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A Quantitative, Sensitive and Rapid Validated Analytical RP-HPLC Method for the Estimation of Dapagliflozin in Bulk and Pharmaceutical Dosage Formulations

Gouru Santhosh Reddy[1], Animesh Bera[1], Madhurima Basak[1], Krishnaveni Nagappan[*1], Ramalingam Peraman[2]

[1]Department of Pharmaceutical Analysis, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Nilgiris, Tamilnadu, India
[2]Division of Pharmaceutical Analysis and Quality Assurance, Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Ananthapuramu-515721, Andhra Pradesh, India

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ABSTRACT

The present study is aimed to develop a linear, precise and accurate RP-HPLC (Reverse Phase High-Performance Liquid Chromatography) method for the determination of dapagliflozin in the formulation. The method was accomplished on a C_{18} column (250×4.6mm; 5μm), & Samples were eluted using acetonitrile: water (40:60%v/v) delivered at a flow rate of 1.0ml/min with a chromatographic run time of 10 min. The eluents were observed utilizing a UV detector with a wavelength set at 277nm. The method that was developed resulted in the retention of dapagliflozin at 7.029minutes. Dapagliflozin through current method has shown linearity (*r2* > 0.999) over the concentration range of 1-16 μg/ml. The percentage recovery was observed to be within the limits of 98-102%, demonstrating the accuracy of the method. Limit of detection (LOD) and limit of quantification (LOQ) were qualified at 0.049μg/ml and 0.1485μg/ml, respectively. A Linear precise, accurate, simple, and rapid RP-HPLC method has been developed and validated for the evaluation of dapagliflozin in bulk drug and tablet dosage forms (5mg &10mg) according to ICH Q2(R1) rules. Additionally, the proposed method could be of use in quality control tests of dapagliflozin in pharmaceutical industries.

*Corresponding Author

Name: Krishnaveni Nagappan
Phone: 9442083447
Email: krisath@jssuni.edu.in

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) was first described as one of the metabolic syndromes in 1988. T2DM (earlier known as non-insulin dependent diabetes mellitus) is the most familiar form of diabetes mellitus characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. T2DM results from the interaction between genetic, environmental and behavioral risk factors (Olokoba *et al.*, 2012).

Sodium-Glucose Transporter (SGLT2) inhibitors were designed to control glucose reabsorption by the kidneys in patients with diabetes. It has become clear that SGLT2 inhibitors shall not only improve the blood glucose level but also show cardiovascular and renal protective effects irrespective of the reduction of blood glucose in patients suffering from type 2 diabetes mellitus (T2DM). The mechanisms underlying cardiovascular and renal protection by SGLT2 inhibitors in T2DM are

complex, multifactorial, and not wholly inferred. A common and perhaps inappreciative feature of T2DM is the chronic activation of the sympathetic nervous system (Sano, 2018).

Dapagliflozin was selected for the study because it is known for keeping side effects related to GIT (Gastro-Intestinal Tract) at bay and side effects due to inhibition of SGLT1 is expected to get minimized. This mechanism is anticipated to be in association with a low risk of hypoglycemia. As shown in Figure 1, Dapagliflozin chemically is (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxy benzyl) phenyl]-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol with molecular formula $C_{21}H_{25}ClO_6$ (Manasa *et al.*, 2014a).

Through a detailed literature survey regarding analytical methods developed for the quantification of drug dapagliflozin individually and in combination with other drugs and formulations, the following methods were found. The maximum absorbance of Dapagliflozin with methanol and water as a solvent was found at 224nm by the UV method (Mante *et al.*, 2017).

Manasa S and co-workers (Manasa *et al.*, 2014a,b) had developed a UV Spectroscopic and RP-HPLC method for the quantification of dapagliflozin in API with a correlation coefficient (r^2) of 0.999 for both methods. A reproducible RP-HPLC Method for the estimation of dapagliflozin in API and pharmaceutical formulations utilizing acetonitrile and dipotassium hydrogen phosphate as a mobile phase by RP-HPLC method was developed and validated by Mitali V and co-workers (Verma *et al.*, 2017). An HPLC method for the quantification of Dapagliflozin in API and pharmaceutical formulations in the presence of degradation products using methol and acetonitrile as mobile phase was developed and validated by M. D Game and co-workers (Game and Naglaxmi, 2018).

Thiyagarajan and his co-workers (Deepan and Dhanaraju, 2018) developed a simultaneous RP-HPLC method for the quantification of dapagliflozin and saxagliptin in API and tablet dosage forms by using Xterra RP18 as a stationary phase with an isocratic elution mode at 248nm utilizing acetonitrile and water as eluents. A more economical method for the simultaneous estimation of dapagliflozin and metformin in pharmaceutical dosage forms by RP-HPLC using methanol and potassium dihydrogen phosphate, over a concentration range of 100-500 μg/ml for dapagliflozin and 1-5 μg/ml for metformin was developed and validated by Nachiket S.D and his co-workers (Nachiket *et al.*, 2019).

