A COMPREHENSIVE REVIEW OF ANALYTICAL METHODS FOR QUANTIFYING TENELIGLIPTIN AND PIOGLITAZONE IN PHARMACEUTICAL FORMULATION

[1]R. Kavitha, [2]C. Kamali*, [3]I. Ponnilavarasan,[4]A. Rego amal,[5]S. Logesh Kumar

[1,2,3,4,5] M. Pharm
[1,2,3,4,5]Pharmaceutical analysis
[1]KMCH college of pharmacy, Coimbatore, India

Abstract: Diabetes Mellitus is a persistent, progressive condition characterized by elevated blood glucose levels. This review highlights the analytical methodologies applied to discern Teneligliptin and Pioglitazone, both individually and in combined pharmaceutical formulations. These drugs assume pivotal roles in managing Diabetes Mellitus, particularly in the combined treatment of Type II Diabetes. The primary focus of this review is to present an updated exploration of the determination techniques for Teneligliptin and Pioglitazone in their raw state and within pharmaceutical formulations. The analytical approaches highlighted encompass Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) and UV-spectroscopic methods. A comprehensive examination is provided, detailing the separation methods employed for Teneligliptin and Pioglitazone in both singular and combined forms. This review is an essential resource for researchers, healthcare professionals, and pharmacists involved in Diabetes Mellitus management

I. INTRODUCTION

AN OVERVIEW OF THE EVOLUTION OF ANALYTICAL METHODOLOGIES.

In contemporary times, a diverse array of analytical methodologies is employed for estimation purposes. Within the realm of analysis, various techniques such as potentiometers, HPLC, and both aqueous and non-aqueous titrations find application. Aqueous and non-aqueous titrations continue to be relevant in analytical processes. Nevertheless, in the current landscape of analytical practices, High-Performance Liquid Chromatography (HPLC) holds significant importance for quantitative determinations.

High-pressure liquid chromatography, commonly referred to as HPLC, is a separation method that relies on a solid stationary phase and a liquid mobile phase. The chromatographic process involves adsorption, a mass transfer mechanism. The active element within the column is the adsorbent, typically composed of solid particles such as silica or polymers. Adsorption, facilitating the movement or separation of substances based on their respective affinities, serves as the fundamental principle for separation in both normal phase and reverse phase modes. In contemporary pharmaceutical analysis, HPLC plays a pivotal role in effectively separating diverse chemicals within complex mixtures [1].

DRUG PROFILE:

Pioglitazone:
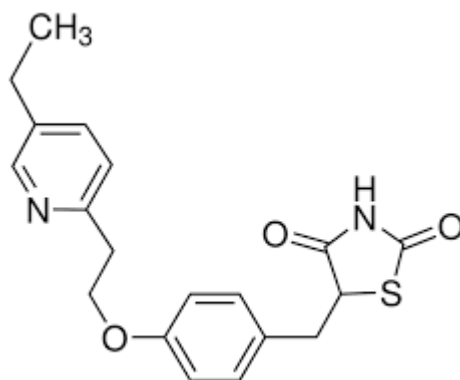
CHEMICAL FORMULA: $C_{19}H_{20}N_2O_3S$

IUPAC NAME: 5-({4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}methyl)-1,3-thiazolidine-2,4-dione
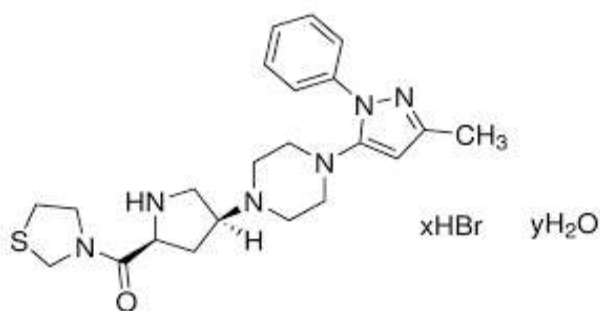
MOLECULAR WEIGHT: 356.439

THE VOLUME OF DISTRIBUTION: 0.63 ± 0.41 L/kg.

Half-life: The mean serum half-life of pioglitazone and its metabolites (M-III and M-IV) range from 3-7 hours and 16-24 hours, respectively.

CLEARANCE: 5-7 L/h

MECHANISM OF ACTION:

 Pioglitazone functions as a discerning agonist specifically targeting the peroxisome proliferator-activated receptor gamma (PPARγ) within insulin-responsive tissues like adipose tissue, skeletal muscle, and the liver. Activation of PPARγ leads to an upsurge in the transcription of insulin-responsive genes, orchestrating the regulation of glucose and lipid production, transport, and utilization. Through this mechanism, pioglitazone not only heightens tissue responsiveness to insulin but also diminishes hepatic glucose production, including gluconeogenesis. Consequently, insulin resistance linked to type 2 diabetes mellitus undergoes improvement without necessitating an elevation in insulin secretion from pancreatic beta cells [2].

