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# Development of a Stability-Indicating RP-HPLC Method for Separation and impurity Profiling of Dapagliflozin and Teneligliptin in Fixed-Dose Combination Tablets

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**Abstract**: *This study aims to develop and validate a simple, accurate, and reliable RP-HPLC method for detecting and quantifying impurities in an anti-diabetic tablet formulation containing dapagliflozin and teneligliptin. The main objective is to separate all impurity peaks clearly from the peaks of both active drugs so that they can be measured accurately during quality-control testing.*

*Key chromatographic conditions, such as mobile-phase composition, pH, flow rate, and detection wavelength, will be optimised using an experimental design approach. The method will be adjusted to give good separation between peaks, proper peak shape, suitable sensitivity, and a reasonable analysis time. Once optimised, the method will be validated for specificity, linearity, accuracy, precision, limit of detection, limit of quantification, and robustness.*

*Blank and placebo samples will be analysed to make sure that excipients or other formulation components do not interfere with the analysis. Recovery studies will be carried out to check the accuracy of the method, while repeatability studies will confirm that it gives consistent results. Small deliberate changes in method conditions will also be studied to assess robustness.*

*Overall, this study will provide a dependable RP-HPLC method for impurity profiling and routine quality control of dapagliflozin and teneligliptin combination tablets*

**Keywords**: Dapagliflozin; Teneligliptin; Anti-diabetic formulation; RP-HPLC; Impurity profiling; Method validation; Experimental design; Quality control.

## I. INTRODUCTION

Impurity profiling is an important part of pharmaceutical analysis that involves the identification, quantification, and structural characterization of impurities present in pharmaceutical substances and drug products. Various analytical techniques, such as spectroscopy and chromatography, are used to detect, measure, and characterize these impurities. The quality of a pharmaceutical product depends not only on the amount of active pharmaceutical ingredient (API) present but also on its purity, formulation, manufacturing process, and storage conditions.

Dapagliflozin and teneligliptin are used together in fixed-dose combination tablets for type 2 diabetes. During storage or manufacturing, medicines may form impurities or degradation products due to heat, moisture, light, oxygen, acid, or alkali.

The purpose of this project is to develop an RP-HPLC method that can separate the two active drugs from each other, from tablet excipients, and from degradation products. A stability-indicating method is important because it proves that the analytical method can detect the intact drugs even when degradation products are present.

Type 2 diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels resulting from insulin resistance and progressive impairment of insulin secretion.

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Effective long-term management often requires combination therapy, as using two medicines with complementary mechanisms can provide better glycemic control than a single drug alone.

This project will provide a reliable analytical method for routine quality-control analysis of dapagliflozin and teneligliptin fixed-dose combination tablets. It will also help in understanding the degradation of both drugs and support the assurance of their safety, efficacy, and quality throughout their shelf life.

## II. MATERIALS AND METHODS

### Materials

**1. Drug Sample**

Marketed tablets containing dapagliflozin and teneligliptin will be used for the study. Before starting the analysis, details such as the brand name, manufacturer, batch number, labelled strength, manufacturing date, and expiry date will be recorded. The tablets will be stored according to the manufacturer's recommendations until they are analysed.

**2. Reference Standards**

Reference standards of dapagliflozin and teneligliptin will be used to compare the analytical results with those obtained from the marketed tablet sample. If specific impurities are to be identified or quantified, suitable impurity reference standards may also be required.

**3. Chemicals and Reagents**

Suitable chemicals and reagents will be selected according to the analytical method used. These may include methanol, acetonitrile, purified water, and appropriate buffer solutions. For forced degradation studies, hydrochloric acid, sodium hydroxide, and hydrogen peroxide may be used to investigate the behaviour of the drugs under different stress conditions.

The grade and purity of each chemical will be selected according to the requirements of the analytical procedure.

**4. Instruments and Laboratory Equipment**

The study may require a UV–Visible spectrophotometer for preliminary drug analysis. If available, an HPLC system equipped with a UV or photodiode-array detector may be used to separate the active ingredients from their related impurities.

Other laboratory equipment may include an analytical balance, sonicator, pH meter, volumetric flasks, pipettes, beakers, and suitable filtration equipment. These instruments will help in accurately weighing the sample, preparing solutions, extracting the drugs, and carrying out the analysis.