Ghadir A Khalil and his co-workers (Ghadir *et al.*, 2018) were the first to report a method for simultaneous determination empagliflozin, canagliflozin, dapagliflozin and metformin using RP-HPLC method. Sayali S.M and co-workers (Sayali *et al.*, 2018) had carried out the simultaneous determination of saxagliptin and dapagliflozin in tablet formulations on Phenomenex hyper clone C_{18} column and estimated the method sensitivity in ranges of 2-12 μg/ml and 4-24 μg/ml with methanol, 20mM phosphate buffer as a mobile phase by RP-HPLC method.

It is known from the literature survey that methods developed for the drug dapagliflozin estimation had utilized with different buffers. The present study aims to develop an RP-HPLC method to achieve a sensitive, precise, accurate, simple and transferable to LC-MS/MS for the estimation of the degraded products through stability studies of drug and shall be utilizable for routine quality control execution of dapagliflozin estimation in bulk and formulations. The method developed for drug dapagliflozin using RP-HPLC had achieved a recovery of 98-102% using isocratic elution with the mobile phase composing acetonitrile & water in the ratio 40:60%v/v which was delivered at 1.0ml/min flow rate through a C_{18} column in isocratic condition. The validation of the developed RP-HPLC was performed as per the ICH guideline Q2R1 (ICH, 2005).

MATERIALS AND METHODS

Chemicals & Reagents

HPLC grade Acetonitrile was obtained from the Merck (Mumbai). HPLC grade water through the Milli Q system is used in the method. Drug dapagliflozin reference standard was procured from Clearsynth, Mumbai. Dapagliflozin formulation was purchased from the local pharmacy in the market area of The Nilgiris, Tamilnadu.

Instrumentation

HPLC autosampler system equipped with an LC-2010A quaternary low-pressure gradient pump & a UV detector (make Shimadzu, Japan) was utilized. A shim pack RP-C_{18} column with dimensions of 250mm$\times$4.6mm, i.d., 5μm was utilized as a stationary phase. Using a Class VP data station, data was processed from obtained chromatograms. UV spectrophotometer (UV-1700 Pharma spec. make Shimadzu, Japan) was utilized to screen the drug for spectroscopic analysis to determine the absorption maxima of analytes.

Preparation of Standard solutions

Standard dilutions of drug dapagliflozin were performed by dissolving 10mg of the drug-using ace-

tonitrile & made up the volume to 10ml to achieve a final concentration of 1.0mg/ml. From the above stock solution, serial dilutions viz., 100μg/ml, 10μg/ml, and 1.0μg/ml were prepared, and each concentration was utilized as percentile concentration.

Assay of the marketed formulations

Ten tablets were weighed and triturated to a fine powder. An equivalent weight of 5mg and 10mg of formulation powder is taken into a 100ml volumetric flask separately. The powdered formulation was dissolved in 75ml of mobile phase and sonicated for 5 minutes using an Ultrasonicator to obtain a homogeneous solution, which was then made up to 100ml with the mobile phase.

Then the above solution was filtered using a 0.45μm nylon filter and diluted appropriately to obtain solutions with a concentration of 5μg/ml and 10μg/ml, respectively. These solutions were injected into the HPLC system through Rheodyne injector repeatedly, and chromatograms were recorded and evaluated to attain mean, standard deviation and coefficient of variance within the acceptable limits.

Validation of Method

A validation protocol was developed for dapagliflozin concerning ICH Q2R1 guideline for measuring the parameters like linearity, specificity, precision, accuracy, the limit of detection, the limit of quantification, and Robustness.

RESULTS AND DISCUSSION

Selection of Wavelength

The drug, dapagliflozin, was screened in the UV spectrophotometer under a band range of 200-400nm and obtained an absorption maximum at 277nm, as depicted in Figure 2. The mobile phase has also been screened at 277nm to ensure the absence of interference at this particular wavelength.

Method development

After passing through several trials to accomplish a symmetric analytical peak at retention time of 7.029±0.2nm with ideal run time at a flow rate of 1.0ml/min using a C_{18} column as a stationary phase, acetonitrile and water (40:60%v/v) as a mobile phase, and 277nm as the detection wavelength, the method was found to be optimized upon obtaining reliable results for the system suitability parameters. Acetonitrile is utilized as a peak modifier and filtered through 0.45μ PTFE (Poly tetra fluoro ethylene) layer channel before being introduced into the chromatographic system as a mobile phase. Data acquisition and integration of chromatograms were performed using the CLASS VP data station. The chromatogram of standard dapagliflozin (10 μg/ml) was depicted in Figure 3.

Method validation

Specificity/ selectivity

The absence of interference at the retention time of dapagliflozin at 7.029±0.2 min after being assessed with diluent, mobile phase, and excipients of the formulation confirms that the method is specific for the determination of dapagliflozin.