TENELIGLIPTIN:

CHEMICAL FORMULA: $C_{22}H_{30}N_6OS$

IUPAC NAME: 1-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-4-[(3S,5S)-5-(1,3-thiazolidine-3-carbonyl)pyrrolidin-3-yl]piperazine.

MOLECULAR WEIGHT: 426.578

VOLUME OF DISTRIBUTION:8.9L/Kg

HALFLIFE: 24 hrs

CLEARANCE:

About 34.4% of teneligliptin is excreted unchanged via the kidney and the remaining 65.6% of teneligliptin is metabolized and eliminated via renal and hepatic excretion; 216 hours after the administration of 14C-labeled teneligliptin (20 mg), the cumulative excretion percentages of radioactive teneligliptin in urine and feces were 45.4% and 46.5%, respectively.

MECHANISM OF ACTION:

Teneligliptin acts by inhibiting DPP-4, extending the activity of incretin hormones like GLP-1. This leads to increased insulin secretion, suppressed glucagon release, and delayed gastric emptying. These actions collectively improve glycemic control in individuals with type 2 diabetes mellitus.

OVERVIEW OF ANALYTICAL TECHNIQUES FOR PIOGLITAZONE:

ESTABLISHED PROCEDURES FOR PIOGLITAZONE ANALYSIS
Liquid chromatography was employed as the official method in the British Pharmacopoeia. The chromatographic conditions included the use of an ODS C18 column (150 × 4.6mm, 5.0 µm), with a mobile phase composed of glacial acetic acid, acetonitrile, and ammonium acetate (in a ratio of 1:25:25 v/v/v). The flow rate was set at 0.7 ml/min, and detection was performed at a wavelength of 269 nm [3].
The official method specified in the Indian Pharmacopoeia utilized liquid chromatography. The chromatographic conditions involved the use of an ODS C18 column (150 × 4.6mm, 5.0 µm), with a mobile phase comprising glacial acetic acid, acetonitrile, and ammonium acetate in a volumetric ratio of 1:25:25. The flow rate was maintained at 0.7 ml/min, and detection occurred at a wavelength of 269 nm [4].

1. RP-HPLC was applied for the examination of pioglitazone hydrochloride, with the chromatographic parameters as follows: a C18 column (250 X 4.6 mm, 5µm), employing a mobile phase consisting of methanol and pH 4.6 buffer adjusted with 0.1 % v/v glacial acetic acid in an 80:20 %v/v ratio. Detection was carried out at a wavelength of 273 nm, with a flow rate set at 1.5 ml/min. The retention time was measured at 3.4 minutes, and the linear range for analysis spanned 5-30 µg/ml [5].

2. RP-HPLC was utilized in the analysis of pioglitazone hydrochloride, employing the following chromatographic conditions: a Hypersil BDS column (250 x 4.6mm, 5 µm), with a mobile phase composed of 0.01M KH2PO4 and acetonitrile in a 40:60 %v/v ratio. Detection was performed at a wavelength of 225 nm, maintaining a flow rate of 1.0 ml/min. The retention time was recorded at 4.726 minutes, and the linear range for analysis extended from 80 to 320 µg/ml [6].

3. The RP-HPLC technique was employed to analyze pioglitazone, utilizing the following chromatographic conditions: a C18 column (300× 3.9 mm, 5 µm), with a mobile phase consisting of acetonitrile and phosphate buffer in a 50:50 %v/v ratio. Detection was conducted at a wavelength of 267 nm, with a maintained flow rate of 1.00 ml/min. The retention time was noted at 8.08 minutes, and the linear range for analysis spanned from 10 to 30 µg/ml [7].

4. The RP-HPLC methodology was employed for the simultaneous analysis of dapagliflozin and pioglitazone hydrochloride. The chromatographic conditions included the use of a Kromstar Vertex C18 column (250 × 4.6 mm, 5µm) and a mobile phase comprising acetonitrile and KH2PO4 Buffer (pH 4)

adjusted with OPA in a 25:75% v/v ratio. Detection was performed at a wavelength of 228 nm, with a consistent flow rate of 1 ml/min. Retention times were observed at 3 minutes for dapagliflozin and 6.5 minutes for pioglitazone. The linear range for analysis was determined as 2-10 µg/ml for dapagliflozin and 3–15 µg/ml for pioglitazone [8].

