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A VALIDATED RP-HPLC METHOD FOR ESTIMATION OF DAPAGLIFLOZIN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Dapagliflozin in pharmaceutical dosage form. Chromatographic separation of Dapagliflozin was achieved on Prominence LC-20A Quaternary Gradient HPLC system, by using Shimpack C-18 (5µm, 4.6 x 250mm) column and the mobile phase containing Methanol and water with 0.1% ortho phosphoric acid with pH of 4.5 in a 80:20v/v ratio. The flow rate was 1.0ml/min; detection was carried out by absorption at 224nm using a UV detector at ambient temperature. LOD and LOQ were found to be 1.279 µg/ml and 3.877 µg/ml respectively and retention time was found to be 3.935mins. The % Recovery was found to be 99.93%-100.29%. The number of theoretical plates and tailing factor for Dapagliflozin were not less than 2000 and not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate and robust method for quantitative analysis of Dapagliflozin.

KEYWORDS: Dapagliflozin, High performance liquid chromatography, Method development, Validation.

INTRODUCTION

Dapagliflozin is a highly selective sodium-glucose cotransporter-2 inhibitor (SGLT2) used for the treatment of type 2 DM. In Europe, oral Dapagliflozin to be taken once daily is approved for use as monotherapy (For diabetic patients who are intolerant of metformin). Dapagliflozin as marketed in 2012, is a new class of anti-diabetic agents that effectively reduce blood glucose levels, body weight, and systolic blood pressure. In addition to that, they have newly shown cardiovascular safety. The glucose-reducing effect of Dapagliflozin has been approved in many randomized controlled clinical trials that showed notable reducing effects of Dapagliflozin in fasting blood glucose, glycosylated hemoglobin (HbA1c), and postprandial blood glucose levels. Furthermore, Dapagliflozin appeared to have a cardio protective effect, by reducing blood pressure, lowering body weight, uric acid, and triglyceride, and enhance insulin resistance.

Dapagliflozin is an oral, selective SGLT2 inhibitor that has displayed a significant improvement in glycemic control. Across universal clinical development programs including analysis of Phase IIB/III trial, treating with

Dapagliflozin alone as monotherapy or in conjunction with pre-existing OADs was linked with a significant lowering in glycosylated hemoglobin (HbA1c), fasting blood glucose and also help in lower or stabilize the body weight and systolic blood pressure (SBP) in patients with T2 diabetes mellitus.

Dapagliflozin inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2), which is responsible for at least 90% of the glucose reabsorption in the kidney. Blocking this transporter causes blood glucose to be eliminated through the urine. It also reduce the body weight, and systolic blood pressure. In addition to that, they have newly shown cardiovascular safety.

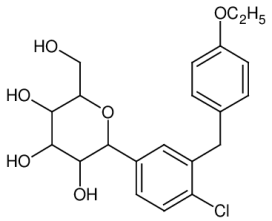

Figure 1: Chemical Structure of Dapagliflozin.

Dapagliflozin is selected in order to assess the potency in pure and tablet dosage form. A thorough review of the literature reveals that only few spectrophotometric techniques[3-10], RP-HPLC[11-21], HPTLC[22] and UPLC[23] are effective for determining the presence of Dapagliflozin alone or in combination in a variety of pharmaceutical formulations and biological fluids, including stability studies. This information provides details about the analyte's synthesis, physical and chemical properties, solubility, and pertinent analytical techniques. For the regular determination of Dapagliflozin in pure and tablet form, newer, easier, more sensitive, quick, accurate, and reproducible spectrophotometric and chromatographical approaches are therefore required.

MATERIALS AND METHODS

Apparatus and Software

Chromatographic separation was performed on a Prominence LC-20A Quaternary Gradient HPLC system as the instrument model and column used is ShimpackC-18 5µm, 4.6 x 250mm.

Chemicals and Reagents

Dapagliflozin pure form was obtained as gifted sample from Althera Laboratories and its pharmaceutical dosage form DAPANORM labeled claim 10mg were purchased from local pharmacy manufactured by alkem laboratories ltd. Methanol, ortho Phosphoric Acid and water obtained from Bharathi college of pharmacy Bharathinagara, K.M. Doddi, Maddur TQ & Mandy dist. India. All the Chemicals used in this investigation were HPLC grade.

