl sulfate: 0.0222 mg per ml

- **Accuracy solution 120%:**

  Weighed and transferred about 60.0 mg of Tiemonium Methyl sulfate API and 115.51 mg of placebo into a vessel of the apparatus, taking care to exclude air bubbles from the surface of the dosage unit, and immediately operated the apparatus at the specified rate given in the dissolution test conditions.

  After 60 minutes, withdrew a 25 ml aliquot from a zone midway between the surface of the Dissolution medium, not less than 1 cm from the vessel wall. Filtered the solution through Whatman# 1 filter paper, discarding the first 5 ml solution.

  Transferred 10 ml of the solution into a 25 ml volumetric flask. Volume was adjusted up to the mark with dissolution media and mixed well. Prepared samples in triplicate (n = 3).

  Concentration of Tiemonium Methyl sulfate: 0.0267 mg per ml

- Measured the absorbance of the following samples using a 1-cm cell at 235 nm.

Sample Name		Number of Measurements
Standard Solution-1		05
Standard Solution-2		02
Accuracy Solution 80%	Sample-01	01
	Sample-02	01
	Sample-03	01
Accuracy Solution 100%	Sample-01	01
	Sample-02	01
	Sample-03	01
Accuracy Solution 120%	Sample-01	01
	Sample-02	01
	Sample-03	01
Standard Solution-1 (End Standard)		01

Table 18: Sequence of Measurements for Determination of Accuracy.

- Acceptance Criteria
  - ✓ The Recovery should be not less than 95.0% and should be not more than 105.0%.
  - ✓ RSD of 9 determination should not be more than 2.0%

4.7.7 Solution Stability

- Procedure:

The solution stability experiments should be performed at intervals of initial and 24 hours. Prepare blank solution, standard solution-1, standard solution- 2 and sample solution for Tiemonium Methyl Sulfate Tablet 50 mg as per method of analysis.

Sample Name	**Number of Measurements**
Standard Solution-1	05
Standard Solution-2	02
Standard Solution at Initial Time Point	01
Sample Solution at Initial Time Point	01
End Standard Solution at Initial Time Point	01
Standard Solution after 3 Hour	01
Sample Solution after 3 Hour	01
End Standard after 3 Hour	01
Standard Solution after 6 Hour	01
Sample Solution after 6 Hour	01
End Standard after 6 hours	01
Standard Solution after 18 Hour	01
Sample Solution after 18 Hour	01
End Standard after 18 hours	01
Standard Solution after 24 Hour	01
Sample Solution after 24 Hour	01
End Standard after 24 hours	01

Table 18: Sequence of Measurements for Determination of Solution Stability.

- Acceptance criteria

  The difference between initial result and time points must be within ±2.0%

Chapter 5. Result & Discussion

5.1 System Suitability

5.1.1 Determination

Standard solutions were measured into the UV system and absorbance were recorded. System suitability as per the test procedure was evaluated.

Solution Name	Absorbance
Standard Solution-1	0.4533
Standard Solution-1	0.4534
Standard Solution-1	0.4533
Standard Solution-1	0.4533
Standard Solution-1	0.4534
Mean	0.4533
Standard Deviation	0.0001
RSD (%)	0.01

Table 19: Summary of Measurements for Determination of Solution Stability.

5.1.2 Acceptance Criteria:

- The %RSD of five replicate measurements must be not more than 2.0%.

- The recovery of standard solution-1 and standard solution-2 must be 98.0% to 102.0%
-

5.1.3 Result:

The RSD of five replicate measurements is 0.01% and recovery of standard solution-1 and standard solution-2 is 99.79%. The above results reveal that the system meets the required system suitability.

5.1.4 Comments

The above data indicates that the system suitability parameters are well within the acceptance limit and the system meets required system suitability.

5.2 Specificity Study

5.2.1 Determination

Blank, placebo solution, standard solution, CRS solution, API solution and sample solution were prepared as per method· Absorbance of these solution were taken separately.

