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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DAPAGLIFLOZIN IN MARKETED FORMULATION BY HPLC

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ABSTRACT

Background: Dapagliflozin, a selective sodium-glucose co-transporter-2 (SGLT2) inhibitor, is widely used in the management of type 2 diabetes mellitus (T2DM). For ensuring therapeutic efficacy and safety, reliable analytical methods are required for its estimation in pharmaceutical formulations.

Objective: The present study aimed to develop and validate a simple, precise, accurate, and cost-effective reverse-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of dapagliflozin in marketed tablet formulations, in accordance with ICH Q2(R1) guidelines.

Methods: Method development was carried out by testing various mobile phase combinations. Methanol and acetonitrile (50:50 v/v) was optimized as the mobile phase, with a flow rate of 1.0 mL/min. The detection wavelength was selected appropriately after scanning. The method was validated for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and assay.

Results: The method showed excellent linearity in the concentration range of 5–25 µg/mL with correlation coefficient (r^2) of 0.999. Recovery studies at 80%, 100%, and 120% levels indicated accuracy within 98.14–98.80%. Precision results, including repeatability and intermediate precision, were within acceptable limits with %RSD <2%. LOD and LOQ were found to be 0.15 µg/mL and 0.45 µg/mL, respectively. Assay of marketed formulation revealed 99.50% of the labeled claim with low variability.

Conclusion: The developed RP-HPLC method is simple, accurate, precise, and sensitive, making it suitable for routine quality control analysis of dapagliflozin in bulk and marketed formulations.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. It is associated with long-term complications such as neuropathy, nephropathy, retinopathy, and cardiovascular disorders, which contribute significantly to morbidity and mortality worldwide (American Diabetes Association; 2014, Fronzo et al., 2015). According to the International Diabetes Federation (IDF), approximately 537 million adults were living with diabetes in 2021, and this number is projected to rise to 643 million by 2030, highlighting the urgent need for effective therapeutic strategies and monitoring tools (International Diabetes Federation; 2021).

Dapagliflozin, a selective sodium-glucose co-transporter-2 (SGLT2) inhibitor, has emerged as an effective oral antidiabetic drug. By inhibiting renal glucose reabsorption in the proximal tubules, it promotes urinary glucose excretion, thereby reducing blood glucose levels independently of insulin secretion (Chao and Henry, 2010). In addition to glycemic control, dapagliflozin provides cardiovascular and renal protective effects, making it a promising therapeutic option in type 2 diabetes mellitus (T2DM) management (Wiviott et al., 2019).

For quality control and regulatory compliance, the development of reliable, sensitive, and validated analytical methods is essential for the estimation of dapagliflozin in bulk and marketed formulations. High-Performance Liquid Chromatography (HPLC) is one of the most widely employed analytical techniques due to its high precision, accuracy, reproducibility, and sensitivity (Snyder; 2010). Moreover, HPLC has been extensively applied for the analysis of pharmaceutical compounds in routine quality control laboratories (Dong; 2010).

Although several analytical methods, including UV-spectrophotometry, LC-MS/MS, and HPLC, have been reported for dapagliflozin, HPLC remains the most cost-effective, accessible, and robust method for routine estimation in pharmaceutical formulations (Venkatesh et al., 2013; Hanchinal and Basavaiah; 2019). Method development involves systematic selection of mobile phase, stationary phase, flow rate, and detection wavelength to achieve optimal separation, while method validation ensures compliance with ICH guidelines in terms of accuracy, precision, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ) (ICH; 2005).

Thus, the present work focuses on the development and validation of a simple, accurate, and precise HPLC method for the estimation of dapagliflozin in marketed formulations, in accordance with ICH Q2(R1) guidelines.

Material and Methods

Material

For the present study, analytical grade (AR) and HPLC grade chemicals were used. Acetonitrile (HPLC grade, Merck), methanol (HPLC grade, Merck), potassium dihydrogen phosphate (AR grade, Rankem), triethanolamine (AR grade, Thomas Baker), and orthophosphoric acid (AR grade, HiMedia) were employed. Milli-Q water was used for the preparation of all aqueous solutions and mobile phases. The marketed tablet formulation containing dapagliflozin 10 mg was procured for assay and validation studies.

