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Original Article

Method Development and Validation of Dapagliflozin Drug in Bulk and Tablet Dosage form by RP-HPLC

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ABSTRACT

A new simple, rapid, efficient and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Dapagliflozin in Tablet dosage form. The separation was effected on a Symmetry C18, 25cm x 4.6mm i.d. 5µm, Particle size column using a mobile phase mixture of Methanol: ACN: %OPA in a ratio of 75:25:05 v/v/v at a flow rate of 1.0ml/min. The detection was maintained at 246nm. The retention time of Dapagliflozin was found to be 2.797minutes. The Dapagliflozin drug was linear in the concentration range of 0-70µg/ml. The method validation parameters were estimated and found in the limit according to ICH guidelines, which indicates the validation of the developed method. The developed method is also found to be simple, precise, accurate, specific, robust and rapid for the estimation of Dapagliflozin in bulk and tablet dosage form.

Key Words: Dapagliflozin, Retention time, ICH Guideline, Validation.

1. INTRODUCTION

Dapagliflozin is used for the treatment of diabetes mellitus type 2 and functions to improve glycemic control in adults when combined with the diet and exercise.[1,2,3] It is a inhibitor of sodium-glucose co-transporter 2, which prevents glucose reabsorption in the kidney. Using Dapagliflozin leads to heavy glycosuria (glucose elimination in the urine), which can lead to weight loss and the tiredness. Dapagliflozin was approved by the FDA on 2014, Jan 08.

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Dapagliflozin is not recommended for patients with diabetes mellitus type 1 or for the treatment of diabetic ketoacidosis. Dapagliflozin [4,5,6] is used for adjunct management of glycemic control in patients with type 2 diabetes mellitus, in combination with diet and exercise.

The chemical name of Dapagliflozin is (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl}-6- (hydroxyl methyl) oxane-3,4,5-triol. The molecular formula [7, 8] is $C_{21}H_{25}ClO_6$. The molecular weight of the Dapagliflozin is 408.873g/mol.

The main objective of this proposed work is to develop a new rapid, simple, precise, accurate and economical analytical method for the estimation of Dapagliflozin in bulk and Tablet dosage form.To validate the developed method in accordance with the USP and ICH guidelines/recommendations for the intended analytical application i.e., to apply the proposed method for analysis of the Dapagliflozin [9,10] in tablet dosage form. The chemical formula of Dapagliflozin is follows.

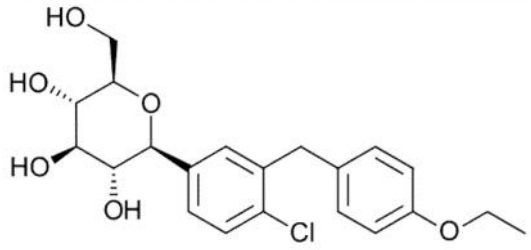

Fig 1: Chemical Structure of Dapagliflozin

2. MATERIALS AND METHODS

Dapagliflozin standard is obtained as a generous gift sample from Syncorp Clincare Technologies Pvt. Ltd., Hyderabad, India. Dapagliflozin tablets labeled to contain Dapagliflozin (5mg) manufactured by Astra Zeneca Pharmaceuticals LP., India, were purchased from local market. All the chemicals used were of HPLC grade, obtained from S D Fine-Chem Limited, Mumbai, India. All HPLC solvents and solutions were filtered through Nylon membrane filter of 0.45µ pore size.

HPLC Instrumentation & Conditions:

The HPLC system employed was WATERS with Empower2 Software with Isocratic with UV-Visible Detector.

Standard & Sample preparation for analysis:

25 mg of Dapagliflozin standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Mobile Phase Preparation

The mobile phase used in this analysis consists of a mixture of Methanol: Acetonitrile: 1% OPA in the ratio of 75:20:05 v/v/v. 750 ml of this methanol solution and 200ml of Acetonitrile was added and properly mixed with 50 ml of 1% orthophosphoric acid and a homogenous solution is achieved. This mobile phase was filled and sonicated for 5 minutes before using in the experiment.

Optimized Chromatographic Conditions:

Column : Symmetry C_{18}, 25cm x 4.6mm i.d. 5µm, Particle size
Mobile Phase : Methanol: ACN: %OPA (75: 25:05)
Flow Rate : 1.0ml/minute
Wave length : 246 nm
Injection volume : 20 µl
Run time : 06 minutes
Column temperature : Ambient
Sampler cooler : Ambient

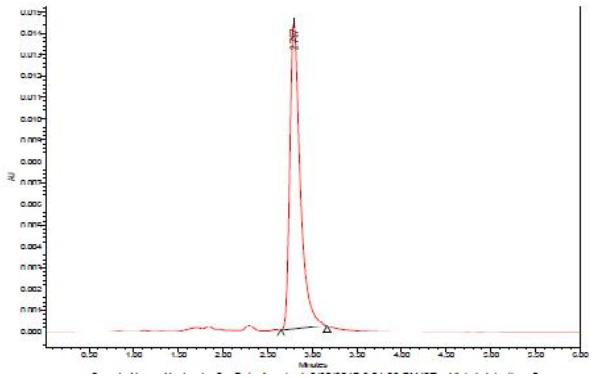

Fig 2: Optimized Chromatographic Condition

3. RESULTS AND DISCUSSION

Method Validation

System Suitability Studies

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system [11,12]. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 50µg/ml. The results which are given in Table were within acceptable limits. System suitability test is a pharmacopoeia requirement.

