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Research Article

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Stability-Indicating RP-HPLC Method Development for Simultaneous Determination and Estimation of Dapagliflozin in Raw and Tablet Formulation

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Abstract A sensitive, feasible RP-HPLC method has developed and validated for the analysis of Dapagliflozin in raw and tablet formulation. Successful separation of drugs products is developed on a C(18) column reversed-phase using mobile phase composition of Methanol: Water (75:25 v/v). The flow rate was adjusted to 1 mL/minute and the absorption maxima were observed at 230 nm utilizing Shimadzu SPD-20A Prominence UV-Vis detector. Good linearity was obtained in the range of 5-25 µg/mL, for Dapagliflozin. The HPLC, Dapagliflozin tablet formulation assay shows percentage purity ranging from 99.98% to 100.12%. The mean percentage purity of Dapagliflozin is 100.22 %. The chromatographic retention time of Dapagliflozin was found to be 3.1 min. The tailing factor was 0.970 respectively. The developed method validated according to the ICH guidelines. The method was found to be applicable for determination and validation of Dapagliflozin in tablet formulation.

Keywords Dapagliflozin (DAP), Methanol, HPLC and UV.

1. Introduction

Dapagliflozin is a tablet medication, Dapagliflozin belongs to a class of medications called sodium-glucose co-transporter 2 (SGLT2) inhibitors, which helps to reduce blood glucose levels by helping the kidneys to remove glucose from the blood and excrete it within urine [1]. Chemically Dapagliflozin is called as (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl) methyl]phenyl}-6-(hydroxymethyl) oxane-3,4,5-triol. The molecular formula is $C_{21}H_{25}ClO_6$. The molecular weight is 408.873 g/mol. The Dapagliflozin helps to support weight loss but may raise the risk of genital thrush and urinary tract infections [1-2].

Dapagliflozin drug work by targeting and helping to stop sodium-glucose transport proteins from allowing glucose that has been filtered out of the blood by the kidneys to be reabsorbed back into the blood [2-3]. The SGLT2 proteins are responsible for 90% of the glucose that is reabsorbed into the blood. By inhibiting the SGLT2 proteins [3]. Dapagliflozin is also used alone or in combination in adults for the treatment of type II diabetes. Dapagliflozin is a relatively new medication, the long term effects of the drug, over several years, are as yet unknown [3].

Literature review shows minimal methods has been developed and reported for Dapagliflozin estimation in biological fluids and there are some methods reported by [4-5], spectroscopy [6], HPTLC HPLC, UPLC and capillary electrophoresis [7-8]. Two methods were reported for estimation of Dapagliflozinin first is UV spectroscopy [9-11] and the other is HPTLC method [11-13]. Method development of HPLC estimation for Dapagliflozinin is new method will fulfil all requirements of validation according to ICH guidelines.

2. Materials and Methods

The working standard of Dapagliflozin was purchased from Sigma, UK. The Marketed sample of Dapagliflozin 10 mg strength, brand Forxiga, manufactured and marketed by Bristol-Myers Squibb and AstraZeneca. Acetonitrile and Methanol HPLC grade was purchased from Merck, Darmstadt, Germany.

2.1. HPLC instrumentation and chromatographic condition

HPLC system of Shimadzu LC-20 AT, with an auto sampler (SIL-20AC HT, Shimadzu, Japan) and SPD-10 detector (SPD- M20A, Japan) was used. For data recording the LC-solution software used. A Zorbax Eclipse Plus, Agilent Technology column (150mm x 4.6mm, 5µm) was used Pore size of the column 95Å. For degassing mobile phase, power sonic 505 ultrasonic baths (Hwashin technology, Seoul, Korea) was used. By using oven (CTO-20AC) column was maintained at a temperature of 37ºC and 1 mL/min was the flow rate. Analysis was carried over with 20µl injection volume using SPD-10 detection at 230nm. 10 minutes was set as run time.

2.2. Preparation of Mobile phase:

Approximately 750 mL of Methanol HPLC (75%) was transferred into 1 litre volumetric flask and water 250 mL of water was added to the volumetric flask (25%), and mixed thoroughly by shaking. The resulting solution was filtered through 0.45µ membrane filters and degassed in an ultrasonic bath for 10 minutes. The final ratio of mobile phase wasMethanol: Water (75:25v/v).