Sl. No.	Name of solution	Absorbance	
		Tiemonium Methyl Sulphate	Placebo Interference
01	Standard Solution-1	Abs. Found at 235 nm	Nill

02	Blank Solution	Not Detected	N/A
03	Placebo Solution	Not Detected	N/A
04	CRS Solution	Abs. Found at 235 nm	Nill
05	API Solution	Abs. Found at 235 nm	Nill
06	Spiked Sample Solution	Abs. Found at 235 nm	Nill
07	Sample Solution	Abs. Found at 235 nm	Nill
08	Standard Solution-1 (End Standard)	Abs. Found at 235 nm	Nill

Table 20: Summary of Measurements for Determination of Specificity Study.

5.2.2 Acceptance Criteria

- Blank solution: No significant absorbance should be found at 235 nm for the blank solution.
- Placebo solution: Interference should not exceed 2.0%.
- Identification: The spectrum of both standard solution and sample solution should correspond to each other.

5.2.3 Results:

From the above data, it is observed that Tiemonium Methyl sulfate gave same response in sample and standards with respect to at 235 nm and no interference due to diluent or placebo.

5.2.4 Comments

Therefore, the method is considered specific.

5.3 Filter Evaluation Study

5.3.1 Determination

The sample solutions were prepared through different filter media as per Protocol. All filtered solution was analyzed for single time. The absolute difference between the unfiltered sample solution and filtered sample solutions were calculated.

Sample Name	Absorbance of Individual Sample	% of Tiemonium Methyl Sulphate	Absolute Difference
Unfiltered	0.4410	96.9	-
Whatman Filter #1	0.4400	96.7	0.21
Whatman Filter #1	0.4398	96.6	0.31

Table 19: Summary of Measurements for Determination of Filter Evaluation.

5.3.2 Acceptance Criteria

The absolute difference between the filtered and unfiltered sample solution should not be more than ±2.0%.

5.3.3 Result

Here, absolute difference between all filtered (Whatman filter paper#1 and Whatman filter paper# 42) and unfiltered sample solution results are within acceptance limit.

5.3.4 Comments

Thus, it is concluded that all above filter can be used for the preparation of sample solution.

5.4 System Precision

5.4.1 Determination

System precision was assessed by performing replicate measurements (n=6) of the standard solution and calculating the % RSD of the measured absorbance of the standard.

Sample Solution	Absorbance
Standard Solution-1	0.4565
Standard Solution-1	0.4568
Standard Solution-1	0.4566
Standard Solution-1	0.4565
Standard Solution-1	0.4565

Standard Solution-1	0.4564
Mean	0.4566
Standard Deviation	0.0001
% RSD	0.03

Table 20: Summary of Measurements for Determination of System Precision.

5.4.2 Acceptance Criteria

RSD of the absorbance of the standard solution must be not more than 2.0%

5.4.3 Result

Here, the RSD of area of six replicated absorbance was 0.03%.

The above results reveal thatthe system meetsthe required system suitability.

5.4.4 Comment

The above data indicates that the system suitability parameters are well within the acceptance limit. Hence, the system is precise.

5.5 Method Precision

5.5.1 Determination

Repeatability was assessed by performing six replicate measurements (n=6) of the dissolution of Tiemonium Methylsulfate Tablet 50 mg as per test method at 100% of the test concentration and calculating the % of RSD of the dissolution of Tiemonium Methylsulfate.

Sl.No.	Absorbance	% of Tiemonium Methyl Sulphate
Sample-1	0.4344	97
Sample-2	0.4344	97
Sample-3	0.4344	97
Sample-4	0.4349	97
Sample-5	0.4346	97
Sample-6	0.4346	97
Mean	N/A	97
Standard Deviation	N/A	0.0000
% RSD	N/A	0.00

Table 21: Summary of Measurements for Determination of Method Precision.

5.5.2 Acceptance Criteria

- Release of Tiemonium Methylsulfate Tablet 50mg in 60 minutes: NL T:75%(Q)
- RSD of release of Tiemonium Methylsulfate must be NMT 5.0%.

5.5.3 Result

The above data show that the % RSD of dissolution of Tiemonium Methy/sulfate of six sample is 0.00% and amount of Tiemonium Methylsulfate are within the acceptance

5.5.4 Comments

Hence, the method meets the requirement of method precision.

