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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND METFORMIN IN BULK AND IN MARKETED FORMULATION

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ABSTRACT

The simultaneous determination of Metformin and Dapagliflozin in synthetic mixtures was successfully achieved through the development of a validated, precise, and simple reverse-phase HPLC method. The therapeutic combination of **Dapagliflozin** and **Metformin Hydrochloride** hasbecome a cornerstone in the management of Type 2 Diabetes Mellitus, as it simultaneously enhances insulin sensitivity and facilitates glucose clearance via renal pathways. Because of this pharmacological synergy, establishing rigorous and dependable analytical frameworks is vital for the concurrent measurement of these drugs in both clinical samples and manufactured tablets. This assessment explores a variety of established analytical techniques used to quantify Dapagliflozin and Metformin, whether they are analyzed individually or as a fixed-dose combination. In the landscape of pharmaceutical analysis, **Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)** is the most widely adopted methodology for this specific drug pair. Its popularity stems from its ability to provide high resolution and reproducible results for molecules with divergent chemical properties. This review offers a detailed summary of the analytical techniques that are currently available and highlights the significance of method validation in achieving accurate and consistent results for the simultaneous measurement of dapagliflozin and metformin hydrochloride.

KEYWORDS: Dapagliflozin, Metformin hydrochloride, RP-HPLC, Diabetes mellitus.

INTRODUCTION

Analytical Method Development

Analytical methods are designed to evaluate the strength, purity, and physical properties of pharmaceutical products, as well as how they remain stable and bioavailable over time. The processes of development and validation serve as documented evidence that a specific procedure is reliable for testing drugs and their core Active Pharmaceutical Ingredients.

Method Validation

The goal of method validation is to provide documented evidence that an analytical technique remains reliable and consistent for its intended application over time. Through a standardized set of procedures, the performance attributes of the method are rigorously tested and verified Method validation comprises the following components.

- Linearity
- Accuracy
- Precision
- Limit of Detection and Limit of Quantification
- System Suitability parameters
- Robustness
- Specificity

DRUG PROFILE: Dapaglifazone

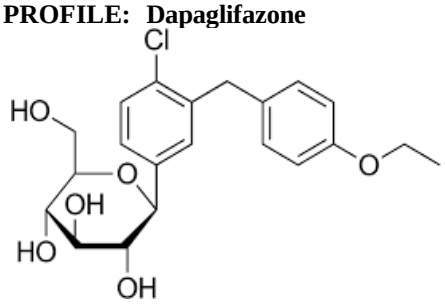


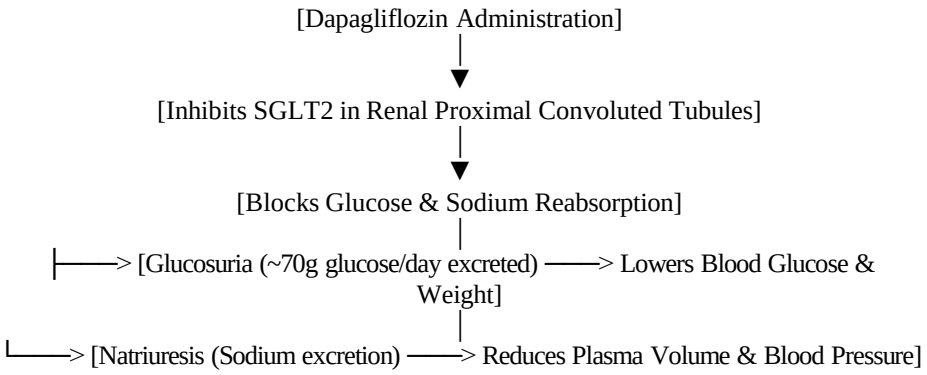
Figure1: Dapaglifazone.

Core Profile & Nomenclature

- **Generic Name:** Dapagliflozin
- **Common Brand Names:** Farxiga (US), Forxiga (EU/India), Oxra
- **Therapeutic Class:** Antidiabetic agent; SGLT2 Inhibitor
- **ATC Code:** A10BK01 (Monotherapy), A10BD15 (Combined with Metformin)
- **Chemical Formula:** C21H25ClO6 (often formulated as a propanediol monohydrate salt)
- **Molecular Weight:** 408.87 g/mol (Base)

Mechanisms of Action (MOA)

Dapagliflozin functions independently of insulin secretion and pancreatic beta-cell function through a distinct, insulin-independent mechanism.



