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Review Article

A Comprehensive Review of Different Analytical Methods for Expiry and Stability Assessment of Dapagliflozin and Pioglitazone.

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ABSTRACT

Dapagliflozin and pioglitazone are widely used oral antidiabetic agents in the management of type 2 diabetes mellitus. The stability of these drugs in pharmaceutical dosage forms is an important factor in maintaining their quality, safety, efficacy, and potency throughout their labelled shelf life. The present study aims to evaluate the stability and expiry-related quality characteristics of marketed dapagliflozin and pioglitazone tablets using suitable analytical methods. The study focuses on assessing the drug content and detecting possible degradation or changes in the pharmaceutical quality of tablets at or around their expiry period. Appropriate analytical parameters such as identification, assay, dissolution, and other relevant quality attributes may be evaluated and compared with the specified pharmacopoeial or manufacturer's limits. A stability-indicating analytical method, particularly reverse-phase high-performance liquid chromatography (RP-HPLC), may be employed for accurate and precise estimation of dapagliflozin and pioglitazone and for monitoring potential degradation products. The study provides information regarding the impact of storage and expiry on the caliber and potency of the selected formulations. Evaluation of expired and unexpired tablets can help in understanding drug stability and the degree of degradation under real storage conditions. The results of this investigation could support the assessment of pharmaceutical quality, development of appropriate storage recommendations, and understanding of the importance of expiry dates for dapagliflozin and pioglitazone tablet formulations.

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INTRODUCTION:

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) specifies the expiration date as a guaranty of the complete potency and safety of the manufacturer's drugs. In 2016, it was observed that pharmaceutical items that are stored in accordance with established standards remain viable and effective long after their expiration dates. The duration of a medication's potency can be influenced by several important elements, including as dosage types, lots, preservatives, and storage conditions.[1]

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Dapagliflozin is a sodium-glucose co-transporter 2 (SGLT-2) inhibitor that is used to treat type 2 diabetes. A proliferator-activator receptor agonist is pioglitazone hydrochloride. The combination of Dapagliflozin and Pioglitazone hydrochloride was investigated in a clinical trial context, and it was observed that the synergistic effect improved glycaemic control while also lowering body weight [1-5]. This combination was effective, well-tolerated, and reduced Pioglitazone-induced weight gain without raising hypoglycemia risk. Pioglitazone (15 mg) and dapagliflozin (10 mg) were the medication dosages, both of which are in phase 3 clinical trials for the treatment of type 1 diabetes (insulin-dependent), a chronic illness in which the pancreas produces little or no insulin [6]

Dapagliflozin is a sodium-glucose co-transporter 2 (SGLT-2) inhibitor used to treat type 2 diabetes. An agonist of the proliferator-activator receptor is pioglitazone hydrochloride. The combination of pioglitazone hydrochloride and dapagliflozin was demonstrated to improve glycemic management and lower body weight in a clinical research setting. Without making hypoglycemia more likely This combination reduced the weight increase caused by pioglitazone and was effective and well-tolerated.[2]

ANALYTICAL METHOD:

According to ICH guidelines, an analytical method must show that it is appropriate for its intended use. Analytical method validation is a crucial stage in the creation of a method from a synthetic combination since it offers information on accuracy, linearity, detection, and quantitation limits. It guaranties that various HPLC analytical procedures produce consistent and reproducible findings.[2]

1. SPECTROSCOPIC TECHNIQUES:

1.1 UV-Visible Spectroscopy:

Ultraviolet spectroscopy has been an important and sophisticated analytical tool in the pharmaceutical industry. The analytical approach is based on measuring monochromatic light absorption by colourless substances in the near ultraviolet portion of the spectrum (200-400 nm). Pharmaceutical analysis includes the techniques necessary to ascertain the "identity, strength, quality, and purity" of such compounds.[3] The amount of visible or ultraviolet light that a sample or substance absorbs substance is the fundamental principle of UV-Visible spectroscopy, which leads to the generation of distinct spectra. The electron percentage in a molecule is excited when UV radiation is absorbed, resulting in an electron's change from a lower to a higher electronic energy level. The ultraviolet emission spectra are the outcome of the contrary type of transition. In UV spectroscopy, the most frequently employed solvents include water, methanol, ethanol, ether, chloroform, carbon tetrachloride, cyclohexane, and dichloroethane. UV spectroscopy is employed for the identification of impurities, conjugation, geometrical isomers, and functional groups.[4]

