INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

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Research Article

Development and Validation of Reverse-Phase High-Performance Liquid Chromatography Method for Estimation of Dapagliflozin in Bulk and Formulation

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ARTICLE INFO

Received: 01 Oct 2023
Accepted: 03 Oct 2023
Published: 10 Oct 2023
Keywords:
Dapagliflozin, HPLC method development, Validation
DOI:
10.5281/zenodo.8428473

ABSTRACT

A new, simple, rapid, accurate and precise high-performance thin layer chromatography (HPLC) method has been developed and validated according to the guidelines of the International Conference on Harmonization (ICH Q2(R1) for the estimation of dapagliflozin in bulk and formulation. The chromatographic analysis was performed by an Agilent HPLC instrument using a BDS Hypersil, C-18, 250mmx4.6mm, 5 µm and mobile phase comprising 0.1% ortho-phosphoric acid in water and acetonitrile (65:35 v/v) at flow rate of 1.0 ml/min. The eluent was monitored at 225 nm for determination of dapagliflozin. The total run time was 10 min and the average retention time of Dapagliflozin was found to be 5.31 min. The calibration curves were linear over the range of 1-20 ng/mL (R2 = 0.999). The intra- and inter-day accuracy and precision values for all the analytes were within the acceptable range. The LOD and LOQ were 0.2076 and 0.9628 ng/mL. The developed method was found to be robust. A simple, precise, accurate, linear and rapid RP-HPLC method was developed and validated as per ICH guidelines. The results suggest that the developed method was found to be robust and it can be applicable in routine analysis and efficiently used for the estimation of dapagliflozin in bulk as well as combined dosage form.

INTRODUCTION

Dapagliflozin is a medication used in the treatment of type 2 diabetes mellitus. It belongs to a class of drugs known as sodium-glucose co-transporter 2 (SGLT2) inhibitors. It is a C-aryl glucoside derivative and is chemically known as (1s)-1, 5-anhydro-1-C- [4- chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-Dglucitol [1-2]. The chemical structure of Dapagliflozin was given in Fig. 1. These medications work by targeting a specific protein in the kidneys responsible for reabsorbing glucose into the bloodstream. By inhibiting this

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

protein, dapagliflozin helps the body excrete excess glucose through urine, thereby lowering blood sugar levels [3-5]. Dapagliflozin is a valuable addition to the arsenal of medications available for managing type 2 diabetes. It operates by a unique mechanism to help control blood sugar levels, offering benefits such as weight loss and potential cardiovascular protection. However, its use should always be under the guidance of a healthcare professional who can tailor the treatment to your specific needs and monitor your progress closely [6-8].

A literature survey on dapagliflozin revealed that, until now only few analytical methods were reported for its estimation of dapagliflozin such as UV-visible spectroscopy, HPLC method in bulk and API form [6-8]. However, the reported RP-HPLC method utilizes complex mobile composition, so there is a need to develop an RP-HPLC method having simple composition of mobile phase. Hence an attempt has been made to develop and validate a novel, simple and sensitive RP-HPLC method in accordance with ICH guidelines for the estimation of dapagliflozin in its bulk and formulation.

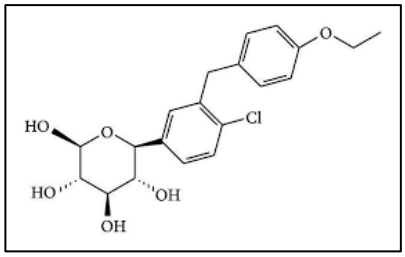

Fig.1- Chemical structure of dapagliflozin

MATERIALS AND METHODS

Chemicals and Reagents

Dapagliflozin (purity 99% by HPLC)) was purchased from TCI Chemicals (India) Pvt. Ltd. All the other chemicals of analytical grade were used for the proposed study.

Instruments

HPLC system of (Agilent Technologies) was used for method development and analysis. The system was equipped with a G1329B auto sampler system. G1315F variable wavelength detector (Agilent Technologies) was used for the analysis. Chromatographic analysis was carried out at room temperature**.** Ultrasonicator (PCi Analyticals) is use for mobile phase degasing. Vibra HT (Essae) analytical balance was used for weighing of chemicals.

Preparation of Mobile Phase

The mobile phase 0.1% ortho-phosphoric acid in water and acetronitrile were mixed in the ratio of 65:35 v/v and filtered through membrane filter (Millipore Nylon disc filter of 0.45 µ). This filtered mobile phase was sonicated for 15 min in ultrasonic bath.