**5. Other Laboratory Materials**

Additional materials, such as a mortar and pestle, sample containers, reagent bottles, and clean glassware, will be used during sample preparation and analysis. Appropriate personal protective equipment, including laboratory gloves and safety glasses, will also be used to ensure safe laboratory practices.

6. Selection of Materials

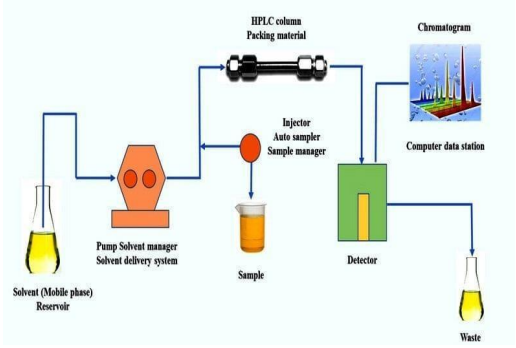
The materials will be selected according to the objectives of the study and the analytical facilities available in the laboratory. Suitable reference standards and properly selected reagents are important for obtaining reliable results.

## III. METHODOLOGY

**1. Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)**

RP-HPLC is a widely used analytical technique for separating dapagliflozin, teneligliptin, and their related impurities. It uses a nonpolar stationary phase and a suitable mobile phase to separate the components of a pharmaceutical formulation. It can be used for impurity estimation and stability studies when the method is appropriately developed and validated.

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[Fig. 1 Schematic Representation of RP-HPLC] [This image belongs to Research gate]

**2. Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS)**

LC-MS/MS combines chromatographic separation with mass spectrometric detection. It is used to investigate unknown impurities and degradation products by providing information about their molecular masses and fragmentation patterns.

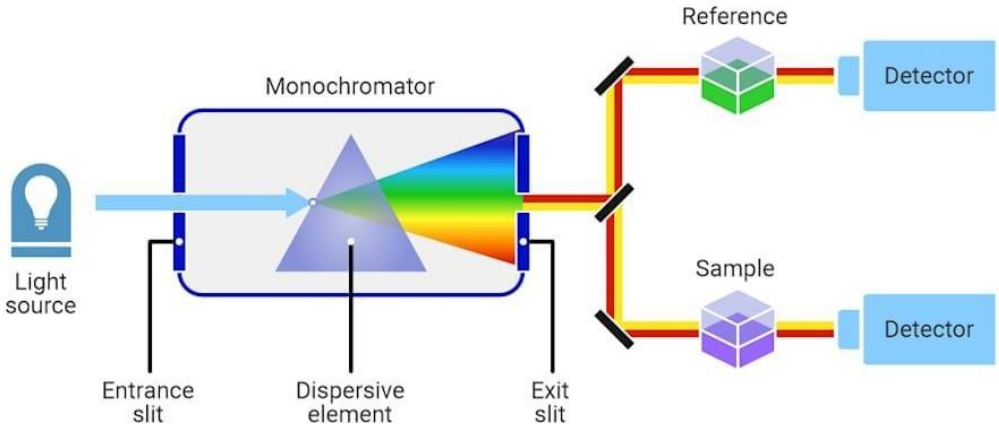
**3. High-Performance Thin-Layer Chromatography (HPTLC)**

HPTLC separates the components of a pharmaceutical sample on a coated plate. It can be used for preliminary impurity screening and quantitative analysis when an appropriate method has been developed and validated.

**4. UV–Visible Spectrophotometry**

UV–Visible spectrophotometry measures the absorption of ultraviolet or visible light by a substance. It can be used for preliminary analysis and estimation of drug concentration.

However, it generally cannot distinguish individual impurities in a combined tablet formulation when their absorption spectra overlap.




[This image belongs to Research gate]

**5. Forced Degradation Studies**

Forced degradation studies evaluate the behaviour of pharmaceutical substances under different stress conditions, including acidic, alkaline, oxidative, thermal, and photolytic conditions. These studies help investigate possible degradation products and assess the stability-indicating capability of an analytical method.

**6. Analytical Method Validation**

Analytical method validation determines whether a method is suitable for its intended purpose. Important parameters include specificity, accuracy, precision, linearity, range, limit of detection (LOD), limit of quantification (LOQ), and robustness, as applicable to the method.