1.2 FTIR Spectroscopy:

A popular analytical method that is renowned for its accuracy, precision, and affordability is Fourier Transform Infrared (FTIR) spectroscopy. Comparative investigation reveals that whereas desktop FTIR instruments provide superior spectrum resolution and sensitivity, portable FTIR provides real-time, on-site analysis with less sample preparation. According to

research, FTIR provides excellent accuracy in pharmaceutical quality control (R2 > 0.999), forensic hematoma analysis (R^2 = 0.88), and food authenticity (R^2 = 0.96).[5]

FTIR spectroscopy in the previously mentioned disciplines. More importantly, a thorough demonstration of the application's validity, reliability, and generality will be provided by a study of the most current studies that have been published in the scientific literature. FTIR spectroscopy has many unquestionable advantages over traditional techniques when used quantitatively in biological, interdisciplinary, and pharmacological fields. An instructive account shall be conducted in this regard.[6]

1.3 NMR:

Nuclear magnetic resonance spectroscopy is one of the most important and frequently used analytical methods in both academic and commercial research. By permitting a distinct and, theoretically, quantifiable measurement of the relative number of molecular groups, it offers a technique to quantify whole molecule structures even in mixes.[7]

For the structural characterisation of unknown synthetic and natural compounds, NMR is a crucial analytical technique that has been recognized as a rapid, useful, reliable, and non-destructive approach for quantitative evaluations.

Nuclear magnetic resonance spectroscopy has enabled most important routine analyses of natural and synthetic medication candidates and related substances. Because nuclear magnetic resonance allows for quality control and simultaneous quantification of different residual solvents, analytes, and contaminants, analysts have continued to use it as a useful quantitative method. Thus, the primary focus of this review is the use and feasibility of this technology for quantitative method validation of medicines and related compounds.[8]

2. CHROMATOGRAPHIC TECHNIQUES:

2.1 Thin Layer Chromatography (TLC):

A very old method for pharmaceutical drug analysis is thin layer chromatography. This approach used two phases both the stationary phase and the mobile phase. The phases utilized in sample preparation are solid phase, adsorbent, and a thin layer of silica gel placed on a glass plate with an aluminum support. This approach was used to analyse both organic and inorganic compounds.[9]

In the examination of medicines, botanicals, food, environmental, and clinical samples, thin layer chromatography—and especially its highperformance application—remains a popular analytical method.[10] The multistage distribution process that forms the basis of TLC includes a suitable "adsorbent" (the stationary phase), solvents or solvent mixtures (the mobile phase or eluent), and the sample molecules.[11]

2.2 Liquid Chromatography– Mass Spectroscopy (LC-MS):

The analytical chemistry method known as liquid chromatography–mass spectrometry (LC–MS) combines the physical separation capabilities of liquid chromatography and the mass analysis capabilities of mass spectrometry (MS). Coupled chromatography: MS systems are widely used in chemical analysis due to the synergistic enhancement of each technique's own capabilities.[12] Currently, the preferred technique for quantitative analysis of chemicals inside biological matrices is liquid chromatography (LC) coupled to tandem mass spectrometric (MS/MS) detection using an air pressure ionization (API) source. The enhanced specificity, sensitivity, and throughput are the primary benefits of employing MS/MS in the selected reaction monitoring (SRM) mode. Because of the aforementioned justifications, analysts have created high throughput techniques with low chromatographic retention and little to no sample preparation.[13] It is an analytical chemistry method that integrates the mass analysis skills of mass spectrometry using liquid chromatography's physical separation capabilities, also referred to as HPLC. (LC-MS/MS) is frequently utilized in labs to analyse pharmacological compounds, drug products, and biological materials both qualitatively and quantitatively.[14]

