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The Review: Development and Validation of RPHPLC Method for Simultaneous Estimation of Ertugliflozin And Metformin in Pharmaceutical Dosage Form.

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ABSTRACT

The accurate and efficient estimation of pharmaceutical compounds is critical in ensuring the quality and safety of medications. Ertugliflozin, a sodium-glucose co-transporter-2 (SGLT2) inhibitor, and Metformin, a widely used biguanide, are frequently combined in the treatment of type 2 diabetes mellitus for improved glycemic control. The development and validation of a robust Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of these drugs in pharmaceutical dosage forms is essential for quality control and regulatory compliance.This review focuses on the principles, methodology, and outcomes associated with the RP-HPLC technique employed for the simultaneous quantification of Ertugliflozin and Metformin. The study explores the optimization of chromatographic conditions, including the selection of the mobile phase, column, flow rate, and detection wavelength, to achieve precise and accurate separation of both analytes. The method development process emphasizes achieving optimal resolution, peak symmetry, and retention time.Validation of the developed method is performed following the guidelines of the International Council for Harmonisation (ICH). Key parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness are systematically assessed. The method demonstrates excellent linearity over a specific concentration range for both analytes, with correlation coefficients exceeding acceptable limits. Precision studies reveal consistent repeatability and intermediate precision, while robustness testing confirms the method's reliability under varied analytical conditions.

The RP-HPLC method described offers a rapid, cost-effective, and reliable approach for the routine quality control of pharmaceutical formulations containing Ertugliflozin and Metformin. The results indicate its suitability for application in pharmaceutical industries and research laboratories for the simultaneous estimation of these drugs in combined dosage forms. Furthermore, the study highlights potential challenges and solutions in method development and validation, contributing to the advancement of analytical methodologies in pharmaceutical analysis.In conclusion, the developed RP-HPLC method is a significant contribution to the field of analytical chemistry, providing a validated protocol for the simultaneous quantification of Ertugliflozin and Metformin in pharmaceutical preparations. The robustness and reliability of the method ensure its utility in routine quality assurance and compliance with regulatory standards.This review aims to provide a comprehensive overview of the RP-HPLC method's development, validation, and application, addressing the critical need for efficient analytical methods in the pharmaceutical sector.

Keywords: RP-HPLC, Ertugliflozin, Metformin, Simultaneous Estimation, Pharmaceutical Dosage Form, Analytical Method Development, Method Validation, ICH Guidelines, Quality Control, Chromatographic Conditions.

INTRODUCTION

The accurate estimation of pharmaceutical compounds is essential to ensure drug quality, safety, and efficacy. Ertugliflozin, a sodium-glucose co-transporter-2 (SGLT2) inhibitor, and Metformin, a first-line biguanide for type 2 diabetes mellitus, are widely used in combination therapies for effective glycemic control. Developing a reliable and efficient analytical method for their simultaneous estimation in pharmaceutical dosage forms is crucial for quality control and regulatory compliance. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is a widely adopted technique due to its high precision, accuracy, and reproducibility, making it ideal for routine analysis of combination drug formulations.

Over 422 million people worldwide suffer from diabetes, with 90–95% of those cases being type 2 diabetes (T2D). Many patients are unable to reach their desired glycemic levels even with better glycemic management. Treatment options include injectable and oral medications, which are selected according to cardiovascular (CV) advantages, patient preferences, cost, hypoglycemia risk, and adverse effects. Ertugliflozin is one of a new class of sodium-glucose cotransporter 2 (SGLT2) inhibitors used to treat type 2 diabetes. Ertugliflozin was approved by the FDA and EMA in 2017 and can be purchased as a monotherapy or in fixed-dose combos with sitagliptin or metformin. Using research and literature from 1995 to 2020, this review looks at its effectiveness, safety, cardiovascular, and renal effects[1] .