2.3. Preparation of Dapagliflozin Stock solution

Accurately 1 mg of raw Dapagliflozin was taken separately in 100 mL volumetric flasks and mixed with 25 mL of mobile phase solution and sonicated for 10 minutes and 75mL of mobile phase was added to the mark and cooled to room temperature. To get the concentration of 5-25 µg/mL of Dapagliflozin, varying quantities of standard stock solution was diluted with mobile phase. The solution was mixed well and filtered through 0.45µ membrane filters The Dapagliflozin powder was freely soluble in methanol and does not have any interference in the absorption peaks.

2.4. Preparation of sample solution

10 tablets of marketed Dapagliflozin was weighed and the tablets were finally powdered and triturated well and powder equivalent of 100 mg of Dapagliflozin transferred into 25 mL volumetric flasks and dissolved with 25 mL mobile phase and the resulting solution was filtered through Whatman 1 filter paper. Further dilutions were made based on the required concentrations.

2.5. Method validation

The present method was preceded to obtain new, sensitive and easy method for simultaneous estimation by HPLC from capsule formulation. According to the ICH guidelines recommendations the experimental was validated and USP-30 for parameters such as, system suitability, accuracy, precision, linearity and specificity.

2.6. System suitability:

System suitability parameters like resolution, retention time, tailing factor and column theoretical plates was performed by injecting six replicates of standards and two replicates of sample preparation at a 100% level to cross verify the accuracy and precision of the chromatographic system.

2.7. Linearity

The chromatographic method linearity was established by plotting a graph to concentration vs peak area of Dapagliflozin standard and determining the correlation coefficients of the compound. For the linearity studies of 5-25 µg/mL of Dapagliflozin respectively were injected into the HPLC system. For 30 minutes column was equilibrated with the mobile phase before injection of the solutions.

2.8. Accuracy

The recovery experiments show the accuracy of the method. The recovery was performed by adding Dapagliflozin working standards to placebo (excipients mixture) in the range of test concentration (60%, 80% and 100 %) and expressed as percent (%) recovered. Three samples were prepared for each recovery level. The recovery statistical results are within the acceptance range (S.D. < 2.0) value for Dapagliflozin.

2.9. Precision

In the proposed method the intraday and interday precision was determined by analyzing the sample responses 4 repeats on the same day and 4 different days of a week for 4 different concentrations of standard solutions of Dapagliflozin. 10-25 µg/mL of Dapagliflozin respectively and results are represented in terms of % RSD.

2.10. Specificity

The analytical method specificity is to measure the compound accurately in presence of interferences like excipients, degradants and matrix components. The HPLC of standard mixture and formulation shows specificity of method. The HPLC method is able to access the analyte in presence of excipients.

2.11. Limit of detection and limit of quantification

To determine the limit of detection (LOD) and limit of quantification (LOQ), serial dilutions of mixed standard solution of Dapagliflozin was made from the standard stock solution and prepared in replicates of three. The samples were injected in HPLC system and measured signal from the samples was compared with those of blank samples.

2.12. Statistical Parameters

The results of assay obtained are subjected to the following statistical analysis, standard deviation, relative standard deviation, coefficient of variation and standard error.

3. Results and Discussion

The HPLC chromatograms of Dapagliflozin raw and tablet formulation are presented in figure 1, 2. The wavelength of 230nm was selected by scanning all standard drugs over a wide range of wavelength 200-400 nm. Linearity was evaluated by plotting peak area as a functional of analyte concentration for Dapagliflozin. The graphical representation was given in figure 3 and data is presented in table 1.

