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Analytical Method Development and Validation of Dapagliflozin in Bulk Drug and Pharmaceutical Dosage Form by U-HPLC Method

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Abstract

The aim of this study is to develop a rapid, precise and accurate UHPLC method for the estimation of Dapagliflozin in bulk and tablet dosage form as per ICH guidelines. The chromatography was carried on AgilentC18 Column (100mm x 4.6mm; 2.5µm) with mobile phase containing. The methanol: 0.1%OPA (60:40) v/v.The detection wavelength was 231nm &flow rate was 0.85 ml/min.

Keywords: Dapagliflozin, UHPLC, Tablet, Validation

Introduction

Dapagliflozin is used for the treatment of diabetes mellitus type 2 and functions to improve glycemic control in adults when combined with diet and exercise. It is an inhibitor of sodium-glucose co-transporter 2, which prevents glucose reabsorption in the kidney[1].Using Dapagliflozin leads to heavy glycosuria (glucose elimination in urine), which can lead to weight loss and tiredness. Dapagliflozin was approved by the FDA on 2014, Jan 08. It was not recommended for patients with diabetes mellitus type 1[2].The chemical name of Dapagliflozin is (2S,3R,4R5S,6R)-2-{4-chloro-3- [(4-ethoxyphenyl) methyl] phenyl}-6-(Hydroxyethyl) oxane-3,4,5-triol. The molecular formula of Dapagliflozin is C21H25CIO6[3].The main objective of this proposed method is to develop a new rapid, simple, precise, accurate and economical analytical method for the estimation of Dapagliflozin.[3]

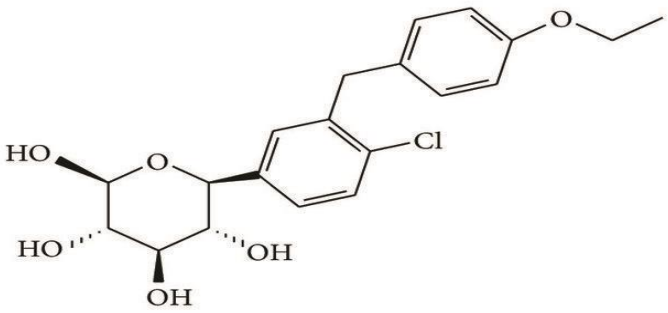

Figure No.1: Structure of Dapagliflozin

Chemical and reagents

Methanol (HPLC grade), 0.1% OPA (HPLC grade), water (HPLC grade) was purchased from Merck Ltd., India.

Instrumentation

UHPLC was selected as analytical technique for estimation of Dapagliflozin .The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector, UV (DAD) & Gradient Detector. Equipped with Reverse Phase (Agilent) C18 column (4.6mm x 100mm; 2.5µm), a 20µl injection loop and UV730D Absorbance detector and running chemstation 10.1 software.

Preparation of Stock Standard Solution

Accurately weight and transfer 10 mg Dapagliflozin working standard into 10 ml volumetric flask as about diluents Methanol completely and make volume up to the mark with the same solvent to get 1000µg/ml standard (stock solution) and 15 min sonicate to dissolve it and the resulting stock solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Methanol: (1 % Acetic Acid)Water, prepared in (6.0 ml MEOH: 4.0 ml (0.1 % OPA)WATER v/v)solvent .

Preparation of standard stock solution for Dapagliflozin

10 mg of Dapagliflozin working standards were weighed and transferred to 10 mL volumetric flask & diluents was added to make up the volume 0.1 ml of this solution diluted upto 10 ml with diluents.

Chromatographic condition
Mobile Phase: Methanol: 0.1% OPA (60:40)
Analytical column : Agilent C18 Column (100mm x 4.6mm), 2.5µm particle size.
Injection volume : 20µl
Flow rate : 0.85 ml/min
Detection : 231 nm
Run Time : 15 min
Detection Wavelength: 231 nm
Validation of method for analysis of Dapagliflozin:
The developed method was validated as per ICH guidelines.

Linearity

An analytical method's capacity to produce test findings that are directly or through a clearly defined mathematical transformation proportionate to the concentration of analyst in samples falling within a certain range is known as linearity.