Preparation of standard stock solution

Stock solutions (1 mg/mL) of dapagliflozin prepared in HPLC grade methanol and filtered through 0.45-m nylon membrane syringe filter.

Preparation of standard calibration curve

Calibration curve was prepared by diluting the stock-I solution to achieve the seven different calibration standards representing 1, 2, 4, 8, 12, 16 and 20 ng/ml strength of dapagliflozin. All these solutions were injected into HPLC column and the peak area of each solution was measured. The standard calibration curves of peak area Vs concentration (ng) were plotted.

Method Validation

The validation of pre-optimized chromatographic method was performed according to the Q2 (R1) guidelines of International Conference on Harmonization (ICH). Various analytical method validation parameters like system suitability, linearity, range, LOD, LOQ, accuracy, precision and stability were assessed [9-10].

System Suitability

Before performing the main analysis, the system suitability test was carried out using freshly prepared standard working solutions of 1.5 ng/mL of dapagliflozin. Standard working solution was repeated analyzed by using proposed HPLC conditions. During analysis, various parameters

viz. retention time, peak area, and the number of theoretical plates were measured. Acceptable upper limit of % RSD for peak area and retention time was set at 2 whereas acceptable lower limit of number of theoretical plates was set at 2000. System was considered to be suitable only when obtained values were within the set limits.

Linearity& Range

Linearity of the proposed method was calculated by using seven different calibration standards of dapagliflozin. The calibration curves were constructed using the Calibration Standards representing 1, 2, 4, 8, 12, 16 and 20 ng/ml strength of dapagliflozin. Concentration vs. peak areas were plotted, subjected to linear regression analysis and linearity in terms of R-squared values and respective range were reported.

Accuracy (% Recovery):

Accuracy of pre-optimized HPLC method was assessed using recovery studies by standard addition method. To the solutions with predefined amount of dapagliflozin (1.5, 10 and 19.5 ng/mL), its 80, 100 and 120 % amount was added externally and the % recovery of the drugs was calculated.

Precision

The precision of the developed method was evaluated by performing Intra-day and Inter-day studies. Intra-day precision study was carried out by analyzing five replicates of three different concentrations (1.5, 10 and 19.5 ng/ml of dapagliflozin) at morning, afternoon and evening time of the same day. Similarly, inter-day precision study was carried out by analyzing the samples on three consecutive days. Intra- and inter-day precision results were expressed in terms of % RSD.

Robustness

Robustness of the proposed HPLC method was evaluated by making slight, deliberate change in chromatographic parameters viz. column temperature, flow rate of mobile phase and the mobile phase composition. Modified chromatographic conditions for the assessment of robustness were ±1°C deviation in column temperature, ±1.0 ml/min deviation in flow rate of mobile phase and ±1 unit deviation in volume of methanol. For the robustness study, a solution (6 ng/ml) was repeatedly (n=5) analyzed for retention time and peak area of dapagliflozin using above mentioned modified chromatographic conditions. Results of the robustness study were expressed in terms of % RSD. Proposed method was considered to be robust only when the % RSD values for both retention time and peak areas were below 2.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. LOQ is the lowest concentration of analyte that can be determined with acceptable accuracy and precision. LOD and LOQ were calculated using following formula

$LOD = 3.3 \times SD/S$

$LOQ = 10 \times SD/S$

Where SD = standard deviation of response (peak area) and S = slope of the calibration curve.

Estimation of Dapagliflozin content in pharmaceutical formulation

Twenty tablets were weighed and powdered finely. A quantity of tablet powder equivalent to 10 mg of dapagliflozin was accurately weighed and transferred to 100 ml volumetric flask, add about 70 mL of diluent vortex and sonicated for 15 min and cool to room temperature make up volume up to the mark with diluent mix well and filtered using 0.45 µm nylon filter. Predefined volume of solution was analyzed using pre-optimized HPLC conditions. Contents of pharmaceutical formulation were calculated by comparing mean peak area of sample with that of the standard.

RESULTS AND DISCUSSION

Optimization of RP-HPLC Method

While developing HPLC method for estimation of dapagliflozin, various mobile phase combinations and the stationary phases were tried. Selection of mobile phase composition and stationary phases was based on the solubility behavior, pKa values and the relative retention of dapagliflozin was optimally resolved (Figure 2) over C-18 HPLC column using combination of 0.1% ortho-phosphoric acid in water and Acetonitrile (65:35 v/v) as a mobile phase. The details of optimized chromatographic conditions are shown in Table No. 1.