5. The RP-HPLC method was utilized for the concurrent analysis of Rosiglitazone and pioglitazone, employing the following chromatographic parameters: an Inertsil ODS column (150x4.6mm, 3.5 µm) and a mobile phase consisting of a buffer with 0.1% formic acid and acetonitrile in a 30:70% v/v ratio. Detection was carried out at a wavelength of 261 nm, with a steady flow rate of 1 ml/min. The retention times were noted at 5.118 minutes for Rosiglitazone and 2.770 minutes for pioglitazone. The linear range for analysis encompassed 1-15 µg/ml for Rosiglitazone and 3-45 µg/ml for pioglitazone [9].

6. In the analysis of Alogliptin and Pioglitazone, an RP-HPLC method was employed using a Develosil ODS C18 column (4.6mm×250mm, 5µm). The chromatographic conditions comprised a mobile phase consisting of acetonitrile, Methanol, and 1% Orthophosphoric acid (50:30:20% v/v), with detection performed at a wavelength of 242 nm. The flow rate was set at 1.0 ml/min, and the retention times were determined as 2.24 min for Alogliptin and 5.44 min for Pioglitazone. The linearity ranges for the analysis were established as 30-70 µg/ml for Alogliptin and 60-140 µg/ml for Pioglitazone. These specified conditions were applied for the accurate assessment of the mentioned pharmaceuticals in the conducted study [10].

7. In the assessment of Metformin and Pioglitazone, an RP-HPLC methodology was applied with the following chromatographic parameters: utilizing a Gemini C18 column (150x4.6mm, 5µm), the mobile phase comprised Acetonitrile and Ammonium Acetate buffer (pH-3) in a proportion of 42:58% v/v. Detection was carried out at a wavelength of 255 nm, and the flow rate was set at 0.3 ml/min. The retention times were determined as 5.17 min for Metformin and 8.1 min for Pioglitazone. The linearity ranges for the analysis were established as 0.5-50 µg/ml for Metformin and 0.3-30 µg/ml for Pioglitazone. These specified chromatographic conditions were instrumental in the precise evaluation of Metformin and Pioglitazone within the context of the conducted review article [11].

8. In the stability indicating RP-UPLC alogliptin and pioglitazone was analysed and the used chromatographic conditions are Column: BEH C18(2.1× 50 mm,1.7 µm) Mobile phase: Phosphate buffer (pH 3): Methanol (45:55 % v/v) Detected Wavelength: 280 nm Flow rate: 0.3 ml/min Retention time: Alogliptin: 0.4 min Pioglitazone: 0.529 min Linearity range: Alogliptin: 6.25–37.5µg/ ml Pioglitazone: 15–90µg/m[11].

9. In the evaluation of atorvastatin and pioglitazone, an RP-HPLC method was employed, utilizing the following chromatographic conditions: a Phenomenex Luna C18 column (250 x 4.6mm, 5 µm) was employed, and the mobile phase consisted of Methanol, Acetonitrile, and KH2PO4 buffer adjusted to pH 2.5 with OPA (60:20:20 v/v). Detection was performed at a wavelength of 233 nm, and the flow rate was set at 1.0 ml/min. The linearity range for the analysis was established as 5-50 µg/ml for both atorvastatin and pioglitazone. These specific chromatographic parameters were crucial in the precise assessment of atorvastatin and pioglitazone within the context of the review article [13].

10. In the analysis of alogliptin and pioglitazone using stability-indicating RP-UPLC, the following chromatographic conditions were applied: a Symmetry C18 column (250 x 4.6mm, 5µm) was utilized, and the mobile phase consisted of phosphate buffer (pH 4, adjusted with OPA) in combination with Acetonitrile (20:80 % v/v). Detection was performed at a wavelength of 278 nm, with a flow rate set at 1.0 ml/min. The retention times were determined as 2.234 min for alogliptin and 3.294 min for pioglitazone. The linearity range for the analysis spanned from 0 to 16 µg/ml for both alogliptin and pioglitazone. These specific chromatographic parameters played a crucial role in the assessment of alogliptin and pioglitazone within the framework of the review article[15].

11. In the evaluation of Pioglitazone Hydrochloride, a UV spectroscopic method was employed, utilizing the following chromatographic conditions: Methanol served as the solvent, and the detection wavelength was

set at 268 nm. The linearity range for the analysis spanned from 10 to 50 µg/ml. These specific conditions were instrumental in the precise assessment of Pioglitazone Hydrochloride within the context of the review article [14].