Oxidative stress brought on by an excess of reactive oxygen species (ROS), mostly from mitochondrial malfunction, is associated with type 2 diabetes (T2D) and hyperglycemia. Oxidative damage, insulin resistance (IR), and consequences such as neuropathy, retinopathy, cardiovascular disorders, and stroke are all caused by elevated ROS levels. For cellular and energy balance, mitochondrial processes such as fusion, fission, and mitophagy are essential; adaptive mitophagy may slow the progression of type 2 diabetes. Over 400 million people worldwide suffer from diabetes, which is on the rise as a result of obesity and sedentary lifestyles. 90% of instances are due to T2D. The gold standard for treating T2D and problems associated to insulin resistance is metformin, which was developed more than 50 years ago[2].

Ertugliflozin Pharmacology :

SGLT2, which is hyperactive in type 2 diabetes (T2D), is the main mechanism by which the kidneys filter and reabsorb glucose, leading to hyperglycemia. By specifically blocking glucose reabsorption, SGLT2 inhibitors, such as ertugliflozin, reduce the renal glucose threshold and encourage urine glucose excretion (UGE). Insulin sensitivity or secretion have no bearing on this process. In addition to diabetic control, SGLT2 inhibitors have anti-inflammatory, natriuretic, decreased plasma volume, and lowered blood pressure actions that enhance cardiovascular (CV) and kidney outcomes. Ertugliflozin works well with insulin, metformin, and sitagliptin, but when used with insulin or sulfonylureas, it may raise the risk of hypoglycemia, thus high-risk patients should reduce their dosage[1].

Pharmacokinetics :

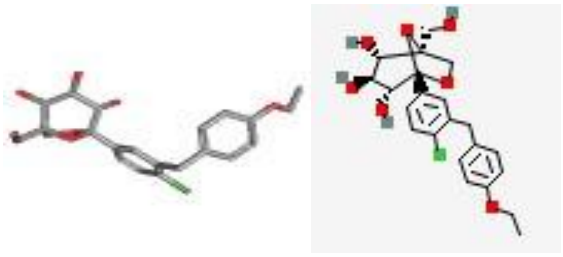
Ertugliflozin is almost entirely bioavailable and reaches its maximum concentration in either one hour while fasting or two hours after eating a high-fat meal. Once-daily dose is supported by its 16.6-hour half-life, and steady-state concentrations are attained after 4-6 days. Ertugliflozin shown decreased efficacy in individuals with mild renal impairment (eGFR <60 mL/min/1.73 m2) and is not advised for usage in this population. It has little cytochrome P450 metabolism and is mostly broken down into inactive glucuronides by UGT1A9 and UGT2B7. It has not been investigated in individuals with severe hepatic impairment, although no dose modification is required in those with mild to moderate hepatic impairment[1].

Pharmacodynamics :

The main effect of ertugliflozin is glucosuria; patients with type 2 diabetes (T2D) exhibit dose-dependent urine glucose excretion (UGE). In one study, ertugliflozin dosages of 1, 5, and 25 mg resulted in an increase in UGE. While systolic blood pressure (SBP) reductions were not statistically significant, body weight and fasting plasma glucose (FPG) reductions were significant at all dosages. Patients with normal renal function had the highest excretion, and UGE decreased as renal function deteriorated. Furthermore, even at supratherapeutic dosages, ertugliflozin did not cause any clinically significant QTc prolongation, according to a study[1].

Other :

A more recent SGLT2 inhibitor, ertugliflozin, is authorized to treat type 2 diabetes mellitus (T2DM). It helps control blood sugar levels and lessen problems such hypoglycemia, diabetic acidosis, and coronary arteriosclerosis. With a 16.6-hour half-life and about 100% bioavailability, it can be taken once day. Uridine diphosphate glucuronosyltransferase (UGT) enzymes break down ertugliflozin to produce inactive glucuronides. It has been demonstrated to lower the cardiorenal risks connected to obesity, hypertension, and chronic hyperglycemia. Based on earlier studies, this review focuses on its function in reducing cardiorenal consequences in type 2 diabetes[3]. Type 2 diabetes mellitus (T2DM) is treated using a class of insulin-independent antihyperglycemic medications called sodiumglucose cotransporter 2 (SGLT2) inhibitors. SGLT2 inhibitors increase urine glucose excretion (UGE) by preventing glucose reabsorption in the kidney, which lowers plasma glucose and HbA1c levels. These medications provide cardiovascular and renal benefits, blood pressure lowering, weight loss, and improved glycemic control. For the treatment of type 2 diabetes, four SGLT2 inhibitors have been approved: dapagliflozin, canagliflozin, empagliflozin, and ertugliflozin. In phase III trials, ertugliflozin has shown efficacy and good safety profiles when used alone or in fixed-dose combos[4].