DRUG PROFILE: Metformin

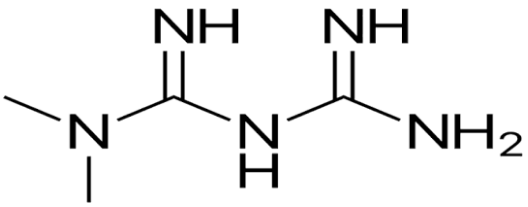


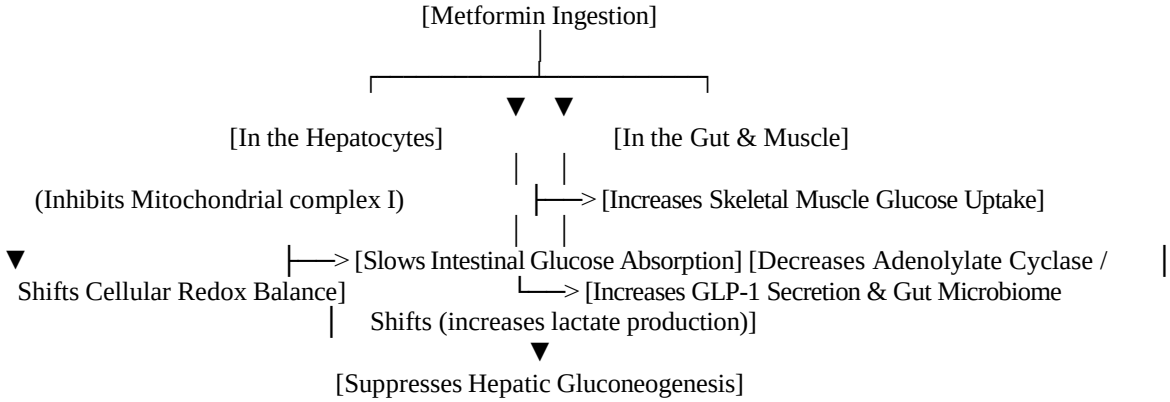
Figure2: Metformin.

Core Profile & Nomenclature

- **Generic Name:** Metformin Hydrochloride
- **Common Brand Names:** Glucophage, Glumetza, Fortamet, Glycomet
- **Therapeutic Class:** Antidiabetic Agent; Biguanide
- **ATC Code:** A10BA02
- **Chemical Formula:** C4H11N5HCl
- **Molecular Weight:** 165.63 g/mol

Mechanisms of Action (MOA)

Metformin is an insulin-sensitizing agent. Rather than stimulating insulin secretion from pancreatic beta cells, it optimizes how the body handles existing insulin through several pathways.



MATERIALS AND METHODS

Preparation of Dapagliflozin Stock Reference Solution

An accurately weighed mass of 10 mg of Dapagliflozin was transferred into a 10 mL volumetric flask. Approximately 75% of the total volume was filled with the designated diluent, and the mixture was subjected to sonication for 10 minutes to ensure complete dissolution. The flask was subsequently diluted to the calibration mark with the same diluent to yield a primary stock solution with a final concentration of 1000g/mL.

Preparation of Metformin Stock Reference Solution

An accurately weighed mass of 10 mg of Metformin was transferred into a separate 10 mL volumetric flask. The matrix was dissolved by adding three-quarters of the total volume of diluent, followed by sonication for 10 minutes. The solution was then brought to volume with the diluent to prepare a primary stock solution with a final concentration of 1000g/mL.

Formulation of Standard Working Solutions

- **Dapagliflozin:** A 0.02 mL aliquot of the primary Dapagliflozin stock solution was precisely transferred into a 10 mL volumetric flask using a micropipette. The volume was adjusted to the mark with the diluent to achieve a working concentration of 0.2 g/mL.
- **Metformin:** A 0.1 mL aliquot of the Metformin stock solution was pipetted into a 10 mL volumetric flask and diluted to volume with the diluent, producing a final working concentration of 10 g/mL.

Preparation of Mixed Standard Working Solution

To replicate the active pharmaceutical ingredient (API) ratio found in the commercial formulation, 1 mL of the 0.2 ppm Dapagliflozin working solution and 1 mL of the 10 ppm Metformin HCl working solution were combined in a 10 mL volumetric flask.