Table 1: System suitability parameters

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	3.15
2	Tailing factor	T ≤ 2	Dapagliflozin =1.78
3	Theoretical Plates	> 2000	Dapagliflozin =3689

Linearity:

To evaluate the linearity, serial dilution of analyte were prepared from the stock (100µg/ml) solution was diluted with mobile phase to get a series of concentration ranging from 20, 30, 40, 50, 60 and 70µg/ml. The prepared solutions were filtered through whatmann filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve [13] was constructed by plotting the mean peak area (Y-axis) against

the concentration (X-axis). The results which are given in Table 6 were within acceptable limits.

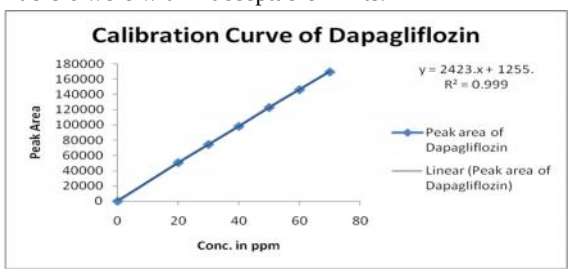

Fig 3: Calibration Curve of Dapagliflozin

Table 2: Linearity Readings for Dapagliflozin

Concentration of Dapagliflozin in ppm	Peak area of Dapagliflozin
0	0
20	50641
30	74567
40	98346
50	123094
60	146541
70	169872

The calibration curve showed good linearity in the range of 0-70µg/ml, for Dapagliflozin (API) with correlation coefficient (r^2) of 0.999 (Fig-10). A typical calibration curve has the regression equation of y = 2423.x + 1255 for Dapagliflozin.

Accuracy:

Recovery study:

To determine the accuracy of the proposed method, recovery studies [14, 15] were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of DAPAGLIFLOZIN were taken and added to the pre-analyzed formulation of concentration 50µg/ml. From that percentage recovery values were calculated. The results were shown in Table-3.

Table 3: Accuracy Readings

Accuracy level	Concentration («g/ml)		Area	% Recovery of Pure drug	Statistical Analysis
	Amount added	Amount found			
80 %	40	40.114	98452	100.285	Mean= 99.96067% S.D. = 0.583546 % R.S.D.= 0.583776
80 %	40	39.715	97485	99.287	
80 %	40	40.124	98476	100.31	
100 %	50	50.186	124384	100.372	Mean= 100.298% S.D. = 0.684009 % R.S.D.= 0.681976
100 %	50	50.471	123548	100.942	
100 %	50	49.790	121897	99.58	
120 %	60	60.787	148542	101.311	Mean= 100.5343% S.D. =0.963591 %R.S.D.= 0.95847
120 %	60	59.674	145847	99.456	
120 %	60	60.499	147845	100.836	

Precision

Repeatability

The precision [16, 17] of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug Dapagliflozin (API). The percent relative standard deviation was calculated for Dapagliflozin are presented in the Table-4.

Table 4: Repeatability Readings for 50ppm 5 injections

Concentration of Dapagliflozin in ppm	Rt of Dapagliflozin	Peak area of Dapagliflozin
50	2.803	117094
50	2.797	118452
50	2.790	116541
50	2.806	115465
50	2.804	116587
50	2.799	118746
AVG	2.799833	117147.5
S.D.	0.005845	1246.803
% RSD	0.20877	1.064302

Intermediate Precision:

Intra-assay & inter-assay:

The intra & inter day variation [18] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Dapagliflozin revealed that the proposed method is precise.

Table 5: Results of intra-assay & inter-assay

Conc. Of Dapagliflozin (API) (µg/ml)	Observed Conc. Of Dapagliflozin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
40	40.005	1.09	40.006	0.29
50	50.003	0.52	49.92	0.45
60	59.84	0.14	59.95	0.14

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness [19] of the method are also in favour of (Table-6, % RSD < 2%) the developed RP-HPLC method for the analysis of Dapagliflozin (API).

Table 6: Result of method robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.57
Flow (0.9 ml/min)	0.59
Temperature (27^{0}C)	0.24
Temperature (23^{0}C)	0.27
Wavelength of Detection (248 nm)	0.91
Wavelength of detection (242 nm)	0.95

LOD & LOQ:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) [20] were found to be 0.04 & 0.12 µg/ml respectively.

STABILITY STUDIES:

The API was subjected to stress conditions [21] in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body.

This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after a long time storage, within a very short time as compare to the real time or long term stability testing.

The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation.

Table 7: Results of force degradation studies of Dapagliflozin API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	90.41	9.59	100.0
Basic Hydrolysis (0.IN NaOH)	24Hrs.	91.63	8.37	100.0
Thermal Degradation (50 ^{0}C)	24Hrs.	93.44	6.56	100.0
UV (254nm)	24Hrs.	96.35	3.65	100.0
3 % Hydrogen peroxide	24Hrs.	92.64	7.36	100.0

Assay of Marketed Formulations:

Table 7: Assay of Dapagliflozin Tablets

Brand name of tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	% Purity
Forxiga tab (Astra Zeneca Pharmaceuticals LP.)	5	4.98 (±0.09)	100.06% (±0.48)

4. CONCLUSION

The results of the analysis of pharmaceutical dosage form by the developed RP-HPLC method are highly accurate, precise and robust and are in good agreement with the labeled claim of the drug.

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Dapagliflozin API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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