2.3 Gas Chromatography:

A popular instrumental analytical method for the division, recognition, and quantitative measurement of volatile and thermally stable chemicals found in complicated mixtures is gas chromatography (GC). In GC,

an inert carrier gas, such as hydrogen, nitrogen, or helium, vaporizes the specimen and moves it through a chromatographic column. Individual components are separated according to how they are distributed between the mobile gas phase and the column's stationary phase. After being separated, each component arrives at the detector at a distinctive retention period that can be utilized for quantitative analysis and identification. [15] GC can be used to directly separate and analyse gaseous materials volatile solids and liquid solutions. GC methods such as derivatization or pyrolysis can be applied if the sample to be examined is non-volatile. When information other than a comparative fingerprint (program) is needed, like for the positive identification of peaks on the chromatogram, gas chromatography, the analytical method used for product identification (under extremely controlled conditions), must be directly connected to a mass spectrometer.[16]

2.4 Ultra Performance Liquid Chromatography:

Gaseous materials can be used to quickly separate and analyze volatile solids, liquid solutions, and gaseous compounds. If the sample to be analyzed is non-volatile, derivatization or pyrolysis GC techniques may be used. Gas chromatography, the analytical method used for product identification (under extremely controlled conditions), must be directly connected to a mass spectrometer when information other than a comparative fingerprint (program) is required, such as for the positive identification of peaks on the chromatogram.[17]

The fundamental idea behind UPLC, a variant of HPLC, is that efficiency and resolution rise as column packing particle size decreases. According to the standard Van Deemter equation, efficiency increases significantly when particle size is reduced to less than 2 µm and does not decline at higher linear velocities or flow rates.[18]

2.5 High Performance Liquid Chromatography:

Among the most important tools in analytical chemistry nowadays is High Performance Liquid Chromatography (HPLC), which was developed from conventional column chromatography. High performance liquid chromatography (HPLC) is a key analytical technology used in all phases of Finding, creating, and manufacturing drugs in the contemporary pharmaceutical industry. HPLC is the preferred technique for determining the peak purity of novel chemical entities, tracking changes in reactions throughout synthetic processes or scaling up, assessing novel formulations, and performing quality control and assurance on the finished pharmaceutical goods.[19]

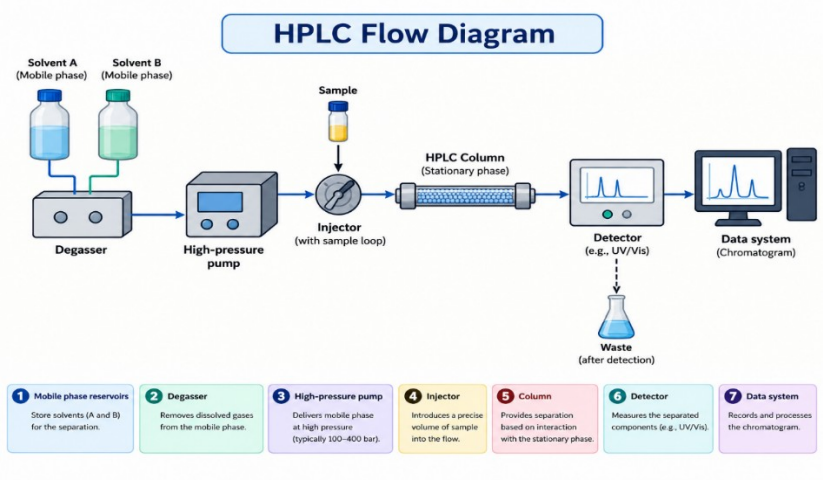


Figure 1: HPLC Flow Diagram

Method Development On HPLC:

A step involved in method development of HPLC is as follows:

- Understanding the Physicochemical properties of drug molecule.
- Selection of chromatographic conditions.
- Optimization of Mobile Phase.