12. In the analysis of Pioglitazone Hydrochloride, a UV spectroscopic method was utilized, with the specified chromatographic parameters being Methanol as the solvent, a detection wavelength set at 270 nm, and a linearity range extending from 10 to 50 µg/ml. These particular conditions played a pivotal role in accurately assessing Pioglitazone Hydrochloride, as outlined in the review article [16].

13. In the assessment of Pioglitazone and Metformin Hydrochloride, a UV spectroscopic method was employed, featuring the following chromatographic conditions: Methanol served as the solvent, and the detection wavelengths were set at 237.4 nm for Metformin Hydrochloride and 225.4 nm for Pioglitazone. The linearity range for the analysis encompassed 5-40 µg/ml for both Metformin Hydrochloride and Pioglitazone. These specific parameters were instrumental in the accurate analysis of Pioglitazone and Metformin Hydrochloride within the framework of the review article [17].

OVERVIEW OF ANALYTICAL TECHNIQUES FOR TENELIGLIPTIN:

1. The analysis of teneligliptin employed a UV spectroscopic method, with the following chromatographic parameters: solvent-distilled water, wavelength - 244 nm, and a linearity range from 5 to 70 µg/ml [18].

2. During the analysis of teneligliptin, an HPLC method was employed with the following chromatographic conditions: utilizing a Protocol C18 ENDURO column (250×4.6mm, 5µm), the mobile phase consisted of methanol and buffer at pH 3.5 in a 72:28% v/v ratio. Detection was performed at a wavelength of 243.5 nm, maintaining a flow rate of 1 ml/min. The retention time was observed at 5.8 minutes, and the linear range for analysis spanned from 10 to 90 µg/ml [19].

3. Stability assessments through RP-HPLC were conducted during the analysis of teneligliptin, employing the following chromatographic parameters: a Kromasil C18 column (250×4.6mm, 5µm), with a mobile phase consisting of pH 6.0 phosphate buffer and acetonitrile in a 60:40 %v/v ratio. Detection was executed at a wavelength of 246 nm, maintaining a flow rate of 1.0 ml/min. The retention time was recorded at 25 minutes, and the linear range for analysis covered 100 to 500 µg/ml [20].

4. In the analysis of teneligliptin, stability investigations were conducted using RP-UPLC, with specific chromatographic conditions as follows: a C8phenomenex column (250 ×4.6 mm, 5 µm), and a mobile phase consisting of formic acid, methanol, and acetic acid in a 25:75:0.1, v/v/v ratio. Detection occurred at a wavelength of 245 nm, with a flow rate set at 0.4 mL/min. The retention time was observed at 4.982±0.02 minutes, and the linear range for analysis extended from 1 to 100 µg/ml [21].

5. Stability investigations utilizing RP-HPLC were conducted during the simultaneous analysis of teneligliptin and metformin, employing the following chromatographic conditions: a Kromasil C18 column (250×4.6 mm, 5 µm), and a mobile phase composed of 0.1% orthophosphoric acid buffer, acetonitrile, and methanol in a 65:25:10, v/v/v ratio. Detection was performed at a wavelength of 254 nm, maintaining a flow rate of 1.0 ml/min. The retention times were recorded at 2.842 minutes for teneligliptin and 2.017 minutes for metformin, with linear ranges for analysis spanning from 5 to 30 µg/ml for teneligliptin and 125 to 750 µg/ml for metformin [22].

6. In the course of analyzing metformin and teneligliptin, stability assessments were conducted using RP-HPLC, with specific chromatographic conditions including a Discovery column (250 X 4.6 mm: 5 µm) and a mobile phase consisting of 0.1% orthophosphoric acid buffer and acetonitrile in a 65:35, v/v ratio. Detection was carried out at a wavelength of 260 nm, maintaining a flow rate of 1 ml/min. Retention times were observed at 2.517 minutes for metformin and 3.687 minutes for teneligliptin, with linear ranges for analysis spanning from 125 to 750 µg/ml for metformin and 5 to 30 µg/ml for teneligliptin [21].

7. For the assessment of teneligliptin, an RP-HPLC method was employed, utilizing the following chromatographic parameters: a Grace SmartC18 column (250 x 4.6mm, 5μm) and a mobile phase consisting of 0.05M KH2PO4 at pH 4.0 with acetonitrile in an 80:20 % v/v ratio. Detection was performed at a wavelength of 242 nm, maintaining a flow rate of 1 ml/min. The retention time was recorded at 7.443 minutes, and the linear range for analysis covered 500 to 3000 μg/ml [24].