After adding approximately 75% of the diluent volume, the mixture was sonicated for 10 minutes and then diluted to the mark. This yielded a combined standard solution containing 0.2 ppm of Dapagliflozin and 10 ppm of Metformin.

Construction of Calibration Curve Serial Dilutions

- **Metformin HCl:** A secondary stock solution of 100 ppm was prepared by transferring 0.1 mL of the primary stock into a 100 mL volumetric flask, adding diluent, agitating thoroughly, and bringing to volume with water. From this 100 ppm intermediate solution, precise aliquots of 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mL were transferred into individual 10 mL volumetric flasks. The final volume of each was adjusted to the mark with diluent to generate a linear calibration range of 2, 3, 4, 5, 6, and 7 ppm, respectively.
- **Dapagliflozin:** Volumetric aliquots of 0.6, 0.9, 1.0, 1.2, 1.5, 1.8, and 2.1 mL were taken directly from the 1000 ppm primary stock solution and transferred into separate 10 mL volumetric flasks. Dilution to the mark with the diluent produced a calibration series of 60, 90, 120, 150, 180, and 210 ppm, respectively.

Preparation of Accuracy Recovery Samples (Spiking Method)

- **Metformin HCl:** Recovery studies were performed by spiking the baseline sample solution (containing 0.2 ppm Dapagliflozin and 10 ppm Metformin HCl) with varying concentrations of Metformin standard. The 50%, 100%, and 150% accuracy targets were achieved by introducing 2 ppm, 4 ppm, and 6 ppm of the Metformin reference standard, respectively.
- **Dapagliflozin:** Similarly, the baseline sample solution (0.2 ppm Dapagliflozin + 10 ppm Metformin HCl) was spiked with Dapagliflozin reference standard to evaluate analytical recovery. Targets for the 50%, 100%, and 150% accuracy levels were prepared by spiking with 60 ppm, 120 ppm, and 180 ppm of the Dapagliflozin standard, respectively.

RESULTS AND DISCUSSION

Analytical Method Validation

The developed High-Performance Liquid Chromatography (HPLC) method was rigorously validated in compliance with standard regulatory guidelines. The assessed validation parameters included system suitability, specificity, precision, linearity, accuracy, sensitivity (LOD and LOQ), and robustness.

System Suitability

To confirm that the chromatographic system was operating optimally prior to analysis, a mixed working standard solution of Dapagliflozin and Metformin Hydrochloride (HCl) was analyzed via five replicate injections. Chromatographic parameters—including peak area, retention time (Rt) tailing factor, and theoretical plate count—were evaluated.

The relative standard deviation (%RSD) for all critical parameters remained well within acceptable regulatory thresholds. The characteristic retention times were resolved at **2.178 minutes** for Metformin HCl and **3.338 minutes** for Dapagliflozin, confirming adequate baseline separation.

Specificity

The specificity of the method was evaluated by injecting a blank matrix sample. The resulting chromatograms exhibited no interfering peaks at the retention windows corresponding to either Metformin HCl or Dapagliflozin, demonstrating that the method is highly selective for the target analytes.

Precision

Method repeatability was established by performing six replicate injections of a combined working standard formulation containing 0.2 ppm Dapagliflozin and 10

ppm Metformin HCl. The calculated %RSD values for peak areas were **0.82%** for Dapagliflozin and **0.63%** for Metformin HCl. Because these values fall safely below the standard 2.0% limit, the method demonstrates excellent instrumental precision and repeatability.

Linearity

The linearity of the assay was evaluated across a concentration range corresponding to 25% to 150% of the target working strength. This encompassed concentrations of 60 ppm to 210 ppm for Dapagliflozin, and 2 ppm to 7 ppm for Metformin HCl.

Calibration curves were constructed by plotting standard concentrations against their respective peak areas. The system exhibited highly reproducible retention profiles across all calibration levels, as detailed below:

- **Metformin HCl:** Retention times remained exceptionally stable across the 25%–150% levels, recorded at 2.172, 2.174, 2.160, 2.170, 2.171, and 2.173 minutes.
- **Dapagliflozin:** The corresponding retention times across the injection levels were registered at 3.336, 3.338, 3.311, 3.330, 3.329, and 3.331 minutes.