- Developing the approach of analysis.
- Sample preparations
- Method optimization
- Method validation

1.Understanding the Physicochemical properties of drug molecule:

When developing a method, a medicinal molecule's physicochemical characteristics are crucial. Studying the drug molecule's physical characteristics, such as its solubility, polarity, pKa, and pH, is necessary for method development. One of a compound's physical characteristics is its polarity. It aids an analyst while choosing the mobile phase and solvent composition.[20]

2.Selection of chromatographic conditions:

Chromatographic modes according to the polarity and molecular weight of the analyte. The most popular method for small organic compounds, reversed phase chromatography (RPC), will be the subject of all case studies. RPC frequently uses buffered mobile phases (to maintain the analytes in a non-ionized condition) or reagents that couple ions to separate ionizable materials (acids and bases).[16]

3.Optimisation Mobile Phase:

3.1 Buffer Selection: The intended pH determines which buffer to use. Reversed phase on silica-based packing often occurs in the pH range of 2 to 8. Given that buffer optimally regulates pH at its pKa, it is essential that the buffer has a pKa that is close to the target pH. Selecting It is recommended to choose a buffer whose pKa value is less than two units of the pH of the planned mobile phase.[20]

3.2 Effect of pH: If analytes are ionizable, the target analyte must be in one predominant ionization state—ionised or neutral—by selecting the appropriate pH of the mobile phase depends on the analyte pKa. One of the best tools in the "chromatographer's toolbox" is the ability to simultaneously alter retention and selectivity between crucial pairs of components by changing the pH of the mobile phase.[16]

3.3 Effect of organic modifier: In reverse phase HPLC, choosing the type of organic modifier is rather easy. Acetonitrile and methanol (occasionally THF) are the typical options. Since it would not be feasible to elute every component Gradient elution is commonly employed with complicated multicomponent samples between k (retention factor) 1 and 10 utilizing a single solvent strength under isocratic conditions.[16]

4 Developing the approach of analysis:

Choosing different chromatographic parameters is the first step in developing the analytical method, including mobile phase, column, flow rate, and pH on RP-HPLC. The system appropriateness parameters are taken into consideration after each of these parameters has been chosen based on testing Retention time should be more than five minutes, theoretical plates more than two thousand, the tailing factor less than two, and the resolution between two peaks more than five, and the percentage R.S.D. of the analyte peak area in standard chromatograms should not exceed two percent. similar to others. When two components are being estimated simultaneously the wavelength of detection is typically an isobestic point.[16]

5 Sample Preparations:

An aliquot of the sample that is comparatively devoid of interferences won't damage the column, and works with the desired HPLC method—that is, without affecting sample retention or resolution, the sample solvent will dissolve in the mobile phase is the goal of sample preparation.[21] In solution (diluent), the drug ingredient under analysis must be stable. The solutions should be prepared in amber flasks throughout the initial phases of method development until it is determined that the active ingredient is stable at room temperature and does not degrade under normal laboratory conditions A filter with a pore size of 0.22 or 0.45 μm is typically advised to remove particles from the sample solution. For HPLC analyses, Filtration is a method of preventive maintenance.[22]

6 Method Optimization:

After obtaining the proper separations, Optimizing the experimental conditions is necessary to obtain the required separations and sensitivity. Through planned and systemic analysis of factors such as gradient, flow rate, mobile phase components and ratio, pH (if ionic),

temperature, sample amounts, injection volume, type of diluent solvent, stability, and assay experimental conditions will be reached.[21]

7 Analytical Method Validation:

The definition of analytical method validation is "establishing a documented proof, it offers a high level of confidence that a particular procedure will reliably yield the intended outcome at its predetermined specifications and quality attributes. ICH Q2 (R1) It is essentially the process of proving that analytical methods support the identification, potency, quality, and purity of the medication ingredients and medicinal items and are suitable for their intended use. Method validation is required when new techniques are developed or when tried-and-true techniques are applied in many labs and by various analysts.[20]

ICH METHOD VALIDATION PARAMETERS:

The validation parameters as per ICH guidelines are described below:

1. Accuracy
2. Precision
3. Specificity/Selectivity
4. Limit of Detection (LOD)
5. Limit of Quantitation (LOQ)
6. Robustness
7. Linearity
8. Forced degradation studies
9. System suitability testing

1 Accuracy:

The degree to which the value and found and the value recognized as either a traditional actual value or a suitable benchmark value is a measure of an analytical method's accuracy. Since almost no measurement procedure is perfect, it is impossible to ascertain the precise value in any given measurement.[22]

2 Precision:

One way to define the precision of an analytical method is the degree of agreement between a set of measurements acquired from multiple sampling of the same standardized sample under the specified conditions. should be investigated using reliable, authentic samples.[23]