5.6 Intermediate Precision

5.6.1 Determination

Intermediate Precision was assessed by performing six replicate measurements (n=6) of the of Tiemonium Methylsulfate Tablet 50 mg as per test method at 100% of the test concentration % on a different day by a different analyst and calculating %RSD of the dissolution of Tiemonium Methyl Sulphate.

Sl.No.	Absorbance	% of Tiemonium Methyl Sulphate
Sample-1	0.4435	97
Sample-2	0.4438	97
Sample-3	0.4440	97
Sample-4	0.4440	97
Sample-5	0.4448	97
Sample-6	0.4444	97
Mean	N/A	97
Standard Deviation	N/A	0.0000
% RSD	N/A	0.00

Table 22: Summary of Measurements for Determination of Intermediate Precision.

5.6.2 Acceptance Criteria

- Release of Tiemonium Methylsulfate Tablet 50mg in 60 minutes: NL T:75%(Q)
- RSD of release of Tiemonium Methylsulfate must be NMT 5.0%.

5.6.3 Result

The above data show that the % RSD of dissolution of Tiemonium Methylsulfate of six sample is 0.00% and amount of Tiemonium Methylsulfate are within the acceptance limit.

5.6.4 Comment

Hence, the method meets the requirement of method precision.

Sample No.	Method Precision	Intermediate Precision
Sample-1	97	97
Sample-2	97	97
Sample-3	97	97
Sample-4	97	97
Sample-5	97	97
Sample-6	97	97
Mean	97	
Standard Deviation	0.0000	
% RSD	0.00	

Table 23: Comparison of results obtained by Analyst 1 & 2

5.6.5 Acceptance Criteria

Cumulative % RSD of 12 determinations of method precision and intermediate precision should not be more than 2%.

5.6.6 Result

The above data show that the Cumulative % RSD of 12 determinations by both analysts is also well within the acceptance limit.

5.6.7 Comment

Hence, the method meets the requirement of intermediate precision/Ruggedness.

5.7Linearity

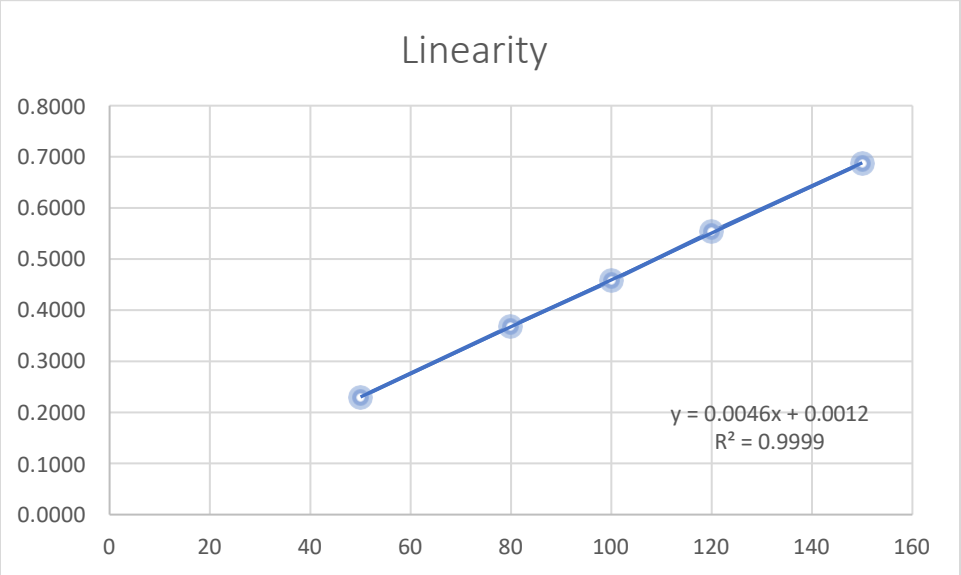
5.7.1 Determination

Linearity study was carried out over a range of 50% to 150% of the 100% test concentration (0.0222 mg/ml of Tiemonium Methylsulfate equivalent) and the correlation coefficient was calculated by plotting the area of the linearity solutions against concentration.