Table No. 1: The optimized chromatographic conditions

Separation variable	Optimized conditions
Chromatography	Agilent
Column	C18-250 mm × 4.6 mm, 5 µ (BDS Hypersil)
Mobile phase	0.1% Ortho-Phosphoric in water and Acetonitrile (65:35 v/v))
Flow rate	1 mL/min
Total Run Time	10 Min
Temperature	40^0C
Detection wavelength	225 nm
Retention time	6.10 min

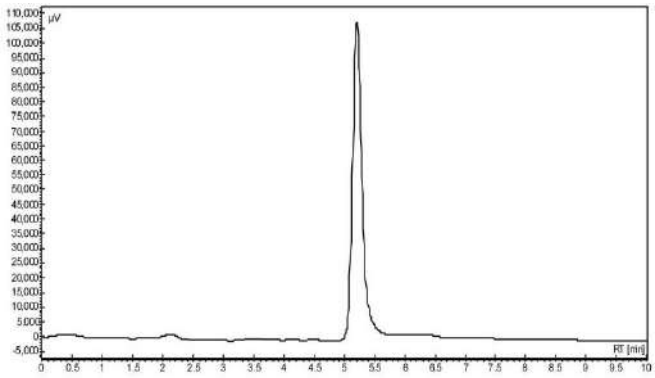

Fig. 2: A typical RP-HPLC chromatogram of Dapagliflozin

System suitability

During system suitability test, RSD of all parameter were calculated to evaluate the suitability of the developed method. From the results, it was found that %RSD for retention time and peak area was less than 2 and the number of theoretical plates were more than 2000 (Table 2). On the basis of obtained results, it was found that system is suitable for the analysis. The details of system suitability results are summarized in Table 2.

Table No. 2: System suitability parameters for Dapagliflozin

Sr.No.	Parameter	Acceptance criteria	Results		
			Dapagliflozin	%RSD	Status
1	Retention Time	%RSD ≤ 2%	5.31	0.4581	Passed
2	Area	%RSD ≤ 2%	20159	0.4938	Passed
3	Theoretical plates	≥ 2000	3015	0.7947	Passed

Method validation

Linearity and Range

Linearity and range are the important parameters of analytical method that demonstrates the limit within which the intended method is to be used for its optimum performance. Considering the prime importance of linearity and the range, seven-point calibration curve of Dapagliflozin (1-20 ng/ ml) were constructed. Different concentrations and peak area values are depicted in Table 3. Calibration curve when subjected to least square regression analysis yielded an equation; y = 10106x – 16.212 with correlation coefficient 0.999 respectively (Fig. 3). From the linearity study, it was revealed that, there is a linear relationship between response and amount of drug within the range 1-20 ng/ml.

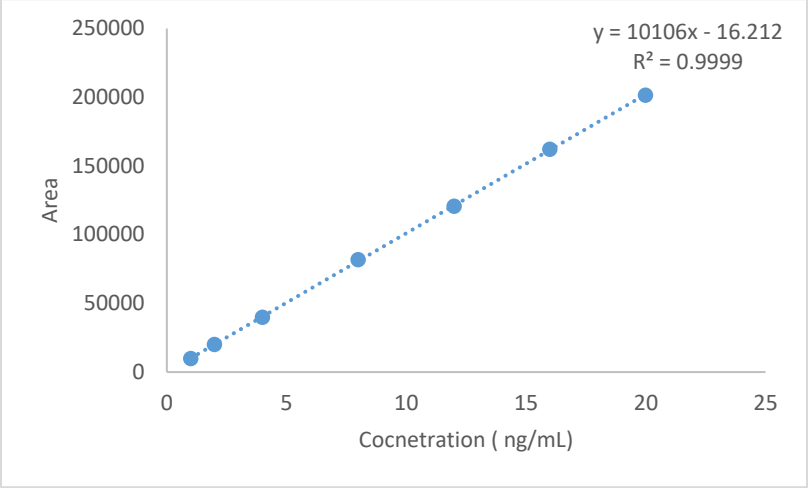

Fig. 3: Calibration curve for Dapagliflozin

Table No 3: Calibration standard data for Dapagliflozin

S. No	Conc. (ng/mL)	Peak Area
1	1	9954 ±0.78
2	2	20159 ±0.49
3	4	39948 ±0.0.61
4	8	81781 ±0.69
5	12	120867 ±0.41
6	16	162278 ±0.58
7	20	201590 ±0.18

Accuracy (percentage Recovery)

Accuracy is the closeness of test results to the true value obtained by proposed method. The accuracy of an analytical method should be established over its calibration range so that at any point of determination, results obtained would be accurate. For Dapagliflozin, accuracy was determined using recovery studies. At 80, 100 and 120 % standard addition, mean recovery of dapagliflozin was found to be in between 99.50 to 100.10 %. The relative standard deviation (% RSD) was found to be less than 2 (Table 4). From the results of accuracy studies, it was concluded that the proposed method is accurate.