Structure of Ertugliflozin [4].

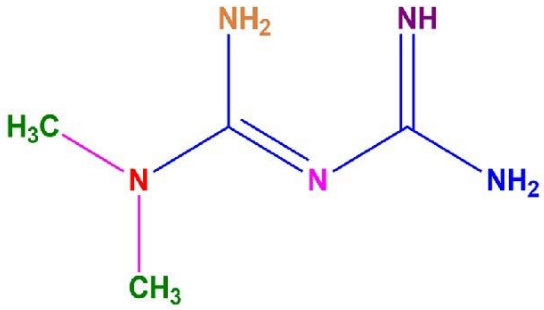
Metformin

For more than 60 years, the main glucose-lowering medication for type 2 diabetic mellitus (T2DM) has been metformin, a synthetic biguanide. Because of its long-term safety, effectiveness, low risk of hypoglycemia, cardiovascular benefits, and moderate weight loss, it is the first-line treatment in many clinical guidelines. Metformin is frequently used in conjunction with other drugs, including DPP-4 inhibitors and sulfonylureas. Polycystic Ovarian Syndrome and gestational diabetes are also treated with it. The medication has flip-flop pharmacokinetics, low oral bioavailability, and is absorbed in the small intestine. Compared to plasma, metformin builds up in tissues, including tumors, at higher amounts[5].

Because it lowers plasma glucose levels, the biguanide derivative metformin has been used as a major treatment for type 2 diabetes (T2D) for almost a century. In addition to diabetes, metformin has demonstrated effectiveness in the treatment of a number of other illnesses, including as cancer, heart disease, liver disease, obesity, neurological conditions, and kidney diseases. By blocking mitochondrial complex I, it triggers AMPK (adenosine monophosphate-activated protein kinase), which impacts homeostasis, glucose and lipid metabolism, and energy metabolism. Metformin also affects IGF receptor signaling and insulin. Although its precise mechanisms in the regulation of disease are still being studied, recent research have demonstrated its influence on a variety of cellular functions[6].

Metformin, either by itself or in conjunction with other glucose-lowering medications, has been demonstrated in numerous studies to be an effective treatment for type 2 diabetes (T2D). Defronzo et al.'s 1995 study showed that metformin lowers HbA1c and fasting plasma glucose levels. The effectiveness of metformin is dose-dependent, according to later research. In comparison to other anti-diabetic medications such as glibenclamide and rosiglitazone, metformin shown moderate efficacy in a 2006 experiment. It has been shown that combination treatments, like metformin with glibenclamide or glimepiride, are more successful in controlling blood sugar. Furthermore, combinations with other medications, such as troglitazone, demonstrated superior outcomes in lowering postprandial and fasting glucose levels[6].

Receiving a type 2 diabetes diagnosis is a life-altering event that necessitates ongoing medical care, understanding long-term risks, and dealing with financial ramifications. According to recent data, prediabetes raises the risk of cardiovascular illness or death, particularly in people who already have atherosclerotic cardiovascular disease. Because of this, there is now more interest in early intervention in the time leading up to a diabetes diagnosis. In 66 nations, metformin is authorized to prevent or postpone type 2 diabetes in people who are at risk. Metformin's importance in preventing diabetes in those with prediabetes or non-diabetic hyperglycemia, particularly those at cardiovascular risk, is now acknowledged by influential guidelines[7].



Structure of Metformin[7].