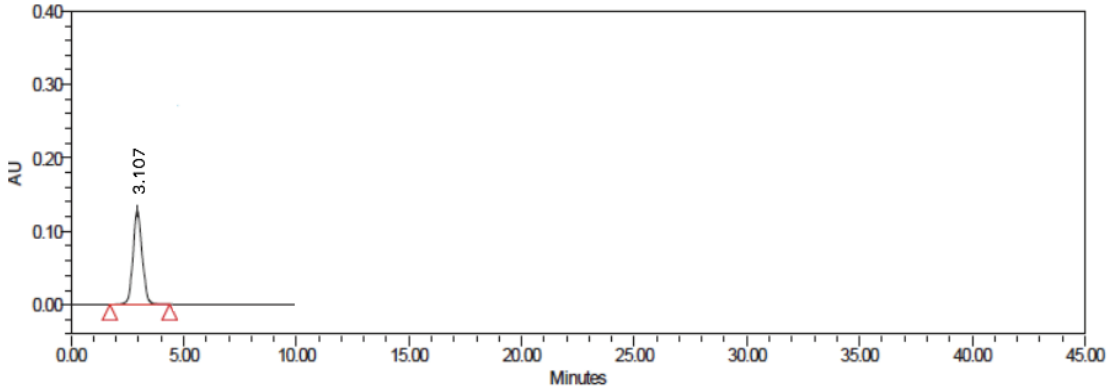

Figure 1: A Typical Chromatogram of Dapagliflozin standard

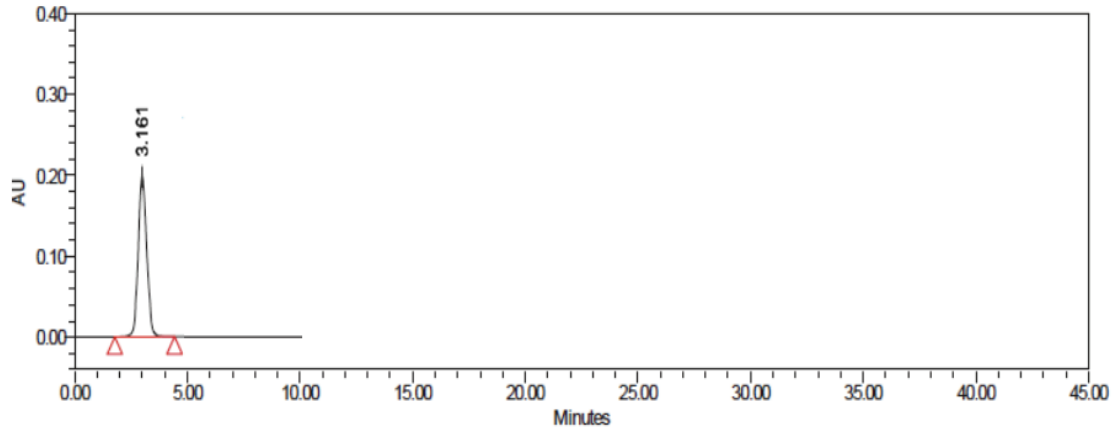

Figure 2: A Typical Chromatogram of Dapagliflozin in tablet formulation

Table 1: HPLC linearity data for Dapagliflozin

S. No.	Concentration (µg/mL) of DAP	Peak area
1	5	3457
2	10	6823
3	15	10044
4	20	13124
5	25	16457

The system suitability parameters like resolution, tailing factor, retention time and theoretical plates for the developed RP-HPLC method data are presented in table 2. The limit of detection and limit of quantification for Dapagliflozin are presented in table 3.

The specific range was determined from linearity studies found to be 5-25 µg/mL of Dapagliflozin. The data was analyzed by linear regression least square fit method. The slop, intercept, correlation coefficient and regression equation were also determined and the data presented in table 4.

Table 2: Results of system suitability parameters

S. No.	Parameters	DAP
1.	Theoretical plates	7867
2.	Tailing factor	0.970
3.	Resolution factor	9.7
4.	Retention time	3.1
5.	Linear dynamic range	5-25 µg/mL

Table 3: Results of Limit of detection (LOD) & limit of quantification LOQ

Parameters	DAP
LOD (µg/mL)	2.5 µg/mL
LOQ (µg/mL)	10.00 µg/mL

Table 4: Results of statistical parameters Statistical parameters

S. No.	Parameters	DAP
1.	Standard deviation (SD)	5.42
2.	Relative standard deviation (RSD)	0.00412
3.	% RSD	0.421
4.	Standard error (SE)	0.02375
5.	Correlation Coefficient (r)	0.9252
6.	Slope (a)	16.423
7.	Intercept (b)	12.234
8.	Regression equation Y = (a X + b)	Y = 160.423X+ 101.234