Methods

Initially, several mobile phase combinations in different ratios were evaluated for the estimation of dapagliflozin. Based on system suitability parameters such as retention time (RT), tailing factor, number of theoretical plates, and height equivalent to a theoretical plate (HETP), the mobile phase consisting of methanol and acetonitrile in the ratio of 50:50 v/v was found to be most suitable for the analysis. The selected mobile phase was filtered through a 0.45 µm membrane filter to remove particulate matter and subsequently degassed before use. The flow rate employed for the analysis was maintained at 1.0 mL/min (Sravya and Kuber, 2023).

Selection of wavelength

100 mg of Dapagliflozin was weighed accurately and transferred to a 100 ml volumetric flask, and the volume was adjusted to the mark with the mobile phase. From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200-400nm), maximum absorbance was found at λ_{max} 224.00 nm (Mohan *et al.,* 2014).

Selection of Separation Variable

Standard drug solution of Dapagliflozin was prepared in different mobile phase and chromatograph was recorded by using different column (5µm) at different chromatographic condition like different flow rate and temperature. Considering the theoretical facts and after several trials separation variables were selected which were constant during whole experiment.

Table 1: Selection of Separation Variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5 µm
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Methanol	50
Acetonitrile	50
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 µl
Detection wavelength	224 nm
Retention time	3.115 ± 0.3 min

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Dapagliflozin 10µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram (Ganthi *et al.,* 2016).

Linearity and Calibration Graph

Preparation of Standard Stock Solution

10mg of Dapagliflozin was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm (Reddy *et al.,* 2017).

Preparation of Working Standard Solution

From stock solutions of Dapagliflozin 1 ml was taken and diluted up to 10 ml from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with methanol, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Preparation of the calibration curves of the drug

Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve. The calibration curve is shown in Figure.

Validation (ICH, 2000)

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in Figure 6.5. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration (Reddy *et al.,* 2012).

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite

concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Desai *et al.*, 2012).

Precision

Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts (Krishna *et al.*, 2010). Statistical analysis was carried out.

Intermediate Precision

Day to Day

The statistical analysis method was carried out and the data is presented in Table.

Analyst to Analyst

The intermediate precision expresses with in laboratories variation (different analysts, different equipment etc) (Desai *et al.*, 2011). The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH and concentration of the mobile phase were made to check the method capacity to remain unaffected (Swartz and Krull, 2006). The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of Dapagliflozin was studied.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in tablet sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 10mg of Dapagliflozin was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 10µg/mL for Dapagliflozin.

Results and Discussion

The HPLC method developed for the estimation of dapagliflozin in marketed formulation was found to be simple, accurate, precise, and reproducible. The chromatograms of blank, standard, and sample (Figures 1–3) clearly indicated no interference at the retention time of dapagliflozin, confirming the specificity of the method.

The method exhibited excellent linearity in the concentration range of 5–25 µg/mL with a correlation coefficient (r^2) of 0.999 (Table 2), indicating a strong linear relationship between concentration and peak area. The slope and intercept values further confirmed the suitability of the calibration curve for quantitative analysis.

Accuracy of the method, determined by recovery studies at 80%, 100%, and 120% levels, was within acceptable limits (98.14–98.80%) with low standard deviation (Table 3). This suggests that the method is free from interference of excipients and can be reliably applied for routine analysis of dapagliflozin in formulations.

Precision studies demonstrated that the method provided highly reproducible results. Repeatability, day-to-day precision, analyst-to-analyst variation, and reproducibility were all within narrow ranges (98.57–99.16%) with low %RSD values (Table 4), signifying robustness of the method.

The sensitivity of the method was evident from the low values of LOD (0.15 µg/mL) and LOQ (0.45 µg/mL) (Table 5), indicating that the developed method is capable of detecting and quantifying very low concentrations of dapagliflozin.

Assay of the marketed tablet formulation (10 mg label claim) revealed 99.50% of the drug content with a %RSD of 0.165 (Table 6). This result falls within the acceptable range prescribed by pharmacopeial standards, confirming the accuracy and suitability of the method for quality control applications.

The results of linearity, accuracy, precision, sensitivity, and assay demonstrate that the developed RP-HPLC method is reliable, sensitive, and validated as per ICH Q2(R1) guidelines. Hence, it can be effectively applied for routine analysis of dapagliflozin in bulk drug and pharmaceutical formulations.