Significance of RP-HPLC in simultaneous estimation of Ertugliflozin and Metformin :

Calibration Curve :

Using a fixed volume of the internal standard solution (20 µL) and varying concentrations of the working standard solutions in 10 mL volumetric flasks, the calibration curves for the quantification of ertugliflozin and metformin in pure form were created. Ertugliflozin's concentrations ranged dynamically from 0.05 to 30.00 µg/mL, Metformin's from 0.05 to 500.00 µg/mL, and the internal standard's from 0.04 to 20.00 µg/mL. After injecting a 10 µL aliquot of each solution, the chromatograms were examined. The average peak area ratio of the analytes to the internal standard was plotted against the corresponding concentrations to create the calibration curves[8].

Preparation of Tablet and Dosage Forms :

For technique validation, laboratory-prepared tablet formulations comprising Ertugliflozin (2 mg), Metformin (500 mg), and other excipients were created. Methanol was used to extract the corresponding medicines from simulated synthetic tablets that were manufactured for the analysis. Similarly, before creating dilution solutions for analysis, commercially available dosage forms like Amaryl M 2/500 and Piompride tablets were ground into powder, soaked in methanol, sonicated, and filtered[8].

Analytical Quality-by-Design (AQbD)

To guarantee the RP-HPLC technique's robustness and dependability, the AQbD approach was applied during method development. In order to identify the Analytical Target Profile (ATP), the first step was to define the Quality Target Product Profile (QTPP). Critical quality characteristics (CQAs) that potentially affect the method's performance were identified through risk analysis utilizing techniques such as the Ishikawa diagram. During the screening phase, a fractional factorial design (FFD) was used to determine which CMPs—such as flow rate, methanol %, column temperature, buffer pH, and buffer concentration— had the most effects on method efficiency[10].

Table 1: Methods reported in the literature for the determination of Ertugliflozin and Metformin

Author Name/ Journal Name	Title of the Journal	Chromatographic Conditions	References
Haritha G , Vijay Aanandi M, Shanmugasundaram P 2021[8]	A quality by design based method development and validation of a high performance liquid chromatography for simultaneous estimation of metformin and ertugliflozin	**Validated as per ICH guidelines.** Mobile Phase – 60: 40 (0.1% OPA Buffer: Acetonitrile) Wavelength- 230 nm Flow rate- 0.98 ml/min Column Temp- 29.15^{0}C LOD- 59, 3.7 LOQ- 77.6, 5.2	**23**
Bhawani Sunkara, **Tulja RaniGampa,** **Mounika Markanti ,** **Ratna Kumari Midthapally** **2021 [7]**	Stability Indicating Method Development and Validation for Simultaneous estimation and qualification of Ertugliflozin and Metformin In Bulk and Tablet Dosage Form Dosage Form	**Validated as per ICH guidelines.** Mobile Phase – 60: 40 (0.1% OPA Buffer: Acetonitrile) Retention Time – 2.226 & 2.955 min Flow rate – 1.0 ml/min. Temperature: 30℃ Injection Vol – 10uL. Column: C18 Wavelength - 220 nm. LOD- 0.2 & 0.10 ug /ml. LOQ- 0.03 & 0.09 ug/ ml.	**22**
Jyosthna Sri Lakshmi Durga **Savala, SK Abdul Rahaman.** **2021 [6]**	RP-HPLC method development and validation for Simultaneous determination of ertugliflozin and metformin in pharmaceutical dosage forms.	**Validated as per ICH guidelines**. Mobile phase 55:45 (v/v) (0.1% OPA :Acetonitrile) Wavelength - 230nm. Retention time- 3.221 & 2.565min. Flow rate – 1 ml/min. Column temp – 30℃ Column – C18 Injection vol. – 10uL. LOD – 0.01 & 0.36 LOQ – 0.03 & 1.08	**21**