8. In the evaluation of teneligliptin hydrobromide hydrate, an RP-HPLC method was applied with the following chromatographic parameters: utilizing a Kromasil C18 column, the mobile phase comprised pH 5.5 phosphate buffer and methanol in a 75:25%v/v ratio. Detection occurred at a wavelength of 270 nm, maintaining a flow rate of 1.2 ml/min. The retention time was observed at 2.51 minutes, and the linear range for analysis extended from 80 to 120 μg/ml [25].

9. In the assessment of teneligliptin, RP-HPLC was employed for analysis, utilizing the following chromatographic conditions: a Kromasil C18 column (150 × 4.6 mm, 5.0 μm), and a mobile phase consisting of (A) acetonitrile: water: trifluoroacetic acid in a 60:1940:2 v/v ratio and (B) acetonitrile: trifluoroacetic acid in a 2000:2 v/v ratio. Detection was performed at a wavelength of 245 nm, with a flow rate set at 1.0 ml/min. The retention time was noted at 11.2 minutes, and the linear range for analysis covered 50-150 μg/ml [26].

10. In the evaluation of teneligliptin, RP-HPLC was utilized for analysis, employing the subsequent chromatographic parameters: a Cosmosil C18 column (250 × 4.6mm, 5.0 μm), and a mobile phase consisting of methanol and phosphate buffer with a pH of 3 in a 70:30 %v/v ratio. Detection occurred at a wavelength of 246 nm, with a flow rate set at 0.8 ml/min. The retention time was observed at 4.2 minutes, and the linear range for analysis spanned from 10 to 50 μg/ml [27].

11. In the assessment of teneligliptin and metformin, RP-UHPLC was employed, with the following chromatographic conditions: utilizing an Eclipse plus C18 column (150 × 4.6 mm, 5 μm), the mobile phase consisted of buffer and acetonitrile in a 65:35 % v/v ratio (pH 3.5 with OPA). Detection was performed at a wavelength of 233 nm, maintaining a flow rate of 0.7 ml/min. The retention times were recorded at 2.81 minutes for teneligliptin and 1.71 minutes for metformin, with a linear range for analysis spanning from 20 to 100 μg/ml [28].

REPORTED METHODS FOR TENELIGLIPTIN PIOGLITAZONE IN COMBINATION:

A highly efficient and reliable thin-layer chromatographic (TLC) method has been developed and validated for the simultaneous determination of teneligliptin hydrobromide hydrate and pioglitazone hydrochloride in tablet formulations, showcasing exceptional sensitivity, precision, accuracy, and robustness. In this methodology, aluminum plates coated with silica gel 60F254 were utilized, and the solvent system comprised a mixture of chloroform, methanol, and ammonia (8:1:0.5 v/v/v). The analysis involved scanning the plate at a wavelength of 229 nm and interpreting the resulting densitograms.

Linear correlation was successfully established across a concentration range of 250–3000 ng/band for teneligliptin hydrobromide hydrate and 187.5–2250 ng/band for pioglitazone hydrochloride. Notably, the method exhibited low detection limits of 21.29 ng/band for teneligliptin hydrobromide hydrate and 25.97 ng/band for pioglitazone hydrochloride, with quantification limits of 64.52 ng/band and 78.71 ng/band, respectively.

Precision parameters demonstrated a consistently low % relative standard deviation of peak area, consistently below 2%, indicative of high precision. Moreover, the accuracy of the method fell within the range of 96% to 103%. Overall, the proposed method outperforms previously reported techniques, addressing limitations and establishing itself as a superior approach for assessing both drugs simultaneously [29].

The amalgamation of multiple pharmaceutical agents in formulations has significantly advanced the treatment of intricate medical conditions, particularly benefiting individuals with type 2 diabetes mellitus. The potent antidiabetic agents, teneligliptin hydrobromide hydrate (TEN) and pioglitazone hydrochloride (PIO), play pivotal roles in blood glucose regulation. This investigation introduces novel approaches for concurrently quantifying TEN and PIO in pharmaceutical formulations, ensuring precision and stability assessment. Our TLC-densitometric method employs a mobile phase comprising Methanol, Toluene, Ethyl Acetate, and Triethylamine (1:7:2:0.1, v/v/v/v) on TLC silica gel plates, followed by densitometric scanning at 268 nm.

Simultaneously, the RP-HPLC method utilizes isocratic elution with acetonitrile and acetate buffer (pH 2.3, 60:40 v/v) on a C18 column, employing diode-array detection at 235 nm. Both techniques demonstrate outstanding accuracy and reliability, serving as invaluable tools for pharmaceutical quality control. Furthermore, our research integrates an environmental impact assessment to align with global sustainability objectives. Factors such as solvent consumption, waste generation, and energy usage are considered, employing assessment tools like the eco-scale assessment, AGREE, Green Analytical Procedure Index (GAPI), and the National Environmental Method Index (NEMI) to evaluate the environmental impact of our methods. By adopting these approaches, pharmaceutical companies can enhance their drug quality control processes while fulfilling environmental responsibilities. Rigorous statistical comparisons, including t-tests and F-tests, validate the outcomes of the TLC densitometric and RP-HPLC methods, affirming their efficacy in drug formulation analysis [29].

