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SIMPLE RP-HPLC METHOD DEVELOPMENT & VALIDATION FOR ESTIMATION OF DAPAGLIFLOZIN AND METFORMIN IN MARKETED FORMULATION

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Abstract

A simple, precise, accurate, and robust RP-HPLC method was developed and validated for the simultaneous estimation of Dapagliflozin (DAPA) and Metformin (MET) in pharmaceutical dosage forms, following ICH Q2(R1) guidelines. Chromatographic separation was achieved with well-resolved peaks, showing retention times of 2.05 min for MET and 9.40 min for DAPA. The method exhibited excellent linearity over the concentration range of 5–25 µg/mL for both drugs, with correlation coefficients (r^2) of 0.999. Accuracy, assessed through recovery studies, ranged from 98.24% to 99.07% for DAPA and 98.34% to 98.85% for MET, with low %RSD values, indicating trueness. Precision studies, including repeatability and intermediate precision, showed %RSD values below 0.2%, confirming high reproducibility. Robustness testing demonstrated that small deliberate changes in chromatographic conditions did not significantly affect the results. The method exhibited high sensitivity, with LOD and LOQ values of 0.25/0.75 µg/mL for DAPA and 0.35/1.05 µg/mL for MET. Application to commercial tablet formulations revealed assay values of 99.80% for DAPA and 99.85% for MET, with %RSD <0.25%. The developed RP-HPLC method is suitable for routine quality control, stability studies, and simultaneous estimation of Dapagliflozin and Metformin in combined dosage forms.

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes mellitus (T2DM) is the most prevalent form, accounting for approximately 90–95% of all diabetes cases worldwide, and is associated with significant morbidity and mortality due to complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy (American Diabetes Association,

2023; Cho et al., 2018).

Dapagliflozin, a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor, reduces renal glucose reabsorption and enhances urinary glucose excretion, providing effective glycemic control with additional benefits including weight loss and blood pressure reduction (Wilding et al., 2019; Devineni et al., 2017). Metformin, a biguanide, is the first-line therapy for T2DM due to its ability to decrease hepatic gluconeogenesis, improve peripheral insulin sensitivity, and exhibit favorable effects on cardiovascular outcomes (Rena et al., 2017; Bailey et al., 2018). The combination of dapagliflozin and metformin is commonly prescribed in clinical practice for synergistic antihyperglycemic effects and improved patient compliance (Scheen, 2015).

Accurate quantification of active pharmaceutical ingredients (APIs) in combination formulations is essential for quality control, regulatory compliance, and therapeutic efficacy. Reverse-phase high-performance liquid chromatography (RP-HPLC) is a widely employed analytical technique due to its sensitivity, precision, reproducibility, and ability to simultaneously separate and quantify multiple drugs in complex matrices (Snyder et al., 2012; Kumar et al., 2015).

Previous studies have reported individual HPLC methods for the estimation of dapagliflozin or metformin; however, simultaneous estimation in marketed combination formulations remains a challenge due to differences in polarity, solubility, and detection characteristics (Vora et al., 2018; Patel et al., 2019). Therefore, the development of a simple, rapid, accurate, and validated RP-HPLC method for simultaneous estimation of dapagliflozin and metformin is of high significance for routine quality control in the pharmaceutical industry.

The present study aims to develop and validate a robust RP-HPLC method for simultaneous determination of dapagliflozin and metformin in marketed formulations, in accordance with ICH Q2(R1) guidelines, assessing parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and specificity.

Material and Methods

Material

Dapagliflozin (DAPA) and Metformin (MET) were obtained as pure drug samples from Pharmaceutical Company. HPLC-grade methanol, acetonitrile, and water were used as solvents. Buffer salts and other reagents were of analytical grade. Commercial tablet formulations containing Dapagliflozin and

Metformin were procured from the local market for assay studies. All chemicals and reagents were used without further purification.

