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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
A simple, rapid, precise, accuracy, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the Dapagliflozin (Dapa) in bulk and pharmaceutical dosage form. Chromatographic separation of Dapagliflozin was achieved on Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size and the mobile phase containing Phosphate Buffer: Methanol in the ratio of 35:65 v/v. The flow rate was 1.0 ml/min, detection was carried out by absorption at 215nm using a UV detector at ambient temperature. The number of theoretical plates and tailing factor for Dapagliflozin were NLT 3000 and should not more than 2 respectively. The proposed method was validated as per ICH guidelines for linearity, precision, accuracy, LOD and LOQ. The linearity of the proposed method was excellent over the range 0-70µg/ml for Dapagliflozin. The correlation coefficient was 0.999. Relative standard deviations of peak areas of all measurements were always less than 2.0%. The proposed method was validated according to ICH guidelines. The result of validation parameters indicates that the proposed method was also found to be accurate, precise, robust and sensitive. It can also be used for routine quality-control analysis of these drugs in commercial tablets.

Key Words: Dapagliflozin, Method Development, Validation, ICH Guidelines.

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1. INTRODUCTION

Dapagliflozin is indicated for the management of diabetes[1,2,3] mellitus type 2, and functions to improve glycemic control in adults when combined with diet and exercise. Dapagliflozin is a sodium-glucose cotransporter 2 inhibitor, which prevents glucose reabsorption in the kidney. Using dapagliflozin leads to heavy glycosuria (glucose excretion in the urine), which can lead to weight loss and tiredness. Dapagliflozin was approved by the FDA on Jan 08, 2014. Dapagliflozin is not recommended for patients with type 1 diabetes[4,5,6] mellitus or for the treatment of diabetic ketoacidosis. Dapagliflozin is indicated for adjunct management of glycemic control in patients

with type 2 diabetes mellitus, in combination with diet and exercise. Dapagliflozin 3-O-glucuronide is the primary metabolite of dapagliflozin, with 61% of the dapagliflozin dose recovered in the urine as this metabolite. The metabolism[7,8] of dapagliflozin is primarily mediated by UGT1A9-dependent glucuronide conjugation. The major metabolite,

dapagliflozin 3-O-glucuronide, is not an SGLT2 inhibitor. A competitive inhibitor[9] of the sodium-glucose transport subtype 2 protein, dapagliflozin blocks glucose reabsorption into the kidney, resulting in the elimination[10]of blood glucose through the urine. The IUPAC[11] Name of Dapagliflozin is (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4 ethoxy phenyl) methyl] phenyl}-6 (hydroxymethyl)oxane-3,4,5-triol. The molecular formula[12] is $C_{21}H_{25}ClO_6$.The Chemical Structure[13] of Dapagliflozin is follows

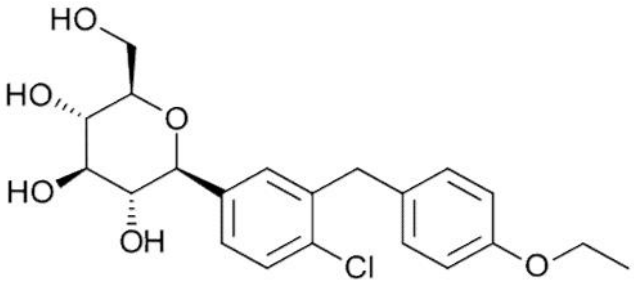

Fig-1: Chemical Structure of Dapagliflozin

2. METHODOLOGY:

2.1 INSTRUMENTS USED

Table-1: Instruments used

S. No.	Name of Instrument
1	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector
2	ELICO SL-159 UV – Vis spectrophotometer
3	High Precision Electronic Balance
4	Ultra Sonicator (Wensar wuc-2L)
5	Thermal Oven
6	Symmetry C_{18} Column, 250 mm x 4.6 mm and 5µm particle size
7	PH Analyzer (ELICO)
8	Vaccum Flitration Kit (Labindia)

2.2 CHEMICALS / REAGENTS USED

Table-2: Chemicals used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Potassium dihydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Dipotassium dihydrogen Peroxide	96%	L.R.	Sd fine-Chem ltd; Mumbai
6.	Ortho phosphoric acid	96%	L.R.	Sd fine-Chem ltd; Mumbai

3. INSTRUMENTATION

The HPLC system used was Waters-717 series, equipped with UV-detector, and auto sampler, Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size.

3.1 Chromatographic Conditions

The analysis was carried on HPLC Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size column with

detection wavelength of 215 nm. The injection volume of 20.0 µL and maintaining a flow rate at 1ml/min.