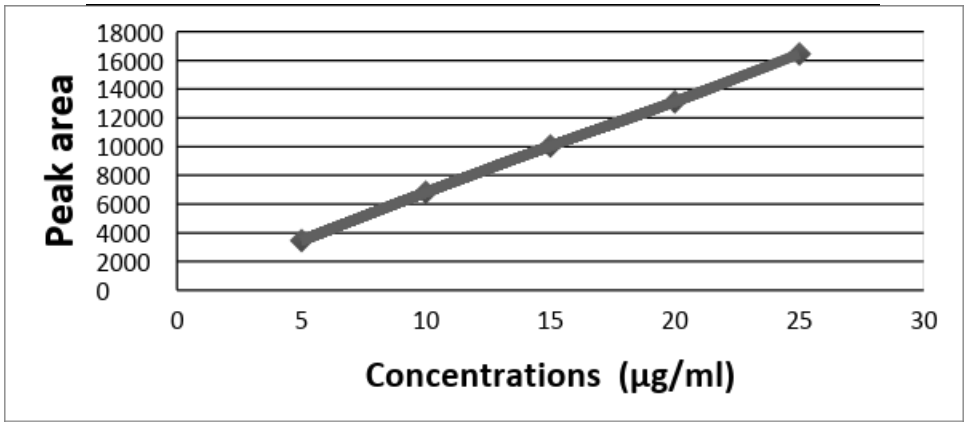

Figure 3: Calibration graph of Dapagliflozin 5-25µg/mL precision

The Dapagliflozin chromatographic retention time found to be 3.1 minutes respectively. This is well within the specific limits of 15 minutes. The high – resolution of Dapagliflozin indicates complete separation of the drugs. The

tailing factor was found to be 0.970. The peaks are symmetrical and theoretical plates for Dapagliflozin were 7869 respectively, which shows the column efficient performance. The quantitative estimation of Dapagliflozin tablet formulation was carried out by RP-HPLC method using Methanol: Water (75:25 v/v) using C18 column as the stationary phase. Chromatogram of Dapagliflozin tablet formulation shown in the figure 2. Quantitative estimation (Assay) data of Dapagliflozin presented in table 5. Recovery studies of Dapagliflozin tablet formulation shown in table 6.

Table 5: Quantitative estimation (Assay) data of Dapagliflozin tablet formulation

S No	Drug	Label claim (mg/Tab)	Amount found (mg/Tab)	Mean amount found (mg/Tab)	Percentage purity (% w/w)	Mean percentage purity (% w/w)	% Deviation
1.	**DAP**	10	10.07	10.22	100.07	100.22	+ 0.7
			10.08		100.08		+0.2
			9.94		99.94		-0.6
			9.98		99.98		-0.2
			10.12		100.12		+0.12

Table 6: Recovery studies of Dapagliflozin tablet formulation

S No	Drug	Amount of Drug in pre-analyzed Sample	Amount of Standard drug (RS) added (µg/mL)	Amount of drug recovered (µg/mL)	% Recovery	Mean recovery in Percentage
1.	**DAP**	10	15.00	25.02	100.02	100.25
			20.00	29.98	99.98	
			25.00	35.74	100.74	

The tablet formulation shows percentage purity ranging from 99.98% to 100.12% for Dapagliflozin. The mean percentage purity is 100.22% for Dapagliflozin respectively. The percentage deviation was found to be -1.0 to +1.0% respectively. The RSD values are below 2% indicating the method precision and the accuracy of the method shown by the low standard error values. This shows a good index of accuracy and reproducibility of the developed method. All the parameters including flow rate, detection wavelength sensitivity was maintained constant.

4. Conclusion

The proposed and developed RP-HPLC method is precise, accurate, and sensitive. The method is rapid, reproducible, and economical and does not have any interference due to the excipients in the pharmaceutical preparations.

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