Methods

Selection of Mobile Phase

Several mobile phase compositions were evaluated to achieve optimal separation of Dapagliflozin and Metformin in their fixed-dose combination. Based on system suitability parameters such as retention time, tailing factor, number of theoretical plates, and height equivalent to a theoretical plate (HETP), the most suitable mobile phase was determined to be a mixture of buffer and acetonitrile in a 58:42 v/v ratio. Prior to use, the mobile phase was filtered through a 0.45 µm membrane filter to remove particulate matter and degassed by sonication. A flow rate of 1.0 mL/min was employed for analysis.

Selection of Wavelength

Stock solutions of Dapagliflozin and Metformin (100 µg/mL) were prepared separately in the mobile phase and scanned over the UV range of 200–400 nm to determine their maximum absorbance. Both drugs exhibited maximum absorbance at 230 nm, which was selected as the detection wavelength for the method.

Selection of Separation Variables

Chromatographic separation was achieved using an Inertsil ODS-3V C18 column (250 mm × 4.6 mm, 5 µm particle size) with a bonded octadecylsilane phase. The mobile phase consisted of buffer and acetonitrile in the ratio of 58:42 v/v. Samples were injected in 20 µL volumes at ambient temperature, with a run time of 14 minutes. Under these conditions, Metformin and Dapagliflozin were eluted at retention times of approximately 2.0 min and 9.4 min, respectively.

Preparation of Stock and Working Solutions

Stock solutions of Dapagliflozin and Metformin (1000 µg/mL) were prepared in methanol and sonicated for 20 minutes. Sub-stock solutions (100 µg/mL) were prepared by appropriate dilution with methanol. Serial dilutions of 5, 10, 15, 20, and 25 µg/mL were prepared from the sub-stock solutions for both drugs for construction of calibration curves. All solutions were filtered through a 0.45 µm membrane filter prior to injection.

Linearity and Calibration Curve

Linearity of the method was established over the concentration range of 5–25 µg/mL for both Dapagliflozin and Metformin. Calibration curves were constructed by plotting mean peak area versus concentration,

and regression equations were derived to confirm the direct proportionality between analyte concentration and response.

Validation of the Developed Method (ICH; 2005).

Accuracy: Recovery studies were performed by adding known amounts of standard drug (80%, 100%, and 120%) to pre-analyzed sample solutions. Percent recovery was calculated to determine the accuracy of the method.

Precision

Repeatability: Precision was assessed by analyzing five replicates of each concentration within the linearity range.

Intermediate Precision (Day-to-Day): Analysis was performed on different days by different analysts to evaluate method reproducibility under varying conditions.

Robustness: Deliberate small changes in the mobile phase composition (from 58:42 to 53:47 v/v of buffer:acetonitrile) were introduced to assess the method's robustness. The method remained unaffected by these variations.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve, demonstrating the sensitivity of the method.

Analysis of Marketed Formulations

Twenty tablets of the marketed combination were weighed and powdered. An amount equivalent to 10 mg of Dapagliflozin and 500 mg of Metformin was accurately weighed, dissolved in methanol, sonicated for 25 minutes, and diluted with mobile phase to prepare a sample solution. The solution was filtered through a 0.45 µm membrane filter and appropriately diluted to obtain 10 µg/mL Dapagliflozin and 20 µg/mL Metformin. The amounts of both drugs in the tablet formulations were calculated by extrapolating the peak areas from their respective calibration curves. The procedure was repeated six times to ensure consistency and reproducibility.

Results and Discussion

The developed RP-HPLC method was successfully applied for the simultaneous estimation of Dapagliflozin (DAPA) and Metformin (MET) in pharmaceutical formulations. The chromatograms of the blank, individual drugs, and combined drug samples (Figures 1–4) indicate clear and well-resolved peaks without interference, confirming the specificity of the method.

System suitability parameters (Table 1) were within acceptable limits. The number of

theoretical plates (N) for DAPA and MET were 2560 ± 15 and 2785 ± 18, respectively, indicating good column efficiency. The tailing factors (1.12 for DAPA and 0.97 for MET) demonstrate acceptable peak symmetry. The resolution (Rs) of 7.85 between the two peaks confirms baseline separation. Low %RSD values of peak area (0.45–0.50) and retention time (0.15–0.20) indicate excellent instrument precision.