Accuracy (Recovery Studies)

To confirm the accuracy of the proposed method, recovery studies were conducted in triplicate by spiking known concentrations of standard drug into the matrix at three distinct levels (50%, 100%, and 150%). The mean percentage recoveries for both drugs fell near the ideal 100% mark, confirming the absence of matrix interferences:

- **Metformin HCl:** Mean recoveries were determined to be 99.83% (at 50% spike), 99.79% (at 100% spike), and 99.75% (at 150% spike).
- **Dapagliflozin:** Mean recoveries were found to be 99.45% (at 50% spike), 99.79% (at 100% spike), and 99.84% (at 150% spike).

Sensitivity (LOD and LOQ)

The sensitivity of the method was determined by calculating the Limit of Detection (LOD) and Limit of Quantitation (LOQ) based on signal-to-noise ratios (S/N):

- **Dapagliflozin:** The LOD was established at 345,000 ppb (345 ppm), with a minimum quantifiable limit (LOQ) of 415,000 ppb (415 ppm).
- **Metformin HCl:** The LOD was determined to be 263,000 ppb (263 ppm), with an LOQ of 324,000 ppb (324 ppm).

Robustness

The capacity of the method to remain unaffected by small, intentional variations in operational parameters was tested by adjusting the mobile phase flow rate by $\pm 0.1 \text{ mL/min}$. The method proved resilient to these deliberate fluctuations.

- **Flow rate reduction (-0.1mL/min):** Resulted in acceptable %RSD values of 0.62% for Dapagliflozin and 0.74% for Metformin HCl.
- **Flow rate acceleration (+0.1 mL/min):** Resulted in %RSD values of 0.86% for Dapagliflozin and 0.52% for Metformin HCl.

Quantitative Assay of Marketed Formulation

The validated HPLC method was successfully applied to quantify Metformin HCl and Dapagliflozin within commercially available pharmaceutical dosage forms. Standard and sample solutions were sequentially analyzed, and their respective chromatograms were compared. By assessing relative peak areas, the percentage recovery (assay purity) of both active pharmaceutical ingredients (APIs) was calculated, confirming that the commercial formulation conformed tightly to labeled claim.

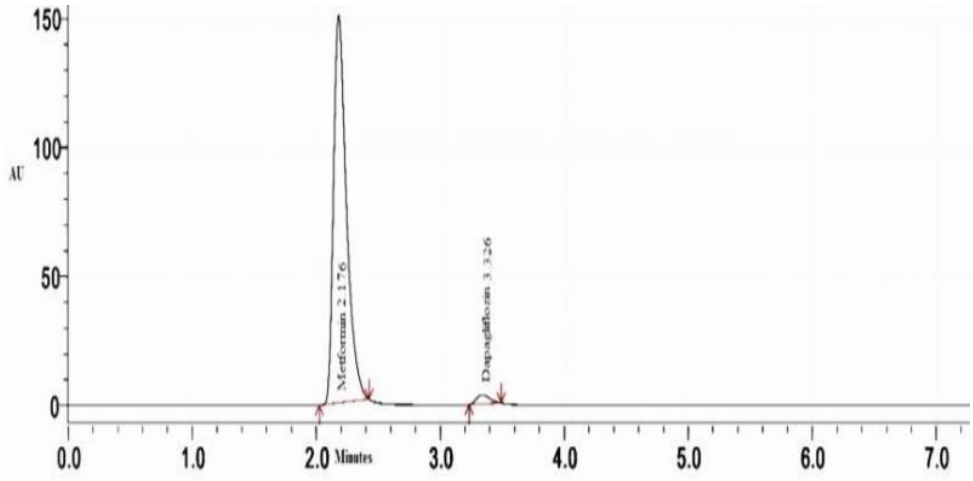


Figure 3: Assay Chromatogram of Standard.