Haritha G, Shanmugasun-daram P **2020** [5]	Stability-indicating RP-UPLC method and Validation for the simultaneously determination of Ertugliflozin and Metformin in Plasma	**Validated as per ICH guidelines**. Mobile Phase -50:10:40 (v/v/v) (NaH2PO4 Buffer (pH-3.5): Methanol: Acetonitrile) . Flow rate – 0.5 ml/min i. Column temp – 25℃ . Wavelength -240 nm Injection vol. – 5 uL. . Column Temp- 25ºC	**20**
Syed Wajahat Shafaat, Aejaz Ahmed, G.J. Khan, Shaikh Anas, Absar A. Qureshi **2020** [4]	Analytical method development and validation for simultaneous estimation of Ertugliflozin and Metformin HCL in Bulk and Pharmaceutical dosage form By HPLC	**Validated as per ICH guidelines.** Mobile Phase – 65: 35 (Buffer- potassium dihydrogen : Acetonitrile) Flow rate – 1.0 ml/min. Injection Vol – 20ul. Column: Inertrsil C18 Wavelength - 220 nm. LOD- 1.04 & 9.16 ug /ml. LOQ- 0.0007 & 0.006 ug/ ml.	**19**
V. Mohan Goud **G. Swapna** **2019** [3]	Stability Indicating method development and validation for the estimation of ertugliflozin and metformin in bulk and pharmaceutical dosage form by UPLC	**Validated as per ICH guidelines.** Mobile Phase – 50: 50 (0.1 % OPA: Acetonitrile) Retention Time – 0.736 & 1.286 min Flow rate – 0.3 ml/min. Temperature: 30℃ Injection Vol – 0.50 ul. Column: HSS C18 Wavelength - 240 nm	**18**

Venkateswara Rao P **Lakshmana Rao A** **Prasad Svum** **2019** [2]	Development and validation of new stability indicating RP-HPLC method for simultaneous determination of metformin HCL and ertugliflozin in bulk and pharmaceutical dosage form	**Validated as per ICH guidelines.** Mobile Phase – 50: 50 (0.1 % OPA: Acetonitrile) Retention Time – 0.736 & 1.286 min Flow rate – 0.3 ml/min. Temperature: 30℃ Injection Vol – 0.50 ul. Column: HSS C18 Wavelength - 240 nm	**17**
A Lakshmana Rao, **U Krishnaveni** **2019** [1]	Stability Indicating RP-HPLC Method for Simultaneous estimation of Metformin and Ertugliflozin	**Validated as per ICH guidelines.** Mobile Phase – 60: 40 (0.01 M KH2PO4: Acetonitrile) Retention Time – 2.357 & 3.209 min Flow rate – 1.0 ml/min. Temperature: 30℃ Injection Vol – 10ul. Column: Denali C18 Wavelength - 224 nm.	**16**

Risk Assessment and Method Optimization

Risk assessment was conducted using the Ishikawa diagram, identifying significant CMPs that could affect the RP-HPLC method's performance. Factors such as flow rate, mobile phase composition, column temperature, and buffer pH were assessed. The Box–Behnken design was used for optimization, focusing on the three most critical CMPs: buffer pH, flow rate, and methanol percentage. The Method Operable Design Region (MODR) was established using regression models, ensuring the method performed optimally under the selected conditions[10].

Diabetes is a rapidly growing health issue in Egypt, with the country ranked ninth globally for diabetes prevalence by the International Diabetes Federation. Metformin (MET), a biguanide, is commonly used to treat type 2 diabetes by reducing liver glucose production and lowering triglycerides and cholesterol. Pioglitazone (PIO), a thiazolidinedione, controls glycemia by reducing insulin resistance and is often used alone or in combination with other antidiabetic medications. Glimepiride (GLM) is a long-acting oral drug used to lower blood sugar, often combined with insulin or other drugs for improved control. Various analytical methods have been developed to estimate MET, PIO, and GLM, both individually and in combinations[8].

For individuals who need more than one medication to control their blood sugar, Tribet-1 and Tribet-2 tablets include 500 mg of MET, 15 mg of PIO, and 1 mg or 2 mg of GLM, respectively. This triple antidiabetic cocktail is frequently analyzed using chromatographic techniques, including RP-HPLC, LC–MS–MS, and HPTLC. However, earlier techniques had drawbacks, such as insufficient validation and system adaptability. By addressing important parameters and guaranteeing performance through risk assessment, Analytical Quality by Design (AQbD) improves the robustness of methods. In order to improve accuracy, sensitivity, and the applicability of biological samples, this study introduces the first AQbD-based RP-HPLC method for concurrently assessing the three medicines[8].