Sl.No.	Name of Solution	Conc. of Standard Solution (mg/ml)	Absorbance	
			Individual Absorbance	Average Absorbance
01	50%	0.0111	0.2297	0.2298
			0.2299	
			0.2298	
02	80%	0.0178	0.3687	0.3687
			0.3687	
			0.3687	
03	100%	0.0222	.4576	0.4575
			.4575	
			.4574	

04	120%	0.0266	0.5534	0.5531
			0.5528	
			0.5530	
05	150%	0.0333	0.6874	0.6875
			0.6876	
			0.6874	

Table 24: Summary of Measurements for Determination of Linearity.



5.7.2 Acceptance Criteria

- System suitability parameters should be within the limits.
- The correlation coefficient for Tiemonium Methylsulfate should not be less than 0.995

5.7.3 Results

The correlation coefficient is well within acceptance limit.

5.7.4 Comment

Thus, the method is considered linear

5.8 Accuracy

5.8.1 Determination

The accuracy study for dissolution test method was determined over a range 80%, 100%and 120% (3 concentration/ 3 replicates each of the total analytical procedure) at 100% of the test concentration.

Sample Name	Absorbance of Tiemonium Methyl Sulphate in sample	Recovery of tiemonium Methyl Sulphate (%)
Accuracy 80%-1	0.3684	101.1
Accuracy 80%-2	0.3663	100.6
Accuracy 80%-3	0.3663	100.5
Accuracy 100%-1	0.4548	99.9
Accuracy 100%-2	0.4543	99.8
Accuracy 100%-3	0.4541	99.8
Accuracy 120%-1	0.5409	99
Accuracy 120%-2	0.5502	100.7
Accuracy 120%-3	0.5425	99.3
Average	N/A	100
Standard Deviation	N/A	0.63924
% RSD	N/A	0.69

Table 21: Summary of Measurements for Determination of Accuracy.

5.8.2 Acceptance Criteria

- The Recovery should not be less than 95% and should not be more than 105%.
- RSD of 9 determinations should not be more than 2.0%

5.8.3 Remarks

The above data show that accuracy in terms of recovery of three different concentration(s) is well within the acceptance limit and RSD of 9 determinations should not be more than 2.0%

5.8.4 Comments

Method meets the requirements of Accuracy.

5.9 Solution Stability

5.9.1 Determination

The standard and sample solution stability study were Performed under room temperature at intervals of initial, 3, 6, 18 & 24 hours as per method.

Sample Name	% of Tiemonium Methyl Sulphate in Standard	Absolute % Difference
Initial	0.4644	N/A
After 3 Hours	0.4653	0.2
After 6 Hours	0.4658	0.9
After 18 Hours	0.467	0.6
After 24 Hours	0.4758	2.5

Table 22: Summary of Measurements for Determination of Solution stability of Standard Solution.

Sample Name	% of Tiemonium Methyl Sulphate in Sample	Absolute % Difference
Initial	0.4509	N/A
After 3 Hours	0.4512	0.1

After 6 Hours	0.4575	1.5
After 18 Hours	0.447	0.9
After 24 Hours	0.4539	0.7

Table 23: Summary of Measurements for Determination of Solution stability of Sample Solution.

5.9.2 Acceptance Criteria

The% difference between initial result and time points must be within± 2.0.

5.9.3 Remarks

The test sample solution is found to be stable up to 18 hours at room temperature.

5.9.4 Comments

Therefore, the test solution can be used up to 18 hours when stored at room temperature.

Chapter 6. Conclusion

In conclusion, the analytical method validation conducted for the Dissolution method of Tiemonium Methyl Sulphate 50 mg tablet has yielded positive and reliable results. The comprehensive evaluation of key validation parameters, including Method Precision, Intermediate Precision, Solution Stability, System Suitability, Specificity, Filter Evaluation, Linearity, and Accuracy, has demonstrated the robustness and accuracy of the Dissolution method. The parameters were found to be well within the acceptable ranges, attesting to the method's precision, reliability, and suitability for its intended purpose. Notably, the extended Solution Stability of up to 18 hours adds an extra layer of confidence in the method's practical applicability. These findings collectively affirm the validity and effectiveness of the Dissolution method for Tiemonium Methyl Sulphate 50 mg tablet analysis, providing a solid foundation for its routine application in pharmaceutical quality control and ensuring the consistency and accuracy of the results over time.

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