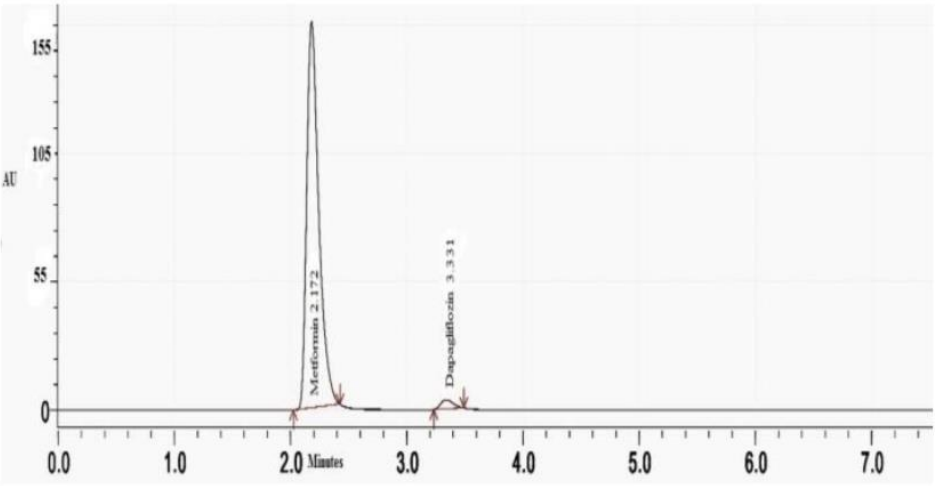


Figure 4: Assay Chromatogram of Sample.

Table 1: %Assay results.

Name of the drug	Metformin HCl	Dapagliflozin
Label claim	500mg	10mg
Assay (%)	99.58	99.41

- **Peak 1 (Metformin Hydrochloride)**
- **Standard Profile (Figure 3):** Resolves at a retention time (Rt) of **2.176 minutes** with a peak height reaching approximately 150AU
- **Sample Profile (Figure 4):** Resolves at an (Rt)of **2.172 minutes** with a peak height of approximately 155AU.
- *Observation:* The minor shift in retention time (Delta Rt = 0.004\min) is negligible and falls perfectly within standard chromatographic system suitability boundaries.
- **Peak 2 (Dapagliflozin)**
- **Standard Profile (Figure 3):** Resolves further down the column at an Rt of **3.326 minutes** with a characteristically shorter peak response (around 50AU due to its lower target concentration ratio).
- **Sample Profile (Figure 4):** Resolves at an Rt of **3.331 minutes**, matching the standard matrix footprint.

Table 2: Summary of Validation Parameters (HPLC).

Validation Parameter	Metformin HCl	Dapagliflozin	Acceptance Criteria
Retention Time (Rt)	2.178 min	3.338 min	For identification
System Precision (%RSD, n=6)	0.63%	0.82%	le 2.0%
Accuracy / % Recovery (Mean)	99.75% – 99.83%	99.45% – 99.84%	98.0% – 102.0%
LOD	263,000 ppb	345,000 ppb	For informational sensitivity
LOQ	324,000 ppb	415,000 ppb	For informational sensitivity
Robustness (%RSD at -0.1 \ mL/min)	0.74%	0.62%	le 2.0%
Robustness (%RSD at +0.1 / mL/min)	0.52%	0.86%	le 2.0%

CONCLUSION

The development and subsequent validation of the Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) method provides a reliable, precise, and highly efficient analytical framework for the simultaneous estimation of Dapagliflozin and Metformin Hydrochloride in both bulk material and commercial tablet formulations.

- **Chromatographic Efficiency:** The method successfully resolved both active ingredients with optimal baseline separation, yielding distinct retention times of **2.178 minutes** for Metformin HCl and **3.338 minutes** for Dapagliflozin.
- **Regulatory Compliance:** Rigorous validation parameters confirmed that the method aligns tightly with established pharmaceutical regulatory standards.
- **Instrumental Precision:** Repeatability testing yielded exceptionally low peak area variance, with %RSD values of **0.63%** for Metformin HCl and **0.82%** for Dapagliflozin, falling safely below the mandatory 2.0%threshold.
- **High Assay Accuracy:** Triplicate recovery studies across 50%, 100%, and 150% spiking levels demonstrated mean percentage recoveries tightly grouped between **99.75%–99.83%** for Metformin

HCl and **99.45%–99.84%** for Dapagliflozin, confirming excellent method accuracy and the absence of matrix interferences.

- **System Robustness:** The analytical procedure proved highly resilient against intentional micro-variations in the operational flow rate (0.1 mL/min) maintaining strict stability across all experimental conditions.

By utilizing a high-key specificity profile that completely eliminates baseline interference, this RP-HPLC methodology offers high resolution and reproducible results for molecules with divergent chemical properties. Given its simplicity, speed, and proven sensitivity parameters (LOD/LOQ profiles), this validated assay serves as an excellent framework for routine quality control testing, bulk chemical analysis, and commercial fixed-dose tablet characterization of this cornerstone Type 2 Diabetes Mellitus combination therapy.

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