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**7. Stability Studies**

Stability studies evaluate changes in the quality of a pharmaceutical formulation under specified storage conditions over time. They can help determine whether the drug content, impurity levels, and other quality attributes change during storage. Suitable validated analytical methods are required to monitor impurities reliably.

8. Impurity Identification and Characterization

Impurity identification involves investigating the chemical identity and possible origin of impurities detected in a pharmaceutical formulation. Techniques such as LC-MS/MS may provide information about molecular mass and fragmentation, while reference standards and other suitable analytical techniques may be needed for confirmation.

**9. Quantitative Impurity Analysis**

Quantitative impurity analysis determines the amount of a specified impurity present in a pharmaceutical sample. It generally requires a suitable analytical method, appropriate reference standards, and adequate sensitivity and specificity.

## IV. RESULT

**• HPLC/RP-HPLC**

Separate and quantify organic impurities main impurity-profiling test.

Reversed-phase HPLC—commonly C8/C18 columns with water–organic mobile phases and UV/DAD detection—is the routine "workhorse" method for assay and related-substances testing. DAD helps assess peak purity, while gradient elution improves separation of structurally similar impurities.

**• UPLC/UHPLC**

Detect trace impurities with better resolution and speed.

Uses sub-2 µm particles and higher backpressure, giving sharper peaks, shorter run times, lower solvent use, and better sensitivity. It is useful for complex chromatograms where many impurities must be resolved.

**• LC-MS/LC-MS/MS**

Identify unknown impurities and degradation products by molecular mass.

LC separates the impurity; MS provides molecular mass, and MS/MS provides fragment ions for structural elucidation. High-resolution MS can propose elemental formulae; LC-MS/MS can quantify impurities at ppb/ppt levels.

**• GC/GC-MS**

Detect volatile impurities and residual solvents.

MS provides mass spectral identification, while headspace GC minimizes matrix interference. It is used for Class 1, 2, and 3 residual solvents and for volatile genotoxic impurities when appropriately validated.

**• ICP-MS**

Detect elemental/metal impurities.

Highly sensitive, multi-element technique capable of detecting metals at very low concentrations. It supports ICH Q3D and USP <232>/<233> control strategies for elements such as Pb, As, Cd, Hg, and catalyst residues.

**• NMR spectroscopy**

Confirm the chemical structure of isolated impurities .

Provides information on hydrogen/carbon environments, connectivity, stereochemistry, and sometimes conformation. It is especially valuable after preparative HPLC isolation of an unknown impurity.

**• FTIR spectroscopy**

Identify functional groups in impurities.

Detects characteristic bonds such as C=O, O–H, N–H, C–N, and aromatic groups. It is rapid and useful for confirming whether an isolated impurity has expected functional groups, but it rarely establishes complete structure alone.

**• TLC/HPTLC**

Quick screening and separation of impurities.

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Low-cost, simple technique for comparing batches, monitoring reactions, and detecting gross impurities. HPTLC offers better reproducibility, densitometric quantification, and parallel analysis of many samples, but has lower sensitivity and resolution than HPLC/UPLC.

**• Capillary electrophoresis (CE/CE-MS)**

Separate closely related or charged impurities.

Stability and safety tests

**• Forced degradation study**

Produce degradation impurities under acid, base, oxidation, heat, humidity and light. Forced degradation, also called stress testing, deliberately exposes the drug substance or product to harsher-than-normal conditions to generate likely degradation products. It helps establish degradation pathways, intrinsic stability, and whether the analytical method is truly stability-indicating—that is, able to measure the API and degradants without interference.