Standard deviation, relative standard deviation, and confidence intervals are commonly used to convey precision data. RSD should be less than 2% to guaranty the method's accuracy for key analytes. RSD of 5–10% is often acceptable for low-level contaminants.[21]

3 Specificity/Selectivity:

Specificity refers to the absence of any interference during the analyte compound's retention period in a blank injection.[20]

Selectivity is the ability of an analytical technique to accurately detect an analyte in the presence of interference, such as synthetic precursors, excipients, enantiomers, and known (or likely) degradation products that would be expected to be present in the sample matrix.[16]

The term "selectivity" is typically more applicable because comparatively few apps. (ICH Q2)

a) Identification tests: To verify the identity of an analyte.

b) Purity tests: To ensure that all analytical procedures —such as testing for heavy metals and associated substances—allow a correct declaration of an analyte's impurity concentration.

c) Assay: to deliver an accurate result that permits an accurate evaluation of the concentration or potency of an analyte in a sample?[16]

4 Linearity:

The capacity of a procedure to produce test findings that are exactly proportionate Linearity refers to the sample concentration over a given range. The linear relationship between sample concentration and detector response (peak area and height) is established for HPLC techniques. Using the suggested methods, the relationship can be directly shown on the drug material by diluting a reference stock or by weighing each part of the sample separately.[21]

5 Limit of Detection (LOD):

Examining samples whose analyte concentrations are known and figuring out how low the analyte can be

consistently detected yield the LOD. The detection limit can be justified with the assistance of the pertinent chromatograms.[22]

The formula for calculating the signal-to-noise ratio is s = H/h. where H is the associated peak's height. h is the largest noise fluctuation from the baseline in the chromatogram of a blank solution.[16]

6 Limit of Quantification (LOQ):

The quantitation limit of a specific analytical procedure is the lowest analyte concentration in the sample that can be quantitatively determined with suitable precision and accuracy..[22]

LOQ is typically calculated using the S/N ratio (10:1) and verified by injecting standards that provide this S/N ratio together with an appropriate percent relative standard deviation.[16]

The limit of quantification is the lowest concentration of analyte in a sample that, under the method's specified operating circumstances, can be quantitatively determined to be measurable with a tolerable degree of accuracy and precision or LOQ. LOQ can change depending on the sample's characteristics and the method used. It is typically employed to identify contaminants or degradation products.[23]

7 Robustness:

An analytical procedure's robustness is a measure of its resilience to minor but deliberate modifications in technique parameters and gives an indicator of its dependability under typical use. It is assessed in part while the technique involves being developed. The objective is to identify the critical operational factors for the method's successful implementation of the robustness study. A precautionary remark should be put in the method documentation and these parameters should be sufficiently managed.

Robustness studies for HPLC methods entail varying method parameters within a appropriate range, such as pH, flow rate, column temperature, and mobile phase composition. To identify the parameter that has a major impact on the approach, the system suitability parameters acquired for each situation are examined.[21]

8 Forced degradation studies:

Stress studies or forced degradation are used to purposefully deteriorate the sample. By producing possible degradation products, these investigations assess an analytical method's capacity to measure an active chemical and the byproducts of its deterioration without interference. Drug substances are subjected to heat, light, acid, base, and oxidizing agents during method validation, resulting in a 10% to 30% deterioration of the active molecule.[22]

9 System Suitability Test (SST):

Tests for system appropriateness are created and applied to improve the capacity to detect subpar performance and confirm that the analytical system and the measurement procedures associated with the analytical method are appropriate for the intended purpose (ICH Q14).

A chromatogram trace comparison with a standard is the most basic type of HPLC system suitability test.[23]

DRUG PROFILE:

NAME: DAPAGLIFLOZIN

DESCRIPTION: It belongs to a class of medications called sodium-glucose co-transporter 2 (SGLT2) inhibitors. It blocks a protein in the kidneys (SGLT2) that normally reabsorbs sugar back into the bloodstream. It forces the kidneys to remove excess sugar and fluid through urine, which lowers blood sugar and reduces strain on the heart and kidneys.