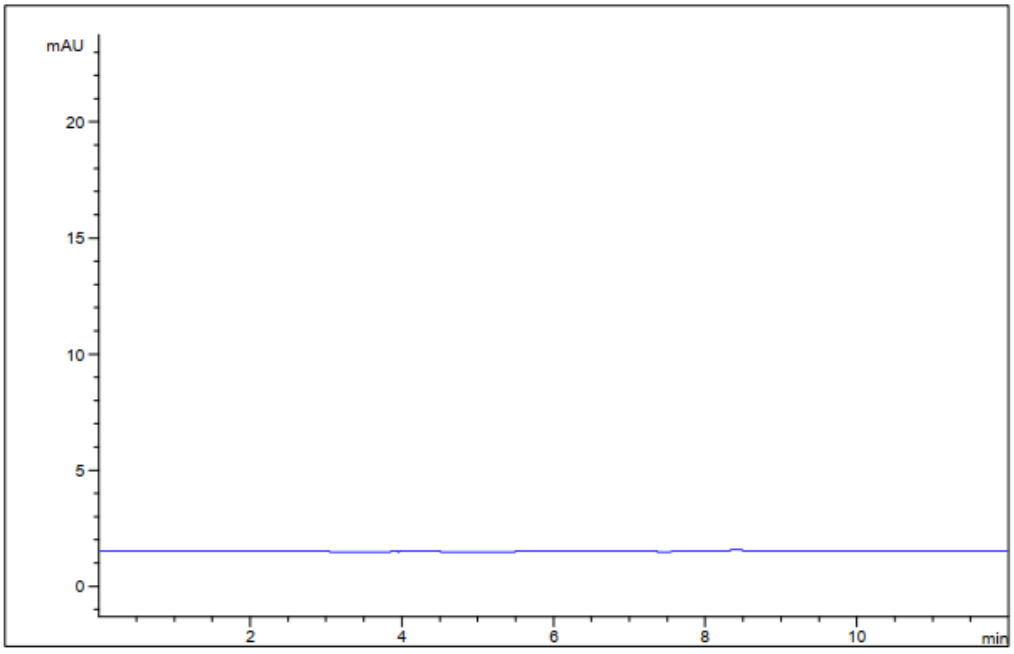

Figure 1: Chromatogram of Blank

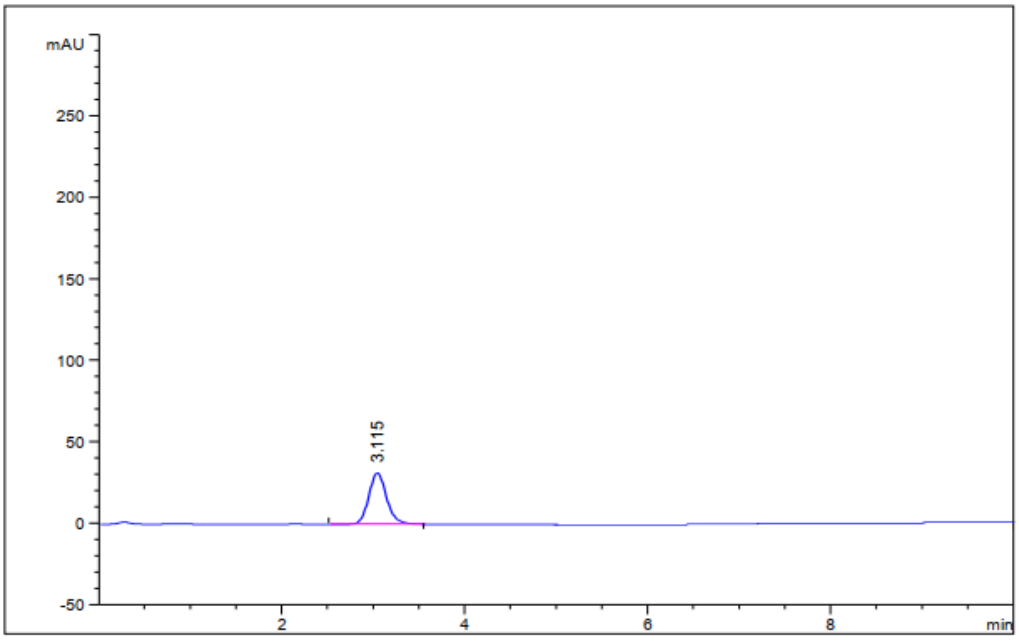

Figure 2: Chromatogram of Standard

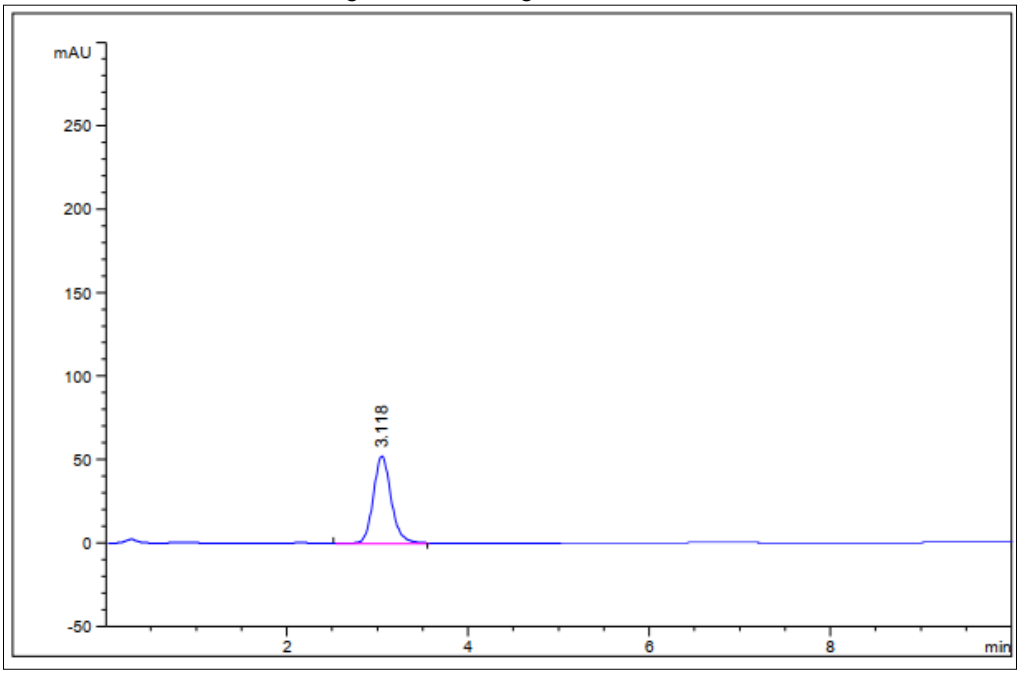

Figure 3: Chromatogram of Sample

Table 2: Results of linearity of Dapagliflozin

Parameter	DAPA
Concentration Range (µg/ml)	5-25
Correlation Coefficient (r^2)	0.999
Slope (m)	102.94
Intercept (c)	3.6614

*Value of three replicate

Table 3: Results of recovery study of Dapagliflozin

% Level	% MEAN±SD*
80%	98.14±1.501
100%	98.80±0.821
120%	98.55±1.037

***** Value of three replicate and five concentrations

Table 4: Results of precision of Dapagliflozin

Parameter	% MEAN±SD*
Repeatability	98.64±0.096
Intermediate precision	
Day to day precision	98.58±0.085
Analyst-to-Analyst	99.16±0.170
Reproducibility	98.57± 0.097

***** Value of five replicate and five concentrations

Table 5: LOD and LOQ of Dapagliflozin

Name	LOD (µg/ml)	LOQ (µg/ml)
Dapagliflozin	0.15	0.45

Table 6: Result of assay of tablet formulation

	Dapagliflozin
Label Claim (mg)	**10mg**
% Found (mg)	9.95
% Assay	99.50
% RSD	0.165

*Average of three determination

CONCLUSION

The present study successfully developed and validated a simple, accurate, precise, and cost-effective RP-HPLC method for the estimation of dapagliflozin in marketed formulations. The optimized mobile phase of methanol and acetonitrile (50:50 v/v) provided sharp and well-resolved peaks with acceptable system suitability parameters. Validation studies confirmed that the method complies with ICH Q2(R1) guidelines, demonstrating excellent linearity, accuracy, precision, sensitivity, and reproducibility. The assay results of marketed tablets were within pharmacopeial limits, confirming the reliability of the method for routine quality control analysis. Hence, the developed method can be effectively applied for regular quality assurance of dapagliflozin in bulk drug and pharmaceutical dosage forms.

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