BIO-ANALYTICAL TECHNIQUES FOR TENELIGLIPTIN:

A straightforward, highly sensitive, precise, and accurate high-performance liquid chromatographic (HPLC) method has been established and validated for the quantification of teneligliptin in rabbit plasma samples. The chromatographic separation utilized a reverse phase Thermo C18 column (4.6×100 mm, 5µ), with the mobile phase consisting of a 60:40 v/v mixture of methanol and 5mm potassium phosphate buffer, flowing at a rate of 1 mL/min. Sitagliptin served as the internal standard, and the retention times for teneligliptin and sitagliptin were determined to be 3.9 and 2.2 minutes, respectively.

The calibration curve demonstrated linearity ($r2 \geq 0.99$) across the concentration range of 7.20 to 470 ng/mL, with a lower limit of quantification at 7.20 ng/mL. Interday precision exhibited a coefficient of variation lower than 5%, and accuracy fell within the range of 90 to 110% in terms of percent accuracy. The mean extraction recovery exceeded 82%. This method was successfully developed and validated in rabbit plasma, showcasing excellent selectivity, accuracy, precision, recovery, and stability. Keywords: HPLC; Teneligliptin; internal standard; Rabbit plasma; Validation

BIOANALYTICAL TECHNIQUE FOR PIOGLITAZONE:

Pioglitazone is extensively utilized in the management of type-II diabetes mellitus. This study aimed to develop a straightforward and cost-effective HPLC method for the quantification of pioglitazone in human plasma. The mobile phase consisted of Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1 v/v/v) at a flow rate of 1.2 mL/min, using a Macherey-Nagel Column C18 (dimensions: 5 µm; 250 × 4.6mm) with a guard column. The UV detector was set at 269nm. Validation of the method, following FDA guidelines, demonstrated excellent linearity (R2=0.9998) within the range of 0.1 to 2.0µg/ml standards, with a detection limit of 0.1 µg/ml. Intra-day accuracy and precision (%CV range: 93.33% to 100.4%, 3.8% to 9.2%) and interday accuracy and precision (range: 94.1% to 102.7%, 4.8% to 9.6%) complied with FDA guidelines. Freeze-thaw stability indicated that plasma samples could be stored for one month at -20oC without appreciable degradation. The method was successfully applied to blood samples from a volunteer following the oral administration of a 30 mg pioglitazone tablet, enabling the calculation of preliminary pharmacokinetic parameters. The study concludes that this method is suitable for routine analysis of pioglitazone in blood samples obtained during pharmacokinetic studies.

GUIDELINES USED IN ANALYTICAL METHOD DEVELOPMENT AND VALIDATION:

Analytical method development and validation in the pharmaceutical industry are crucial steps to ensure the accuracy, reliability, and reproducibility of analytical procedures. Several guidelines provide recommendations and requirements for these processes. Here are some key guidelines used in analytical method development and validation, along with considerations on how to select them:

1. ICH Q2(R1) - Validation of Analytical Procedures:

- This guideline, published by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), is a fundamental reference for the validation of analytical methods. It covers parameters such as specificity, accuracy, precision, linearity, range, and robustness [30].

2. USP General Chapter <1225> - Validation of Compendial Procedures:

- The United States Pharmacopeia (USP) guides the validation of compendial procedures. It complements ICH Q2(R1) and is particularly relevant when using compendial methods [31].

3. USP General Chapter <1226> - Verification of Compendial Procedures:

- This chapter in the USP focuses on the verification of compendial procedures, emphasizing the need for users to ensure that the compendial methods are suitable for their intended use [31].

4. EP General Chapter 2.2.46 - Validation of Analytical Procedures:

- The European Pharmacopoeia (EP) provides guidelines similar to ICH Q2(R1) for the validation of analytical procedures. It is an essential reference for pharmaceutical analysis in European markets [35].

5. FDA Guidance for Industry - Analytical Procedures and Methods Validation:

- The U.S. Food and Drug Administration (FDA) issues specific guidance documents that provide additional insights and expectations for analytical method development and validation. These can be found on the FDA website [33].

6. AOAC International Guidelines:

- AOAC International provides guidelines for method validation in the field of analytical chemistry, including those relevant to the pharmaceutical industry [31].