Table No. 4: Recovery studies of Dapagliflozin

Sr. No	Sample	Spiked level	Theoretical Concentration (ng/mL)	Practical Concentration (ng/mL)	% Recovery	Mean % Recovery	% RSD
1	Dapagliflozin	80%	1.2	1.194	99.50	99.86	0.57
		100%	10	10.01	100.10		
		120%	23.4	23.398	99.99		

Precision

Precision was studied by analysis LQC, MQC and HQC STDs of the dapagliflozin at concentrations covering the entire calibration range**.** The results expressed in terms of % RSD for the intra- and inter-day precision study (Table 4 and 5). Percent RSD values of intra-day precision study were found to be in between 0.1120 to 0.9890, whereas inter-day precision was found to be in between 0.6875 to 0.8738. It was concluded that the analytical technique showed good repeatability.

Table 5: Intra-day precision data for Dapagliflozin

Sr. No.	Dapagliflozin			
	Amount present (ng/ml)	Amount recovered (ng/ml)	% Assay	% RSD
1	1.5	1.499	99.93	0.1120
2	10	9.998	99.98	0.9890
3	19.5	19.511	100.05	0.4975

Table 6: Inter-day precision data for Dapagliflozin

Sr. No	Dapagliflozin			
	Amount present (ng/ml)	Amount recovered (ng/ml)	% Assay	% RSD
1	1.5	1.497	99.80	0.8738
2	10	9.996	99.96	0.7444
3	19.5	19.497	99.98	0.6875

Robustness

An analytical method is considered to be robust when the small, internal changes in method parameters did not alter the final results significantly. Robustness of the proposed method was established by slightly changing the column temperature, mobile phase flow rate and mobile phase composition. It was found that, slight change in internal method parameters did not alter the final result (retention time and peak area) significantly. The % RSD values were found to be less than 2 (Table No.7). Thus, proposed method was found to be robust.

Table No. 7: Robustness study for Dapagliflozin

Sr.	Parameter	Setting	Dapagliflozin			
			RT	% RSD	Peak Area	% RSD
1	Mobile phase flow rate (ml/min)	0.9	5.302	0.98	20148	0.5747
		1	5.310	0.46	20431	0.5897
		1.1	5.214	0.49	20512	0.8941
2	Mobile phase composition (%, v/v)	64.5:35.5	4.608	0.49	20189	0.4589
		65:35	4.612	0.34	20497	0.6928
		65.5:34.5	4.615	0.58	20486	0.9835

LOD and LOQ

LOD and LOQ of proposed HPLC method was found to be 0.2076 and 0.9628 ng/ml. Lower LOQ value indicated that proposed method would be sensitive enough to quantify the Dapagliflozin content of samples at its lower level.

Estimation of Dapagliflozin content in pharmaceutical formulation

Proposed validated analytical method was successfully applied to the determination of dapagliflozin in pharmaceutical formulation. By proposed HPLC method, Dapagliflozin content in the tablet formulation was found to be 99.18 ± 0.015 %. Further, it was found that proposed HPLC method is specific for the dapagliflozin.

CONCLUSION

An accurate, precise, sensitive yet robust HPLC method was developed and validated for the determination of dapagliflozin in bulk and formulation. Proposed HPLC method was found to be specific for dapagliflozin and was free from any interference of formulation excipients. Proposed HPLC method can be used for routine analysis of dapagliflozin in bulk as well as formulation.

ACKNOWLEDGEMENT

The extra-mural grant support of DST-DPRP, Govt. of India (Ref:-VI-D&P/626/2018-19/TDT) sanctioned to P.I. Dr. Sachin S. Bhusari for the proposed research work is highly acknowledged.

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HOW TO CITE: Sachin Bhusari*, Pritesh Jawale, Pravin Wakte, Development and Validation of Reverse-Phase High-Performance Liquid Chromatography Method for Estimation of Dapagliflozin in Bulk and Formulation, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 10, 78-84. https://doi.org/10.5281/zenodo.8428473