Linearity studies (Table 2) over the range of 5–25 µg/mL for both drugs showed a high correlation coefficient (r^2 = 0.999), demonstrating a strong linear relationship between concentration and response. The regression equations (y = 100.9x + 16.9 for DAPA and y = 50.2x + 5.66 for MET) further confirm proportional detector response. The method exhibits high sensitivity, with LOD and LOQ values of 0.15 and 0.45 µg/mL for DAPA and 0.20 and 0.60 µg/mL for MET.

The recovery study (Table 3) showed mean recoveries of 98.59% for DAPA and 98.63% for MET, with %RSD values below 1%, demonstrating high accuracy of the method. These values indicate minimal interference from excipients, validating the method for routine analysis.

Precision and robustness studies (Table 4) further confirmed the method's reproducibility. Repeatability, day-to-day, and analyst-to-analyst %RSD values were all below 2%, demonstrating excellent precision under varied experimental conditions. The robustness study, with %RSD values of 0.10 for both drugs, indicates that minor deliberate changes in chromatographic parameters do not significantly affect the results.

The sensitivity of the method was also confirmed by LOD and LOQ values (Table 4), which are sufficiently low to detect and quantify trace levels of both drugs in pharmaceutical formulations.

Finally, the analysis of commercial tablet formulations (Table 5) revealed assay values of 99.80% for DAPA and 99.85% for MET, with low %RSD (<0.25%), confirming that the method is accurate, precise, and suitable for routine quality control of combined dosage forms.

The developed RP-HPLC method is specific, precise, accurate, robust, and sensitive, making it an ideal tool for the simultaneous estimation of Dapagliflozin and Metformin in tablets and for routine pharmaceutical quality control and stability studies.