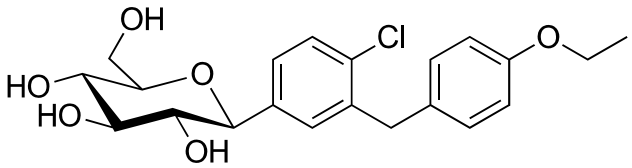


Figure 2: Structure of Dapagliflozin

IUPAC NAME: (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-6-(hydroxymethyl)oxane-3,4,5-triol

CHEMICAL FORMULA: $C_{21}H_{25}ClO_6$

MOLECULAR MASS: 408.88 g·mol−1

MECHANISM OF ACTION: Dapagliflozin inhibits the sodium-glucose cotransporter 2 (SGLT2), which is

mostly found in the nephron's proximal tubule. Since SGLT2 is responsible for 90% of the kidneys' reabsorption of glucose, its inhibition permits the excretion of glucose in the urine. For those with type 2 diabetes mellitus, this excretion enables improved glycemic management and possibly weight loss.

METABOLISM: The uridine diphosphate-glucuronosyltransferase-1A9 (UGT1A9) enzyme conjugates dapagliflozin extensively into the inactive metabolite dapagliflozin 3-O-glucuronide. Enzymes called cytochrome P450 (CYP) are barely involved in its removal. The medication is primarily eliminated by renal excretion and has a plasma half-life of roughly 13 hours.

NAME: PIOGLITAZONE

DESCRIPTION: Pioglitazone is an oral antidiabetic drug belonging to the thiazolidinedione (TZD) class. It is used as an adjunct to diet and exercise for the management of type 2 diabetes mellitus. Pioglitazone improves insulin sensitivity by activating peroxisome proliferator-activated receptor-gamma (PPAR-γ), thereby enhancing insulin-mediated glucose utilization and reducing insulin resistance.

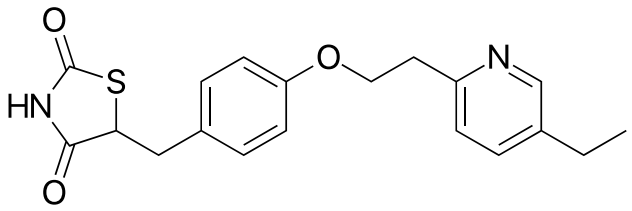


Figure 3: Structure of Pioglitazone

IUPAC NAME: (RS)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione

CHEMICAL FOEMULA: $C_{19}H_{20}N_2O_3S$

MOLECULAR MASS: 356.44 $g \cdot mol^{-1}$

MECHANISM OF ACTION: In target tissues for insulin action, such as the liver, skeletal muscle, and adipose tissue, pioglitazone selectively activates peroxisome proliferator-activated receptor-gamma (PPARγ). PPARγ activation increases the transcription of insulin-responsive genes that control glucose and fat synthesis, transport, and utilization. Because pioglitazone both increases tissue sensitivity to insulin and decreases the hepatic production of glucose, it addresses insulin resistance associated with type 2 diabetes mellitus without increasing insulin release by pancreatic beta cells. (i.e., gluconeogenesis).

METABOLISM: Pioglitazone is an oral thiazolidinedione antidiabetic medication that is extensively metabolized in the liver, mostly via the cytochrome P450 (CYP450) enzyme system.

Table 1: Different Analytical Techniques of Anti-Diabetic Drugs.

Sr. No.	Drug Name	Analytical Techniques	Description of Techniques	Author &Year
1.	Dapagliflozin	RP-HPLC	Column: Shimadzu C18 (150 × 4.6 mm, 5 µm). Mobile phase: Buffer: Acetonitrile (65:35 v/v), buffer pH 3.8. Flow rate: 0.8 mL/min. Detection: 205 nm. Injection volume: 10 µL. Retention time: 6.986 min. Linearity: r^2: 0.99985 for standard and 0.99947 for test solution. Assay recovery: 99.10–101.71%.	Chaudhary A, Saini R, Patil S, 2025