3.2 Preparation of Phosphate buffer

Perfectly weigh 6.8 grams of Potassium dihydrogen orthophosphate and transferred into a 1000ml beaker, dissolved and diluted to 1 liter with HPLC Grade water. pH was adjusted to 5.2 with Orthophosphoric acid.

3.3 Mobile Phase Preparation

A mixture of above prepared phosphate buffer 350mL (35%) and 650 mL of methanol HPLC (65%) were mixed together and degassed in ultra sonication water bath for 15 minutes and filtered through 0.45 µm filter under vacuum filtration.

3.4 Preparation of Diluent

Mobile phase used as diluent[15].

3.5 Preparation of Standard Solution:

Working concentration should be about 40µg/ml. Correctly weigh around 25mg of Dapagliflozin working standard, poured into a clean and dry 25 ml volumetric flask. Then dissolved and diluted to volume with the mobile phase to obtain a solution having a known concentration of about 1000 mcg/ml or 1000ppm. Further dilutions have been made to get the final concentration of 40µg/ml.

3.6 Preparation of Test Solution

Quantitatively diluted accurately measured volume of labial claim solution with the diluents in order to obtain a solution containing around a linear range.

4. METHOD VALIDATION

1. Accuracy:

Recovery study:

In order to determine the accuracy of the proposed method, % recovery[16,17] studies was performed by adding different amounts (80%, 100%, and 120%) of pure drug of DAPAGLIFLOZIN was taken and added to the pre-analyzed formulation of concentration closely 40µg/ml. From that % recovery values were calculated. The obtain results was shown in Table-3.

Table-3: Accuracy[18, 19] Readings

Sample ID	Concentration («g/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Injected	Amount Recovered			
S_1 : 80 %	32	31.825	107846	99.453	Mean= 99.672% S.D. = 0.388589 %R.S.D.= 0.3899
S_2 : 80 %	32	32.039	108564	100.121	
S_3 : 80 %	32	31.822	107835	99.443	
S_4 : 100 %	40	40.138	135687	100.345	Mean= 100.61% S.D. = 0.351266 %R.S.D.=0 .349105
S_5 : 100 %	40	40.406	136584	101.015	
S_6 : 100 %	40	40.199	135891	100.497	
S_7 : 120 %	48	47.766	161234	99.512	Mean= 99.91033% S.D. = 0.405165 %R.S.D. = 0.405528
S_8 : 120 %	48	48.157	162541	100.322	
S_9 : 120 %	48	47.951	161854	99.897	

2. Precision

The precision[20,21] of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Dapagliflozin (API). The percent relative standard deviation[22] was calculated for Dapagliflozin are presented in the Table-4.

Table-4: Repeatability Readings

HPLC Injection Replicates of Dapagliflozin	Peak Area
Replicate – 1	123731
Replicate – 2	121238
Replicate – 3	121622
Replicate – 4	122392
Replicate – 5	123119
Replicate – 6	123435
Average	**122589.5**
Standard Deviation	**1009.938**
% RSD	**0.823838**

2.2 Intermediate Precision:

2.2.1 Intra-assay & inter-assay:

The intra & inter day[23,24] variation 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
32	32.08	0.96	32.03	0.97
40	40.04	0.40	40.03	0.42S
48	47.97	0.33	47.95	0.14

3. Linearity[25] and Range

The calibration curve[26] showed good linearity in the range of 0-70 µg/ml, for Dapagliflozin (API) with correlation coefficient (r2) of 0.999 (Fig-2). A typical calibration curve has the regression equation[27] of y = 3349x + 1263 for Dapagliflozin.

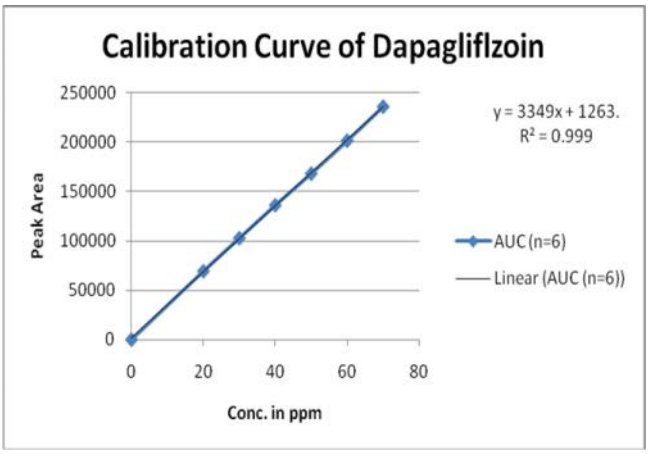

Fig-2: Calibration curve for Dapagliflozin

Table-6: Linearity Results for Dapagliflozin

CONC.	AUC (n=6)
0	0
20	69231
30	102677
40	136028
50	168014
60	201329
70	235798

4. Method Robustness[28]:

Influence of little changes in optimized chromatographic conditions like changes in flow rate (±0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) and Methanol content in mobile phase (±2%) studied to measure the robustness of the method are also in favour of (Table-7, % RSD < 2%)

the developed RP-HPLC method for the analysis of Dapagliflozin (API).