Accuracy

A method's accuracy (recovery) is how closely test results obtained using that approach match the true value. By using known additional amounts of analyte in the experiment, accuracy is sometimes stated as a percentage of recovery.

Analytical methods are applied to analyzed samples that have known amounts of analyte added in order to assess their correctness. The percentage of analyte recovered by the assay is used in the test findings to calculate accuracy.

Repeatability

The system's accuracy was assessed using a sample. Peak areas were assessed and %RSD was determined after two replicates of the sample solution containing 20 g/ml of Dapagliflozin were injected.

Precision

When a procedure is routinely used on several Samplings of a homogenous sample, precision is the degree of agreement among individual test findings. Standard deviation or relative standard deviation is typically used to express the accuracy of an analytical technique. Additionally, one-way ANOVA was used to compare the data, and the F-test was used to determine the within-day mean square and between-day mean square.

Rubustness

The flow rate of Methanol was altered in the mobile phase composition at a rate of (1 ml/ min1) and the detection wavelength was changed at a rate of (1 ml/ min1) before the results were reviewed. Dapagliflozin 30 g/ml solution was used in triplicate for the experiment.

Limit of Detection

The detection limit (DL) was computed as follows based on the S.D. of the response and the slopslop

LOD = 3.3(Sy/S)

Limit of Quantitation

The quantitation limit (QL) was computed as follows using the S.D. of the response and the slope of the calibration curve.

LOQ = 10(SD/S)

Results and discussion

UV Spectroscopy

UV absorption of 20 µg/mL solution of Dapagliflozin in methanol was generated and absorbance was taken in the range of 200-400 nm. Λmax

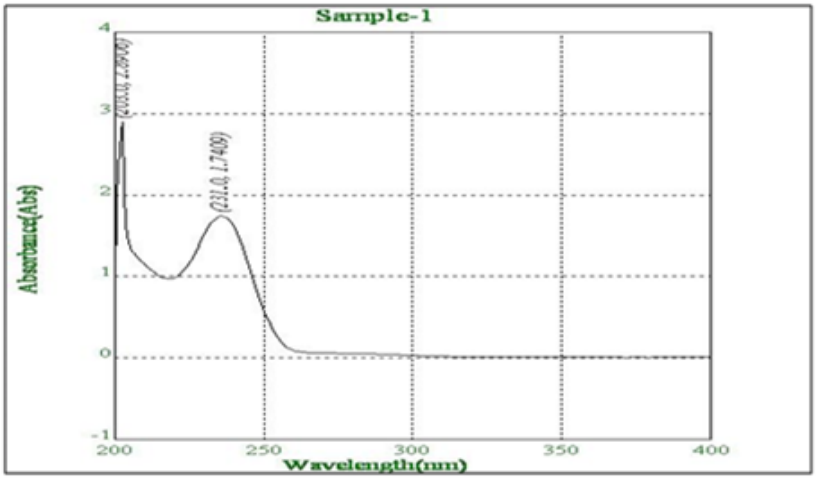

Figure No. 2: UV spectrum of Dapagliflozin

Table No.1: Different Trials of Chromatographic Condition

Fig No	Column used	Mobile phase, Flow Rate and Wavelength	Inj. Vol.	Observation	Conclusion
1	AGILENT C-18 (100 ×4.6mm, 2.5µ)	ACN+ (H2O)Water (90+10% v/v)231 nm0.6ml/min. C18 Column (100mm x 4.6mm), 2.5µm ,231 nm	20µl	Sharp Peaks were not obtained	Hence rejected
2	AGILENT C-18 (100 ×4.6mm, 2.5µ)	Acetonitrile+Water(80+20 % v/v)231 nm0.6 ml/min.	20µl	Sharp Peaks were not obtained	Hence rejected
3	AGILENT C-18 (100 ×4.6mm, 2.5µ)	Acetonitrile+ (1 % ACETIC ACID pH4 Water (80+20% v/v)231 nm,0.7 ml/min	20µl	Sharp Peaks were not obtained	Hence rejected
4	AGILENT C-18 (100 ×4.6mm, 2.5µ)	Acetonitrile+(1 % ACETIC ACID pH4.0)Water (70+30% v/v)231 nm0.7ml/min	20µl	Sharp Peaks were not obtained	Hence rejected
5.	AGILENT C-18 (100 ×4.6mm, 2.5µ)	Methanol+(0.1%OPA)Water (60+40% v/v)231 nm1 ml/min.	20µl	Sharp Peaks were obtained	GOOD PEAK Hence selected