Sr. No.	Drug Name	Analytical Techniques	Description of Techniques	Author &Year
2.	Dapagliflozin	Bioanalytical RP-HPLC	Column: Kromasil C18 (250 × 4.6 mm, 5 µm). Mobile phase: Acetonitrile:0.1% Orthophosphoric acid (50:50 v/v). Flow rate: 1.0 mL/min. Detection:224 nm. Run time: 15 min. Retention time: Dapagliflozin 4.6 min Linearity: 1.50–60 µg/mL. r^2: 0.9994. LOQ: 1.50 µg/mL.	Dighe PR, Kumbhare MR, 2025
3.	Dapagliflozin	RP-HPLC	Column: Kromasil 100-5-C8 (100 × 4.6 mm). Mobile phase: Acetonitrile:Water (52:48 v/v). Flow rate: 1.0 mL/min Wavelength: 224 nm. Retention time: 1.67 min. Forced degradation: acid, alkaline, oxidative, thermal, neutral and photolytic conditions.	Chaudhari U, Sahu JK, Dande PR, 2023
4.	Dapagliflozin	RP-HPLC	Column: Inspire column (4.6 × 150 mm, 5 µm). Mobile phase: methanol:water (80:20, v/v). Flow rate: 1 mL/min. Detection: 235 nm. Retention time: 4.422 min. Linearity: 50–90 µg/mL	Gaikwad AV, Gawade AS, Huppa-rage VB, et al. 2022
5.	Dapagliflozin	RP-HPLC	Column: Kromasil C18 column mobile phase:methanol:water (75:25, v/v) Flow rate: 1 mL/min. UV detection: 223 nm. Retention time: 5.27 min.	Borse LB, Wagh MS, Borse SL, et al. 2022
6.	Dapagliflozin	RP-HPLC -stability indicating	Column ZORBAX C18 (250 × 4.6 mm, 5 µm). Mobile phase: phosphate buffer:acetonitrile:methanol (55:40:05, v/v/v). Flow rate: 1 mL/min. Detection: 225 nm. Retention time: 2.12 ± 0.05 min. Linearity: $r^2 = 0.9997$.	Murugesan A, Annapurna MM. 2022
7.	Dapagliflozin	RP-HPLC	Column: Hypersil BDS, 250 × 4.6 mm, 5 µm. Mobile phase: 0.1% orthophosphoric acid buffer:acetonitrile (50:50, v/v). Flow rate: 1 mL/min. Detection: 245 nm. Injection volume: 10 µL. Linearity: 25–150 µg/mL. Retention time: 2.226 min. Recovery: 100.12%.	Jeyabaskaran M, Rambabu C, Dhanalakshmi B. 2013
8.	Pioglitazone HCL	RP-HPLC	Column: Thermo Hypersil BDS C18 (250 × 4.6 mm, 5 µm) Mobile phase: Ethanol:phosphate buffer pH 4.0 (50:50 v/v) Flow rate: 0.7 mL/min Detection: 269 nm Linearity: Pioglitazone 25.67–77.03 µg/mL LOD: 3.78 µg/mL LOQ: 11.47 µg/mL	Tahani Y. A. Alanazi et al., 2026

Sr. No.	Drug Name	Analytical Techniques	Description of Techniques	Author &Year
9.	Pioglitazone HCL	RP-UPLC	Column: C18 BEH (50 × 2.1 mm, 1.7 µm) Mobile phase: Phosphate buffer pH 6 and acetonitrile (55:45 v/v) Flow rate: 0.4 mL/min Detection: 225 nm Injection volume: 2 µL Retention time: 1 min	Bilgiç Karaağaç et al., 2025
10.	Pioglitazone HCL	TLC-Densitometry / RP-HPLC	TLC: Silica gel plate mobile phase methanol:toluene:ethyl acetate:triethylamine (1:7:2:0.1 v/v/v/v) detection 268 nm. RP-HPLC:Column: Shimpack C18 (250 × 4.6 mm, 5 µm); Mobile phase : acetonitrile:sodium acetate buffer pH 3.6 (60:40 v/v) flow rate 1 mL/min; detection 235 nm run time 10 min.	Ashok H. Akabari et al., 2024/2025
11.	Pioglitazone HCL	RP-HPLC	Column: Hypersil BDS C18 (250 × 4.6 mm, 5 µm) Mobile phase: 0.01 M potassium dihydrogen phosphate:acetonitrile (40:60 v/v) Flow rate: 1.0 mL/min Detection: 225 nm Retention time: 4.726 min Linearity: 80–320 µg/mL r^2: 0.999.	Pramila T. Alka Agarwal, Maya Sharma, 2020
12.	Pioglitazone HCL	LC-MS/MS	Column: YMC Pro C18 (100 × 4.6 mm, 3 µm) Mobile phase: 0.1% formic acid:acetonitrile (5:95) Flow rate: 0.7 mL/min Injection volume: 10 µL Internal standard: Rosiglitazone Detection: Triple-quadrupole MS/MS, ESI positive mode Linearity: 1–500 ng/mL LOD: 0.5 ng/mL LLOQ: 1 ng/mL	Kusuma Kumari G. et al., 2021
13.	Pioglitazone HCL	HPLC	Column: Macherey-Nagel C18 (250 × 4.6 mm, 5 µm) Mobile phase: Acetonitrile:0.1 M ammonium acetate:glacial acetic acid (25:25:1 v/v/v) Flow rate: 1.2 mL/min Detection: 269 nm Linearity: 0.1–2.0 µg/mL r^2: 0.9998 LOD: 0.1 µg/mL	Syed Imran Ali et al., 2018