Table-7: Result of Method Robustness[29] Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.02
Flow (0.9 ml/min)	0.08
Temperature (27^0C)	0.04
Temperature (23^0C)	0.16
Wavelength of Detection (217 nm)	0.05
Wavelength of detection (213 nm)	0.07

5. Limit of detection (LOD) & Limit of quantification (LOQ):

The detection limit[30] (LOD) and quantization limit (LOQ)[11] may be expressed as:

L.O.D. = 3.3 (SD/S).

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Result & Discussion

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.36 µg/ml respectively.

6. Assay[32,33] of Dapagliflozin in dosage form

Estimation of Dapagliflozin in Tablet Dosage Form

Each tablet contains: 5 mg

Twenty tablets were taken and the I.P. method was follow to calculate the average weight[34]. Above weighed tablets were finally powdered and triturated well. Some quantity of powder which is equivalent to 25 mg of drug was transferred to a clean and dry 25 ml volumetric flask, make and solution was sonicated for fifteen minutes. Then the volume was made up to 25 ml with the same Mobile Phase[35]. Then 10 ml of the prepared above solution was diluted to 100 ml with the help of mobile phase. The resulted solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The final solution prepared was injected in 5 replicates into the HPLC system and the s are record the observations.

Some duplicate injections[36] of the standard solution were also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-8.

ASSAY:

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with sample preparation

AS = Peak Area of drug obtained with stock preparation

WS = Working standard weight of taken in mg

WT = Sample weight of taken in mg

DS = Standard solution dilution

DT = Sample solution dilution

P = Working standard Percentage purity

Assay was performed as described in previous chapter. Results obtained are tabulated below:

Table-8: Assay of DAPAGLIFLOZIN Tablets

Brand name of tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
FORXIGA (Dapagliflozin Tab, AstraZeneca Pharmaceuticals LP.	5	4.98 (±0.06)	99.6 (±0.48)

Result & Discussion: The assay of FORXIGA Tablets containing Dapagliflozin was found to be 99.6 %.

8. STABILITY STUDIES

Following protocol was strictly adhered to for stability studies[37,38] of Dapagliflozin Active Pharmaceutical Ingredient (API).

The APIs of Dapagliflozin was subjected to different stability conditions[26] in various ways to observe the rate and extent of degradation occur in the course of storage 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.

Results of degradation studies:

The results of the stress studies indicated the specificity of the method that has been developed. Dapagliflozin was stable in photolytic & thermal stress conditions. The results of forced degradation studies are given in the following Table-9.

Table-9: Results of forced degradation studies of Dapagliflozin.

Stress condition	**Time (hours)**	**Assay of active substance**	**Assay of degraded products**	**Mass Balance (%)**
Acid Hydrolysis (0.1N HCl)	24Hrs.	92.51	7.49	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	93.63	6.37	100.00
Thermal Degradation (50 ^{0}C)	24Hrs.	95.01	4.99	100.00
UV (254nm)	24Hrs.	98.26	1.74	100.00
3% Hydrogen peroxide	24Hrs.	94.34	5.66	100.00

9. RESULTS & DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Dapagliflozin, different chromatographic conditions were applied & the results observed are presented in previous chapters.

Out of the two elutions the Isocratic elution is simple; require only one pump & flat baseline (Stabilization) separation for easy and reproducible results. So, it was favored for the present study over gradient elution.

In case of RP-HPLC various stationary phases are available, but here Symmetry C18 Column, 250 mm x 4.6 mm i.d. and 5µm particle size was chosen because using this column shape of peak, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of

API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl).

The drug was found to be highly soluble in Acetonitrile, methanol, DMSO and DMF. Drug was practically insoluble in water. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Dapagliflozin it is evident that most of the HPLC work can be accomplished in the wavelength range of 200-250 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis.

The results show the developed method is yet another suitable method for assay, stability and purity which can help in the analysis of Dapagliflozin in different formulations.

10. CONCLUSION

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.

This proposed method can be used for the further analysis of Dapagliflozin in the future.

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