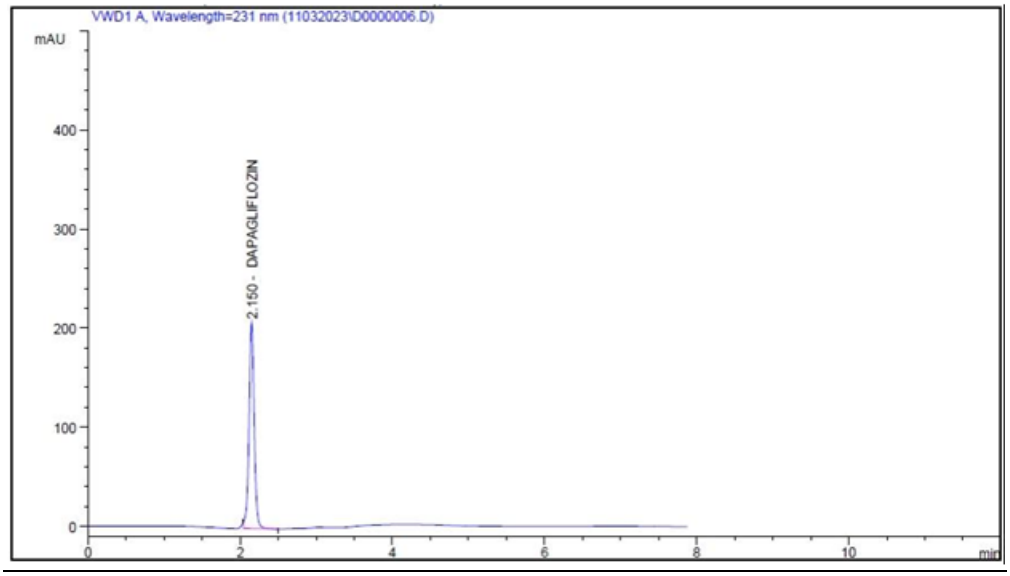

Figure No. 3: Result of chromatogram trial

Method and Validation

Linearity

From Dapagliflozin standard stock solution, different working standard solution (10-50µg/m) were prepared in mobile phase 20µl of sample solution was injected into the chromatographic system using mixed volume loop injector. Chromatograms were recorded. The areas for each concentration were recorded. The Calibration curves are shown in fig

Table No.2: Result of linearity studies

Regression Equation Data Y=mx+c	
Slope(m)	101.1
Intercept(c)	8.928
Correlation Coefficient	0.999