Sr. No.	Drug Name	Analytical Techniques	Description of Techniques	Author &Year
14.	Dapagliflozin + Pioglitazone	HPTLC	Stationary phase: Silica gel 60 F254. Mobile phase: Toluene:ethyl acetate:methanol:glacial acetic acid (5:3:1:0.5 v/v/v/v). Detection wavelength: 280 nm. Rf: Dapagliflozin 0.37 and Pioglitazone 0.73. Linearity: R^2 0.9994 and 0.9993 for Dapagliflozin and Pioglitazone, respectively. Accuracy: 98–100% recovery. Precision: %RSD <2%.	Tathe SV, Gosavi A, Bohara P., 2026
15.	Dapagliflozin + Pioglitazone	UPLC	Column: Acquity BEH C18 (2.1 × 100 mm, 1.7 µm). Column temperature: 45°C. Mobile phase: Ethanol:9 mM ammonium formate buffer (43.7:56.3). Flow rate: 0.246 mL/min. Linearity: 1–50 µg/mL for both drugs. R^2: 0.9997 for Pioglitazone and 0.9998 for Dapagliflozin. Detection: 223 nm LOD: PIO 1.2, DAPA 0.9 µg/mL; LOQ: PIO 3.5, DAPA 2.6 µg/mL; RT: 4.62 and 6.21 min	Elzayat EM, Sherif AY, Attwa MW, Altamimi MA., 2024
16.	Dapagliflozin + Pioglitazone	RP-HPLC	Column: Kromstar Vertex C18 analytical column. Mobile phase: Acetonitrile:potassium dihydrogen phosphate buffer (pH 4) (25:75 v/v). Flow rate: 1 mL/min. Detection: 228 nm. Retention time: approximately 3–3.5 min for Dapagliflozin and 6–6.5 min for Pioglitazone HCl. Linearity: Dapagliflozin 2–10 µg/mL; Pioglitazone HCl 3–15 µg/mL. LOD: 0.041 µg/mL and 0.105 µg/mL, respectively. LOQ: 0.13 µg/mL and approximately 0.32–0.34 µg/mL, respectively	Patel T, Parikh R., 2022

CONCLUSION:

The stability and expiry assessment of dapagliflozin and pioglitazone tablet formulations is essential for ensuring their quality, safety, efficacy, and potency throughout their intended shelf life. Different analytical techniques, including UV spectroscopy, FTIR spectroscopy, and particularly stability-indicating RP-HPLC, UPLC can provide reliable information regarding drug identification, assay, purity, and possible degradation. Evaluation of marketed formulations at or around their expiry period can help determine changes in drug content and other critical quality attributes under real storage conditions. A validated analytical method with suitable parameters such as resilience, linearity, LOD, LOQ, accuracy, precision, and specificity is important for obtaining accurate and reproducible results. Overall, stability studies of dapagliflozin and pioglitazone formulations can support the assessment of pharmaceutical quality and provide a deeper comprehension of the significance of expiry dates and appropriate storage conditions.

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