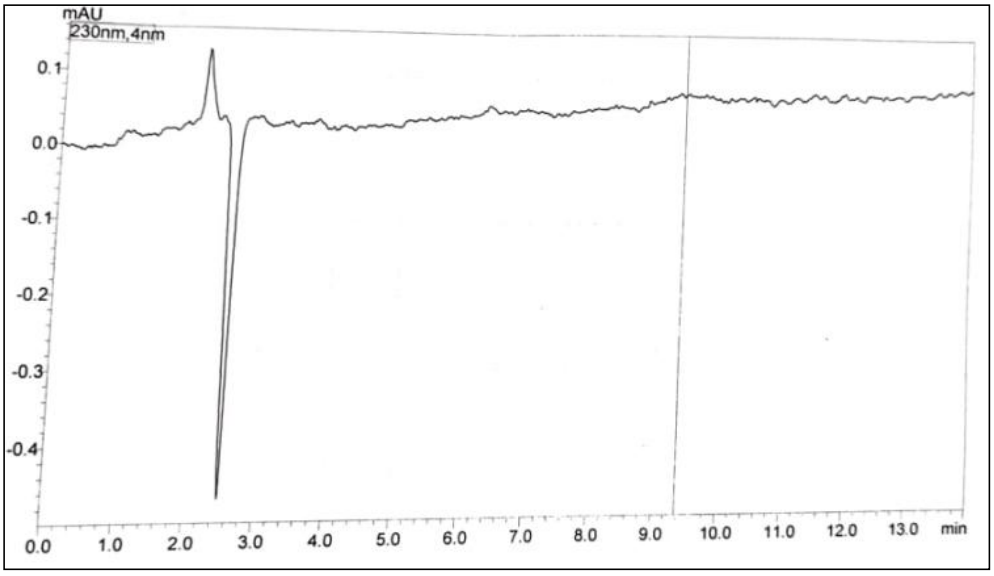

Figure 1: Chromatogram of Blank

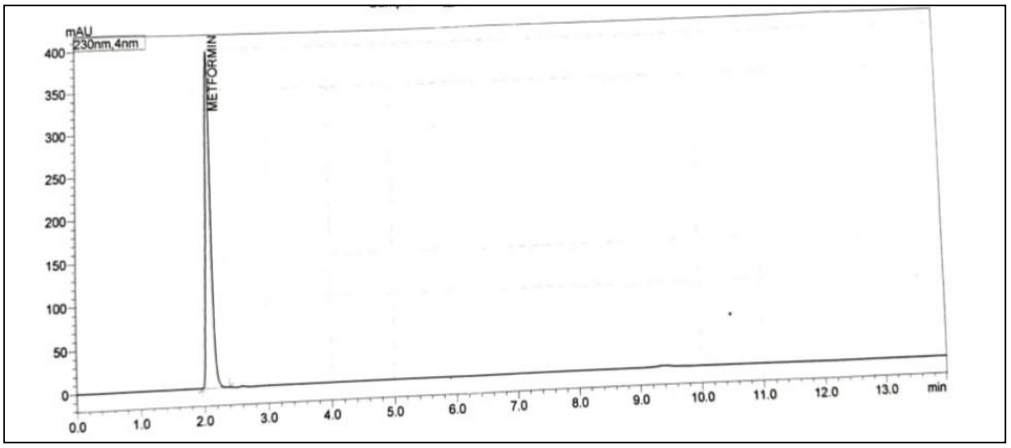

Figure 2: Chromatogram of Metformin

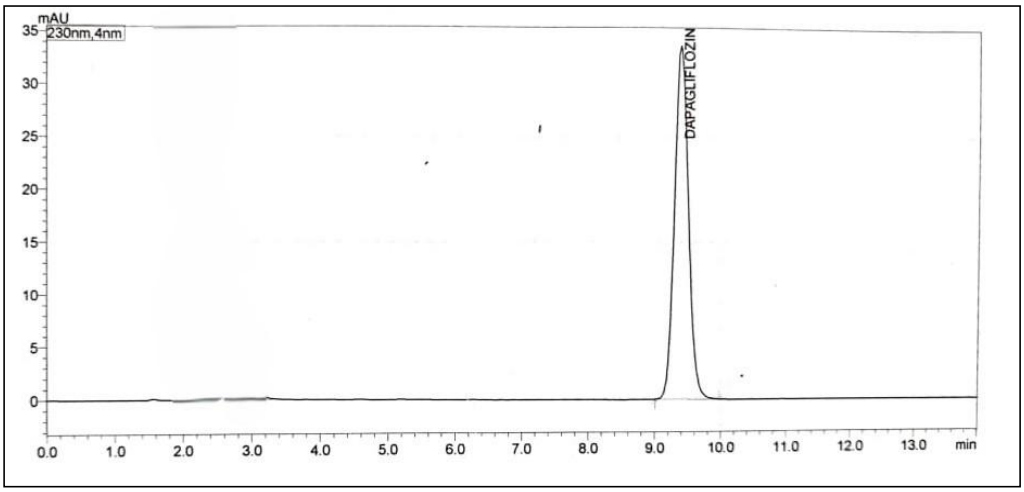

Figure 3: Chromatogram of Dapagliflozin

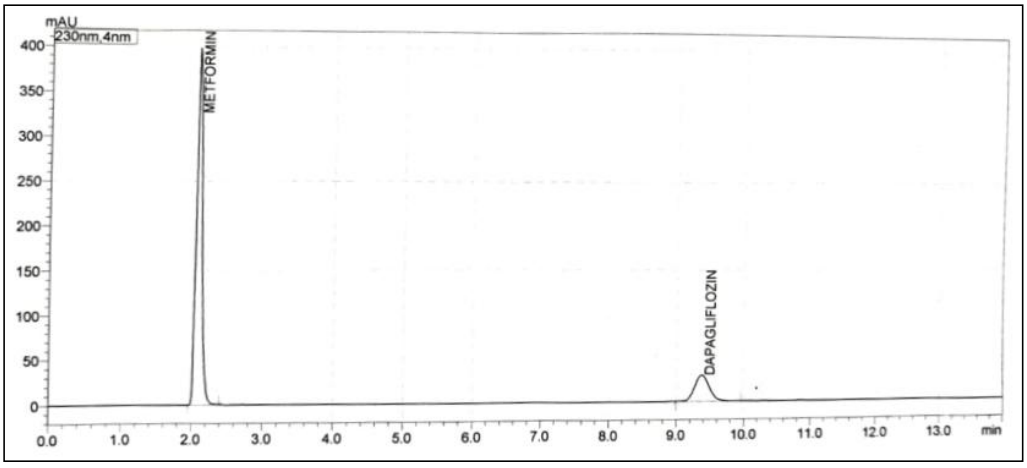

Figure 4: Chromatogram of Metformin and Dapagliflozin

Table 1: Results of System Suitability parameters

Parameters	Metformin (MET)	Dapagliflozin (DAPA)
Number of Theoretical Plates (N)	2560 ± 15	2785 ± 18
Tailing Factor (T)	1.12 ± 0.02	0.97 ± 0.03
Retention Time (Rt, min)	2.05 ± 0.01	9.40 ± 0.01
Resolution (Rs)	7.85 ± 0.12	-
Capacity Factor (k')	1.35 ± 0.04	4.12 ± 0.05
Selectivity Factor (α)	3.05 ± 0.06	-
%RSD of Peak Area (n=5)	0.45	0.50
%RSD of Retention Time (n=5)	0.20	0.15

Table 2: Results of linearity of Dapagliflozin and Metformin

Parameter	Dapagliflozin (DAPA)	Metformin (MET)
Concentration range (µg/mL)	5–25	5–25
Correlation coefficient (r^2)	0.999	0.999
Slope (m)	100.9	50.2
Intercept (c)	16.9	5.66
Regression equation	y = 100.9x + 16.9	y = 50.2x + 5.66
%RSD of response	0.34	0.42

LOD (µg/mL)	0.15	0.20
LOQ (µg/mL)	0.45	0.60

Table 3: Results of recovery study of dapagliflozin and metformin

% Level	Dapagliflozin (DAPA) % Mean ± SD	Metformin (MET) % Mean ± SD
80%	98.24 ± 0.42	98.34 ± 0.68
100%	98.47 ± 1.10	98.70 ± 0.23
120%	99.07 ± 0.47	98.85 ± 0.22
Overall Mean Recovery	98.59 ± 0.66	98.63 ± 0.38