Selection of mobile phase

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases Methanol and water with 0.1% ortho phosphoric acid with pH of 4.5 in a 80:20v/v ratio with detection wavelength of 224nm, since it gave sharp peak with good symmetry within limits.

Buffer Preparation

1ml of phosphoric acid is dissolved in 1litre of HPLC grade water. Filter through FCP-305µ membrane filter, after that adjust its pH to 4.5 with ortho phosphoric acid Preparation of mobile phase:
Mobile phase was prepared by mixing Methanol and water with 0.1% ortho phosphoric acid with pH of 4.5 in a 80:20v/v ratio. It was filtered through FCP-305µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of standard stock solution

Accurately weigh and transfer 100mg of Dapagliflozin working standard into a100ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (100ppm of Dapagliflozin).

Preparation of sample solution

Accurately weighed and transfer 100mg of Dapagliflozin sample into a 100ml clean dry volumetric flask add diluent and sonicate it up to 30min to dissolve, and centrifuge for 30min to dissolve it completely and make volume upto the mark with the same solvent. Then it is filtered through 0.2µ Whatman Uniflo Nylon filter (Stock solution). Further pipette 1ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent (100ppm of Dapagliflozin).

Flow rate selection

Different flow rates were studied. A flow rate of 1.0ml/min gave an optimal signal to noise ratio with a reasonable separation time.

Validation of Analytical Method

The method is validated according to the ICH guidelines; Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics are expressed in terms of Analytical parameters.

System suitability

10µl of the standard solution was injected under optimized chromatographic conditions to evaluate the suitability of system. Parameters such as number of theoretical plates (N), tailing factor (T), retention time (tr), asymmetry and area were determined. The obtained values indicate good performance of system Fig: 1, the values of system suitability parameters were shown in

Table: 1.

Solution stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24hr at room temperature. The results show that for solutions, the retention time and peak area of Dapagliflozin remained almost unchanged (% RSD less than 2.0).

Specificity

Specificity of the HPLC method was checked for interference of impurities, degradants or excipients in the analysis of sample solution and was determined by injecting a volume of 10µl of sample solution and the chromatogram was recorded. There is no interference of impurities, excipients peak on the peak of Dapagliflozin, indicating the high specificity of method. Which are shown in Fig: 2 and 3.

Linearity and Range

The linearity of the method was demonstrated over the concentration range of 3- 18µg/ml of the target concentration. Aliquots of 3, 6, 9, 12, 15 and 18µg/ml were prepared from above prepared stock solution. Different concentrations of the pure drug were injected into the chromatographic system. Calibration curve of Dapagliflozin was constructed by plotting peak area v/s applied concentration of Dapagliflozin. The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range & it has shown in Fig: 4. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in Table: 2, and their calibration parameters were shown in Table: 3.

Precision

The precision of the analytical method was determined by intra-day and inter- day precision Table: 5 and Table: 6, respectively the sample solution was prepared as per the test method. In intra-day precision, the same concentration of sample solution was injected 6 times in the same day and in inter-day precision, injecting six solutions of same concentration for six different days in a week. The average and standard deviation of mean area were taken and %RSD was calculated and reported. %RSD values were within the limits and the method was found to be precise.

Accuracy

The accuracy of the method was determined by recovery studies by the determination of % mean recovery of the drug at three different levels (80%, 100% and 120%). At each level, three determinations were performed. A known amount of standard pure drug was added to pre analyzed tablet powder and the sample was then analyzed by developed method. Results of recovery studies were reported Table: 7, the observed data were within the range, which indicates good recovery values.

Robustness

Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at by changing parameters like change in Flow rate of the Mobile phase and change in organic phase, and the results were shown in Table: 8. The method has no effect on the retention time and chromatographic response of the 6 solutions indicating that the method was robust.

Limit of detection

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The results of LOD were shown in Table: 9.

Limit of quantitation

Limit of quantitation is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably Quantitate. The LOQ can also be calculating based on the LOD strength, the LOD values were multiplied by three times to get LOQ. The results of LOQ were shown in Table: 9.