Linearity graph of Dapagliflozin

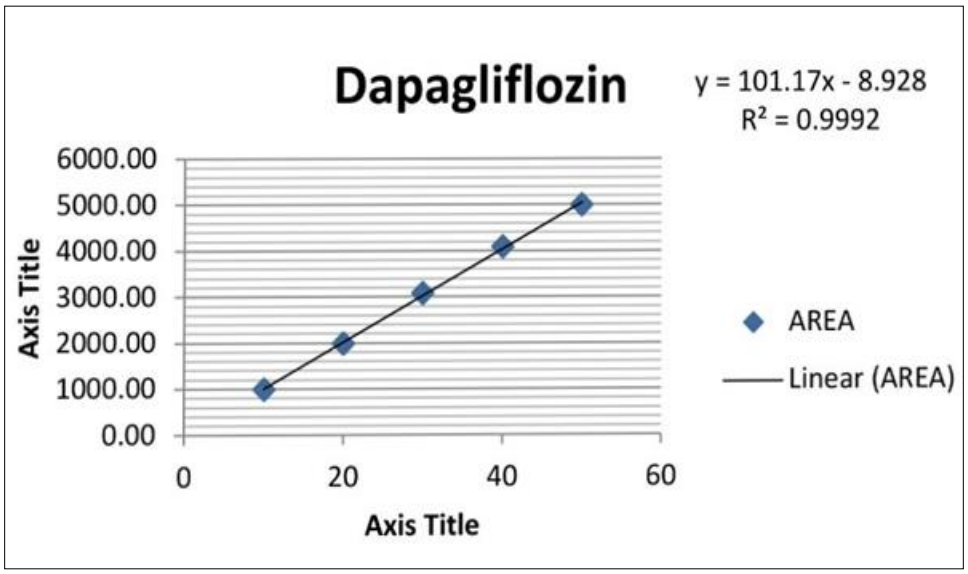

Figure No. 4: Linearity graph of Dapagliflozin

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed Tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Table No.39). Statistical validation of recovery studies shown in table

Table No.3: Result of Accuracy

Level of Recovery (%)	Mean % Recovery	Standard Deviation*	% RSD
80%	99.36	0.34	0.35
100%	101.82	0.98	0.96
120%	101.61	0.07	0.07

Repeatability

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Dapagliflozin system suitability parameters were studied. The result shown in below table

Table No.4: Result of Repeatability

Hr	Concentration of Dapagliflozin(mcg/ml)	Peak area	Amount found (mg)	% Amount found
1	30.00	3012.556	29.69	98.98
2	30.00	3009.287		
	Mean	3010.92		
	SD	2.31		
	%RSD	0.08		

Precision

The method was established by analyzing various replicates standards of Dapagliflozin. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in table

Table No.5: Result of Intraday Precision and Interday Precision

Conc^{n} (µg/ml)	Intraday Precision			Interday Precision		
	Mean± SD	%Amt Found	%RSD	Mean± SD	%Amt Found	%RSD
10	1028.49±0.10	102.61	0.48	1030.5±3.01	101.05	0.29
20	2050.48±2.15	101.85	0.46	2054.19±9.01	101.15	9.01
30	3015.65±1.74	99.72	0.21	3016.83±1.98	99.17	0.07

Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in (±1 ml/min-1) proportion and the flow rate was varied by of optimized chromatographic condition. The results of robustness studies are shown in (Table No.51).Robustness parameters were also found satisfactory; hence the analytical method would be conclude

Table No.6: Result of Precision

Parameters	**Conc.(µg/ml)**	Amount of detected(mean ±SD)	**%RSD**
Mob-phase composition 59+41ml) Methanol + 0.1% (Buffer)water	30	2925.3±14.27	0.49
Mob-phase composition (61ml+39ml) Methanol + 0.1%(Buffer)water	30	2928.40±6.85	0.23
Wavelength change230nm	30	2866.4±27.56	0.96
Wavelength Change 232nm	30	1224.41±4.24	0.35
Flow rate change(0.8ml)	30	3097.1±2.12	0.07
Flow rate change(0.9ml)	30	2953.20±7.02	0.24

Limit Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope the limit of detection (LOD) may be expressed as:

$$LOD = 3.3\ (SD)/S$$

$$= 3.3 \text{ X } 3.82/\ 101.1$$

$$= 0.1242$$

Where, SD = Standard deviation of Y intercept

S = Slope

The LOD of Dapagliflozin was found to be 0.1242 (µg/mL) analytical methods that concluded.

Limit Quantification

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

$$LOQ = 10 (SD)/ S$$

$$=10 X 3.82/ 101.1$$

$$= 0.3776$$

Where, SD = Standard deviation Y intercept

S = Slope

The LOQ of Dapagliflozin was found to be 0.3776 (µg/mL) analytical methods that concluded

Conclusions

The method provides selective quantification of Dapagliflozin This developed RP-HPLC method for estimation of Dapagliflozin is accurate, precise, robust and specific.

The method has been found to be better than previously reported method, because of its less retention time, isocratic mode and use of an economical and readily available mobile phase, readily available column, UV detection and better resolution of peaks.

The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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