**• Accelerated stability study**

Check impurity information during storage. Accelerated stability studies evaluate the product under more stressful but controlled storage conditions to predict its behaviour during the proposed shelf life. They provide early evidence of physical and chemical stability and help identify impurities that form during storage

**• Photostability testing**

Detect impurities formed by light exposure. Photostability testing determines whether exposure to light causes chemical degradation, colour change, or loss of potency. ICH Q1B divides it into forced degradation testing and confirmatory testing; the latter uses standardized light exposure to decide whether light-resistant packaging or a "protect from light" label is needed

**• Ames test**

check whether an impurity is mutagenic result is positive or negative. The Ames test is a bacterial reverse-mutation assay used to determine whether an impurity can cause point mutations in DNA. It is the principal in-vitro screen for mutagenic potential and is conducted under ICH S2(R1) and OECD Test Guideline 471 principles

## V. DISCUSSION

The fixed-dose combination of dapagliflozin and teneligliptin is widely used for the management of type 2 diabetes mellitus, as both drugs act through complementary mechanisms to improve glycaemic control. Since both active ingredients are present in a single tablet, a reliable analytical method is required to quantify them accurately and to ensure that impurities or degradation products do not affect the quality, safety, and efficacy of the formulation.

The proposed work aims to develop and validate a stability-indicating RP-HPLC method for the simultaneous estimation of dapagliflozin and teneligliptin in tablets. Chromatographic conditions such as column type, mobile phase, pH, flow rate, and detection wavelength will be optimised to achieve good resolution, symmetrical peaks, and short analysis time. The method will then be subjected to forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions to confirm that degradation products do not interfere with the analyte peaks.

The developed method will be validated according to ICH guidelines for parameters including specificity, linearity, accuracy, precision, robustness, LOD, LOQ, and system suitability. Impurity profiling may also be carried out to identify major degradation products and understand possible degradation pathways. Overall, the study will provide a simple, precise, and robust analytical method suitable for routine quality control and stability assessment of dapagliflozin–teneligliptin fixed-dose combination tablets.

## VI. CONCULSION

This project will develop a simple, accurate, and stability-indicating RP-HPLC method for the separation and impurity profiling of Dapagliflozin and Teneligliptin in fixed-dose combination tablets. The method will separate both drugs from each other as well as from their degradation products and related impurities.

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Forced degradation studies will be carried out under acidic, alkaline, oxidative, thermal, and photolytic conditions to check whether the method can detect impurities formed during storage. The developed method will be validated as per ICH guidelines for specificity, linearity, accuracy, precision, LOD, LOQ, and robustness.

The results will prove that the proposed RP-HPLC method is suitable for routine analysis of Dapagliflozin and Teneligliptin tablets. This method will be helpful in quality-control laboratories and pharmaceutical industries for checking the purity, stability, and safety of the fixed-dose combination formulation.

## VII. FUTURE PERSEPECTIVES

The developed stability-indicating RP-HPLC method for Dapagliflozin and Teneligliptin in fixed-dose combination tablets has good potential for further use and improvement. The present work gives a reliable way to separate and estimate both drugs, but in the future it can be extended for more detailed quality control, stability, and impurity studies.

In the future, this method can be used for routine quality control analysis in pharmaceutical industries. Once it is completely validated, it can help manufacturers check the assay, purity, and content uniformity of Dapagliflozin–Teneligliptin tablets before the product is released into the market. This will help maintain consistent product quality and ensure patient safety.

The method can also be applied for long-term and accelerated stability studies. By keeping the tablets under different temperature and humidity conditions, researchers can observe how the drug combination behaves over time. This will help in deciding the proper storage conditions and shelf life of the formulation.

Further studies can be carried out to identify and quantify the impurities and degradation products of both drugs. The present method can be improved to separate even very small amounts of impurities. This will help in understanding how the drugs degrade and may support the development of a more stable formulation.

In future work, the HPLC method can be combined with advanced techniques such as LC-MS/MS. This will help in identifying the exact structure of unknown degradation products and impurities. Such information can be useful for safety evaluation, regulatory documentation, and improving the manufacturing process.

There is also scope to make the method faster and more economical. By optimizing the mobile phase, flow rate, column, and detection wavelength, the analysis time can be reduced and the resolution can be improved. Reducing the use of organic solvents can also make the method more environmentally friendly and cost-effective.

With slight modification and validation, the method may also be applied to other formulations containing Dapagliflozin and Teneligliptin, such as tablets of different strengths or new fixed-dose combinations. This can make the method more useful and widely applicable.

After proper validation according to ICH guidelines, the method can support regulatory work, stability testing, and quality assessment of the formulation. Overall, the present research can act as a strong base for future analytical studies and can help in ensuring the quality, safety, and stability of Dapagliflozin–Teneligliptin combination products.

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