RESULTS AND DISCUSSION

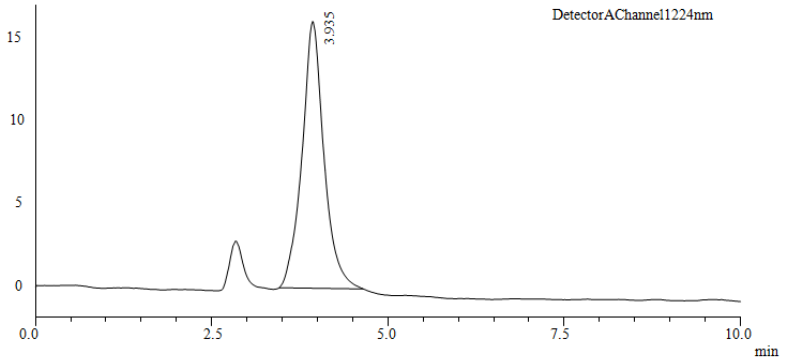

Fig. 1: Chromatogram of Dapagliflozin.

Table 1: Optimized chromatographic conditions.

Optimized conditions	Values
Column	ShimpackC-18(5µm,4.6x250mm)
Mobile phase	Methanol and water with 0.1% ortho phosphoric Acid with pH of 4.5 in a 80:20v/v ratio
Flow rate	l.0ml/min
Injection volume	20µl
Wavelength	224nm
Temperature	30ºC
Retention time	3.935min
Run time	10min
Elution	Isocratic

Table 2: System suitability studies of Dapagliflozin by RP-HPLC method.

System suitability Parameters	Acceptance criteria	Results
Tailing factor	T≤ 2	1.091
Theoretical plates	N≥ 2000	2832
Retention time	-	3.935
Area	-	277322

1. Specificity

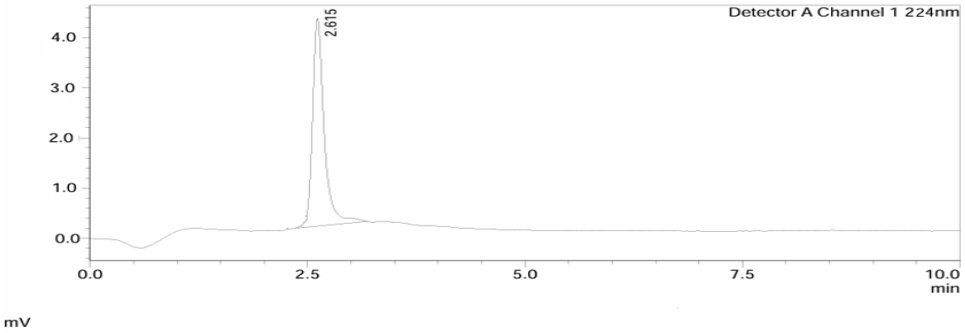

Fig. 2: Chromatogram of Blank.

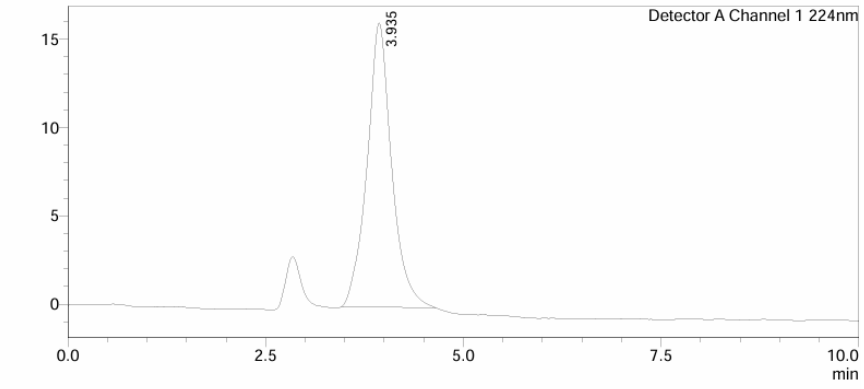

Fig. 3: Chromatogram of sample.

Table 3: Calibration data of Dapagliflozin by RP-HPLC method.