CONSIDERATION FOR SELECTING GUIDELINES:

1. Geographic Region:

- Consider the regulatory requirements of the region where your product will be marketed. Different countries may have specific guidelines, and compliance with local regulations is crucial.

2. Type of Method:

- Different guidelines may be more specific to certain types of methods (e.g., chromatographic, spectroscopic). Select guidelines that are most relevant to your analytical technique.

3. Pharmacopeial Requirements:

- If your method is intended for use in a pharmaceutical monograph, refer to the relevant pharmacopeia (e.g., USP, EP) for guidance.

4. Industry Best Practices:

- Stay informed about industry best practices by reviewing literature, attending conferences, and engaging with professional organizations.

5. Product Development Stage:

- Consider the stage of product development. Early-stage development may involve more flexibility, while late-stage development and commercialization require adherence to established guidelines.

6. Specific Requirements of the Analyte:

- Some guidelines may be more applicable to specific types of analytes or products. Consider the characteristics of the substance being analyzed.

CONCLUSION:

Numerous published methodologies exist for the determination of Teneligliptin and Pioglitazone, pivotal medications in Diabetes Mellitus. These drugs are available in diverse formulations and combinations, addressing various dosage requirements. RP-HPLC methods, applied either individually or in combination, are commonly employed for the estimation of Teneligliptin and Pioglitazone. Notably, RP-HPLC techniques demonstrate enhanced resolution when the mobile phase comprises Acetonitrile, water, Methanol, and Phosphate buffer. Additionally, UV-photo spectroscopy methods, predominantly employing Methanol as the solvent, have been reported. Overall, these methodologies are recognized for their simplicity, accuracy, and cost-effectiveness.

REFERENCES:

1. Monike et al., A Review on Analytical Methods for Estimation of Teneligliptin and Pioglitazone in Pharmaceutical Dosage Form. International journal of pharmacy and pharmaceutical research.27;1:2023

2. Drug bank. Pioglitazone: Uses, Interactions, Mechanism of Action | DrugBank Online

3. British Pharmacopeia; Govt. of British Ministry of Health and Family Welfare, 2nd volume; The British Pharmacopoeia commission, 2020, 2601-2603.

4. Indian Pharmacopeia; Ministry of Health and Family Welfare, 2nd volume; The Indian Pharmacopoeia Commission, 2010, 1916-1917.

5. Kommana R, Rebecca S D. Development and validation of HPLC and UV spectrophotometric method for determination of pioglitazone hydrochloride in bulk and its formulations. Pharm Lett. 2013; 5(1):269-78.

6. Agarwal P T, Sharma M. Development and validation of Pioglitazone hydrochloride in bulk and pharmaceutical formulations by RP-HPLC method. World J. Pharm. Res, 2020; 9(8): 1966-1977.

7. Maste M M, Gawas N S, Shashtri U, Shelar P. RP-HPLC Method Development and Validation for Pioglitazone in Bulk and Marketed Formulation. Der Pharma Chemica, 2021, 13(6): 6-15.

8. Ronak P. Method Development, Validation and Forced Degradation Studies of Dapagliflozin and Pioglitazone Hydrochlorides in Synthetic Mixtures by RP-HPLC. International Journal of Trend in Scientific Research and Development. 2022; 6(6):1858-69.

9. Rafi S, Rambabu K. New Validated Method for the Estimation of Pioglitazone and Rosiglitazone Using RP-HPLC. Journal of Pharmaceutical Research International, 2021; 33 (47A): 254-262.

10. Kandala M, Mounika R, Sultana S, Hogue N, Ali S, Prashanthi S, Islam S, Aktar SN, Kabir S. Method development and validation of Alogliptin and pioglitazone by RP-HPLC method in bulk and its marketed dosage form. World J. Pharm. Res. 2021 Jun 10;10:782-98.

11. Lakshmi K S, Rajesh T, Sharma S. Simultaneous determination of metformin and pioglitazone by reversed-phase HPLC in pharmaceutical dosage forms. Int. J. Pharm. Pharm. Sci. 2009 Oct;1(2):162-6.

12. Dhani R, Guptha H K, Rajamanickam D. Development of stability indicating method for the simultaneous estimation of alogliptin and pioglitazone in bulk and tablet dosage form by reversed-phase ultra-performance liquid chromatography method. J. Appl. Pharm. Sci. 2019 Dec 3; 9(12):051-6.

13. Nizami T, Shrivastava B, Sharma P, Darwhekar G N, Sharma P. Spectrophotometric and Reversed-Phase High-Performance Liquid Chromatographic methods for simultaneous determination of atorvastatin and pioglitazone in combined tablet dosage form. J. drug delivery. ther. 2017 Dec 22; 7(7):116-7.