The study's materials included MET, LIN, PIO, GLM, and a variety of excipients from Sigma for the pharmaceutical industry, including lactose, cellulose, and microcrystalline hypromellose. The blood bank at Tanta University Hospital supplied samples of human plasma. Amaryl M 2/500, Bioglita Plus, and Piompride 30/4 were among the dosage formulations available in Egyptian markets. The investigation used TEA, potassium dihydrogen phosphate, orthophosphoric acid, and HPLC-grade methanol from different vendors. A Dionex UltiMate 3000 RS system was used for chromatographic separation, while Chromeleon 7.1 software was used for data processing. Derringer's desirability algorithm and a Box-Behnken design served as the foundation for the optimization[8].

Working standard solutions were created by dilution for calibration, and stock solutions of MET, PIO, GLM, and LIN were prepared. In both pure and spiked human plasma, calibration curves were created for a dynamic concentration range. Protein precipitation, centrifugation, dilution, and filtration

were used to process human plasma samples. Both commercial dose forms and synthetic laboratory tablets were removed for analysis. Using risk assessment techniques such as the Ishikawa diagram, analytical quality-by-design (AQbD) identified the critical method parameters (CMPs) influencing the method's performance. Fractional factorial and Box-Behnken designs were used to optimize CMPs for effective RP-HPLC analysis[8].

Ertugliflozin (and metformin (MET) in bulk and tablet form were to be simultaneously estimated using a reverse-phase high-performance liquid chromatography (RP-HPLC) approach that is straightforward, accurate, specific, and stability-indicating. Specificity, linearity (5-30 µg/mL for TEN and 125-750 µg/mL for MET), accuracy, precision, and robustness were among the factors evaluated during the method's validation in accordance with ICH recommendations. The stability-indicating nature of the approach was confirmed by forced degradation studies carried out under a variety of settings. The technique has been effectively used to estimate TEN and MET in pharmaceutical formulations[9].

The paper discusses the development and validation of an RP-HPLC method for the simultaneous estimation of Metformin (MET) and Glimepiride (GLM) in pharmaceutical formulations. The method was optimized with an isocratic mobile phase consisting of methanol, acetonitrile, and phosphate buffer (pH 3.0), with a flow rate of 1.0 mL/min. The method was validated for linearity, accuracy, precision, LOD, LOQ, robustness, and ruggedness. Forced degradation studies were conducted to ensure stability-indicating properties. The method was efficient, with a short run time (less than 6 minutes) and good resolution for both drugs, offering potential for quality control in pharmaceutical industries[10].

Result and Discussion

Important Results from the Development and Validation of the Method

A robust and dependable chromatographic technique was established as a result of the development and validation of the RPHPLC method for the simultaneous quantification of metformin and ertugliflozin in pharmaceutical dosage forms. With a reasonable resolution between the analytes and the internal standard, the approach produced distinct, well-resolved peaks for both metformin and ertugliflozin. The RP-HPLC method satisfies the necessary criteria, such as linearity, precision, accuracy, selectivity, and robustness, across a broad range of concentrations, according to method validation. For both intra- and inter-day precision experiments, the approach demonstrated strong repeatability with low relative standard deviations (RSD), and the recovery rates for the medicines were constant[8].

Discussion of Chromatograms, Retention Times, and Resolution

Examining Chromatograms, Resolution, and Retention Times Both pure and spiked human plasma chromatograms for metformin and ertugliflozin had distinct, symmetrical peaks. Ertugliflozin and metformin had retention periods of 3.4 and 5.2 minutes, respectively. These are comparable to current techniques for comparable analytes, demonstrating the method's time-efficiency and reproducibility. A good degree of separation between the analytes was shown by the resolution between metformin and ertugliflozin being greater than 2. The method's selectivity was guaranteed by the absence of interference in the resolution between the analytes and internal standard. For precise quantification in intricate pharmaceutical matrices like plasma and tablet formulations, this is essential[8].