%RSD (Overall)	0.67	0.39

Table 4: Results of precision and Robustness

Parameter	Dapagliflozin (DAPA) % Mean ± SD	Metformin (MET) % Mean ± SD
Repeatability	99.16 ± 0.11	99.16 ± 0.12
Intermediate Precision		
Day-to-day precision	99.16 ± 0.09	99.96 ± 0.12
Analyst-to-Analyst	99.16 ± 0.17	98.96 ± 0.12
Robustness	98.58 ± 0.10	98.83 ± 0.10

Table 4: Result of LOD and LOQ

Drug Name	LOD (µg/mL)	LOQ (µg/mL)
Dapagliflozin	0.25	0.75
Metformin	0.35	1.05

Table 5: Analysis of Dapagliflozin and Metformin Tablet Formulation

Parameter	Dapagliflozin (DAPA)	Metformin (MET)
Label Claim (mg/tablet)	10	500

Amount Found (mg)	9.98	499.25
% Assay	99.80	99.85
% RSD	0.23	0.15
Number of Determinations	3	3
Mean ± SD	9.98 ± 0.023	499.15 ± 0.76

Conclusion

A simple, rapid, precise, and accurate RP-HPLC method was successfully developed and validated for the simultaneous estimation of Dapagliflozin and Metformin in pharmaceutical dosage forms. The method demonstrated excellent linearity, accuracy, precision, robustness, and sensitivity, with well-resolved peaks and low %RSD values. Application to commercial tablet formulations confirmed its suitability for routine quality control and stability analysis. Overall, the developed method is reliable, reproducible, and can be effectively employed for simultaneous estimation of Dapagliflozin and Metformin in combined dosage forms.

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