Concentration (µg/ml)	Peak area* (mv)
3	121682
6	191961
9	249052
12	299328
15	357620
18	420524

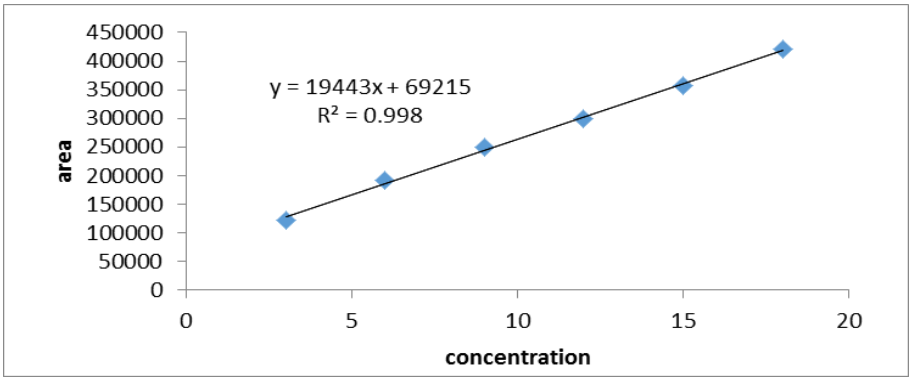

Fig. 4: Calibration curve of Dapagliflozin by RP-HPLC.

Table 4: Regression parameters table of Dapagliflozin by RP- HPLC Method.

Optimized conditions	Values
Linearity range(µg/ml)	3-18µg/ml
Regression equation(Y*)	Y=19443x + 69215
Correlation Coefficient(r^2)	0.998
Slope(a)	69215
Intercept(b)	19443

***Y=bX+a, where X is the concentration of compound in mcg/ml and Y is the peak area.**

Table 5: Intra-day Precision results for Dapagliflozin by RP-HPLC.

SL NO	Concentration (µg/ml)	Area	Concentration Found (µg/ml)	Mean* µg/ml	±SD	%RSD
1	10	265200	10.08	10.10	0.0129	0.12
2	10	265589	10.10			
3	10	265784	10.11			
4	10	265395	10.09			
5	10	265589	10.10			
6	10	265978	10.12			

***Average of six determination**

Table 6: Inter-day precision results for Dapagliflozin by RP-HPLC.

SL NO	Concentration (µg/ml)	Area	Concentration Found (µg/ml)	Mean* (µg/ml)	±SD	%RSD
1	10	266000	10.12	10.11	0.0106	0.10
2	10	265800	10.11			
3	10	265600	10.10			
4	10	265900	10.11			
5	10	265700	10.10			
6	10	266100	10.13			

***Average of six determination**

Table 7: Accuracy results for Dapagliflozin by RP-HPLC.

SL NO	Spiked level	Amount of Standard (µg/ml)	Amount of sample (µg/ml)	Total amount of drug (µg/ml)	Total amount of drug found (µg/ml)	% Recovery	Mean*	±SD	%RSD
1	80	100	80	180	180.8	100.44	100.29%	0.108	0.107
					180.36	100.2			
					180.40	100.22			
2	100	100	100	200	199.38	99.69	99.93%	0.185	0.185
					200.29	100.14			
					199.95	99.97			
3	120	100	120	220	220.03	100.01	99.94%	0.101	0.101
					219.60	99.80			
					220.05	100.02			

***Average of three determination**

Table 8: Robustness results for Dapagliflozin by RP-HPLC.

Parameters	Level	Factor	Mean area ±SD	%RSD
Flow rate (1ml/min)	-2	0.8ml/min	279841±653.19	0.233
	+2	1.2ml/min	265595±896.53	0.337
Wavelength (224nm±2)	-2	222nm	256722.7±612.82	0238
	+2	226nm	275906±1550.52	0.561
Column oven temperature (30˚C±2)	-2	28˚C	233383±1007.55	0.431
	+2	32˚C	233112.3±1087.30	0.466

Table 9: Determination of LOD and LOQ results of Dapagliflozin by RP-HPLC.

Sl. No	Parameters	Values
1	**LOD (3.3×SD of Intercepts/average of slopes)**	1.279µg/ml
2	**LOQ (10×SD of Intercepts/average of slopes)**	3.877µg/ml

****Mean value obtained from six calibration curves.**

CONCLUSION

The current analytical method satisfies the acceptance requirements and has been validated in accordance with ICH recommendations. The new analytical approach was shown to be simple, sensitive, accurate, and cost-effective. It may be applied to the regular analysis of Dapagliflozin in pharmaceutical dosage forms and bulk drug.

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