14. Bhavyasri K, Chandana R S, Sumakanth M, Swethasri R. Analytical Method Development and Validation for The Estimation of Pioglitazone Hydrochloride in Bulk and Formulation by UV-Spectrophotometry. Am. J. PharmTech Res. 2019; 9(04):110-117.

15. . Vasanthi R, Noori K, Sundar P S, Raja M A, Dutt K R, Rao K N, Mahesh M, Charlapally N. Development of rapid stability indicating method for simultaneous estimation of alogliptin and pioglitazone in bulk and combined dosage form by RP-HPLC method. Indo. Am. J. Pharm. 2017; 3: 234-44

16. Doredla N R, Yengisetty B, Bojjagani R, Madasu S V. Method development and validation of forced degradation studies of pioglitazone hydrochloride by using UV spectroscopy. Int. J. Pharm Tech Res. 2012; 4(4):1750-7

17. Joshi R S, Nangare A K, Sanap D S, Sase S M. Development and Validation of UV-Spectrophotometric Method for Estimation of Metformin Hydrochloride and Pioglitazone in Tablet Dosage Form. J. drug deliv. ther. 2019 Aug 30; 9(4-A):381-4

18. Yadav N, Goyal A. Method development and validation of Teneligliptin in pharmaceutical dosage form by UV spectrophotometric methods. Int J Pharm Chem Anal. 2017;4(3):54-8

19. Gaikwad D D. Analytical method development and validation of Teneligliptin hydrobromide in pure form by HPLC. World J. Pharm. Pharm. Sci. 2017 Sep 29:37-48.

20. Kumar T G, Vidyadhara S, Narkhede N A, Silpa Y S, Lakshmi M R. Method development, validation, and stability studies of teneligliptin by RP-HPLC and identification of degradation products by UPLC tandem mass spectroscopy. Journal of analytical science and technology. 2016 Dec; 7:1-2.

21. Annapurna M M, Almas S, Rajasree B, Narendra A. Stability indicating ultrafast liquid chromatographic method for the estimation of Teneligliptin (An Anti-diabetic agent). Asian J. Pharm. 2018 Apr 1;12:S477-83. 15. Jinal Alkesh Gheewala*, Dr. Dilip Girish Maheshwari, Development and Validation of UV Spectrophotometric Method and RP – HPLC Method for Simultaneous Estimation of Teneligliptin and Pioglitazone In Synthetic Mixture, Asian Journal of Pharmaceutical Technology & Innovation, 2017, 05 (23); 66-78.

22. Vetapalem R, Yejella R P, Atmakuri L R. Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of teneligliptin and metformin. Turk. J. Pharm. Sci. 2020 Apr;17(2):141.

23. Swetha A, Kuber B R. A novel stability-indicating reverse phase liquid chromatographic method for the simultaneous estimation of metformin and teneligliptin in pure and pharmaceutical formulations. Int J App Pharm. 2018;10(5):274-80.

24. Patel B D, Nidhi J D, Ankit C. Development and Validation of RP-HPLC Method for Estimation of Teneligliptin and its Impurity in Tablet. Int. J. Pharm. Sci. Rev. Res,(2021) August; 69 (2): 127.;133.

25. Dahikar G D, Bobade G. Development and Validation of Stability Indicating RP-HPLC method for Teneligliptin Hydrobromide Hydrate. Am. J. Pharm Tech Res. 2020;11(1).

26. Biswas B, Kumar M, Sharma J B, Saini V, Bhatt S. Method Development and Validation for Estimation of Teneligliptin in Tablet Dosage Form by RP-HPLC. Res J Pharm Technol. 2020;13(4):1774-8.

27. Lokhande D P. Analytical method development and validation of Teneligliptin by using RP-HPLC with ICH guidelines. Int. J. Trend. Sci. Res. Dev. 2019;3:259-63.

28. Patel V, Pandya C, Patel Z, Patel D, Pandya A. Isocratic RP-UHPLC method development and validation of stability-indicating for simultaneous determination of teneligliptin and metformin in fixed-dose combination. Current Chemistry Letters. 2021;10(4):503-16.

29. Ashim *et al* Densitometric simultaneous assessment of teneligliptin hydrobromide and pioglitazone hydrochloride in combined tablet **Volume 7, Issue 1** **2300139**

30. Ashok *et al.,* Stability-Indicating TLC-Densitometric and HPLC Methods for Simultaneous Determination of Teneligliptin and Pioglitazone in Pharmaceutical Dosage Forms with Eco-Friendly Assessment. research square, 9,93;2023.