Comparison with Existing Methods

Comparing with Current Approaches The new RP-HPLC approach has a number of advantages over current techniques for the simultaneous measurement of metformin and ertugliflozin. This approach employs a quick isocratic separation with an analysis time of less than six minutes, in contrast to earlier techniques that depend on single analyte assays or longer run durations. Because it doesn't call for pricey chemicals or sophisticated equipment, the approach is also economical. The developed method guarantees little matrix interference and excellent specificity, which makes it appropriate for routine quality control and therapeutic medication monitoring, in contrast to other existing methods that may suffer from reduced sensitivity or interference from excipients[15].

Statistical Analysis and Data Interpretation

Interpretation of Data and Statistical Analysis Several validation factors were used to statistically examine the data gathered from the technique validation procedure. Correlation coefficients (r2) for both metformin and ertugliflozin were used to evaluate the linearity of the calibration curves. They were found to be above 0.999, indicating excellent linearity over the tested concentration range. Recovery experiments were used to assess the accuracy, and the results consistently fell within the acceptable range (98– 102%). Intra- and inter-day variability were used to measure precision, and the percentage RSD values were less than 2%, indicating high reproducibility. In order to verify the method's durability, minor changes were made to the chromatographic settings (such as the flow rate and the composition of the mobile phase). The method remained unchanged, proving its resilience under many experimental circumstances[8].

Uses for the Developed RP-HPLC Technique

Ertugliflozin and metformin were successfully measured simultaneously in pharmaceutical formulations and in human plasma that had been tampered with using the RP-HPLC method. The technique demonstrated excellent specificity and accuracy in tablet analysis, with recoveries falling within reasonable bounds. The technique showed adequate sensitivity for therapeutic concentration levels for spiking plasma samples, free from influence from plasma constituents.

For both medications, linear calibration curves with high correlation coefficients were produced. The precision, robustness, and appropriateness of the approach for pharmaceutical quality control and bioanalytical research were confirmed by recovery rates that varied from 94.3% to 105.7% with low percentage RSD values[11].

For the simultaneous measurement of metformin and ertugliflozin in combined pharmaceutical dosage forms, the established RPHPLC method is straightforward, accurate, precise, and dependable. The robustness and reproducibility of the approach were confirmed by the RSD values for each validation parameter falling within the specified ranges. Furthermore, the method's specificity is demonstrated by the lack of interference from tablet excipients, which makes it ideal for routine quantitative analysis in pharmaceutical formulations with several component[12].

7. FUTURE DIRECTIONS

1.Emerging Trends in Chromatographic Techniques for Simultaneous Estimations

Future research may explore advanced chromatographic techniques such as ultra-high-performance liquid chromatography (UHPLC) or two-dimensional liquid chromatography (2D-LC), which could enhance resolution and reduce analysis time.[46], [47] These methods may offer improved sensitivity and separation capabilities for complex biological matrices.

2.Potential Modifications to Enhance Method Efficiency

Modifications such as optimizing the mobile phase composition or utilizing different stationary phases could further enhance method efficiency. Implementing automation and integrating with mass spectrometry could also provide real-time analysis capabilities, allowing for quicker decision-making in clinical and manufacturing environments.[24]

Conclusion

The development and validation of an RP-HPLC method for the simultaneous estimation of Ertugliflozin and Metformin in pharmaceutical dosage forms is critical for ensuring quality, efficacy, and safety. By optimizing chromatographic conditions, such as the choice of stationary phase, mobile phase composition, flow rate, and detection wavelength, a precise and accurate analytical method can be established. Validation parameters, including specificity, linearity, accuracy, precision, LOD, and LOQ, confirm the method's reliability and robustness. Adherence to ICH guidelines ensures reproducibility and regulatory compliance. This method provides a reliable tool for routine quality control and formulation analysis in pharmaceutical industries

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