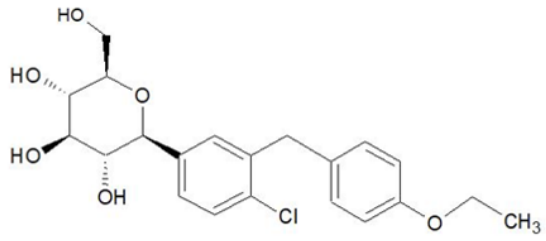

Figure 1: Chemical structure of Dapagliflozin

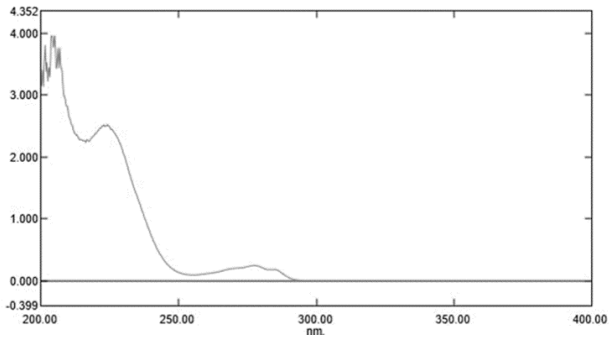

Figure 2: UV spectra of Dapagliflozin

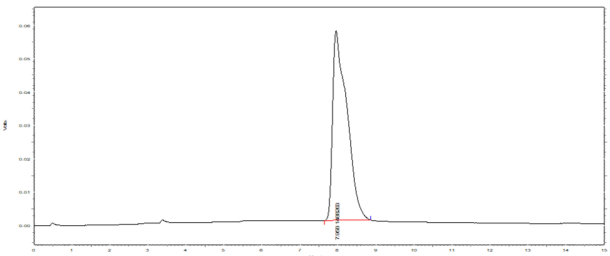

Figure 3: Chromatogram of the standard solution (Dapagliflozin - 10μg/ml)

Accuracy and Precision

Selected median concentrations were spiked into the formulations and were analysed to study the recovery. Recoveries for the drug were reported in Tables 1 and 2 and concluded to be within the range of 98-102% in concurrence of three replicates for each concentration. The chromatogram of the sample solution extracted from the tablet dosage form

Table 1: Accuracy studies of Dapagliflozin

S. No	Actual concentration (μg/ml)	Recovered concentration (μg/ml) ± SD; %RSD(n=3)	Percentage Recovered
1.	1	0.98±0.0057;0.5796	98.3%
2.	4	3.96±0.01;0.2525	99.0%
3.	16	15.97±0.02;0.1252	99.81%

Table 2: Assay of marketed Formulations

S.No	Sample	Label Claim	Amount present (mg/Tablet) ± SD; %RSD (n=3)
1.	Formulation-1	5mg	4.83±0.0513;1.062
2.	Formulation-2	10mg	9.84±0.0472;0.4796

Table 3: Precision studies of Dapagliflozin

S.No	Concentration(μg/ml)	Intraday Mean ±SD; %RSD (n=6)	Interday Mean ±SD; %RSD (n=6)
1.	1 (LQC)	1.0867±0.0015;0.1425	1.0871±0.0015;0.1437
2.	4 (MQC)	3.1783±0.0304;0.9564	3.1732±0.0239;0.7554
3.	16 (HQC)	15.4133±0.0472;0.3066	15.4133±0.0472;0.3066

Table 4: System Suitability parameters

S.No	Parameters	Dapagliflozin
1.	Retention Time (min)	7.029min
2.	Theoretical plates (N)	3162.03
3.	Tailing Factor (Tf)	0.83
4.	Asymmetry Factor (As)	1
5.	Regression Coefficient (r2)	0.9993
6.	Regression Equation	Y=8400.5x +1090.1
7.	Linearity and Range	1-16μg/ml
8.	Detection Limit (LOD)	0.049μg/ml
9.	Quantification Limit (LOQ)	0.1485μg/ml

Table 5: Robustness studies

Parameters	Retention Time
Mobile phase ratio (% v/v)	
58:42	7.031±0.2
60:40	7.029±0.2
62:48	7.025±0.2
Wavelength (nm)	
272	7.022±0.2
277	7.029±0.2
282	7.025±0.2
Flow rate (ml/min)	
0.9	7.031±0.2
1	7.028±0.2
1.1	7.025±0.2

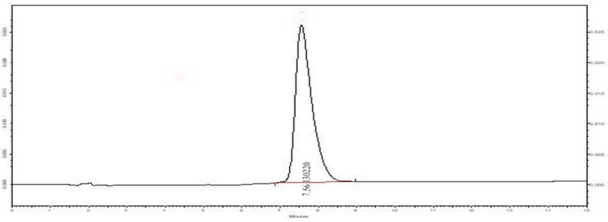

Figure 4: Chromatogram of the sample solution extracted from the tablet dosage form

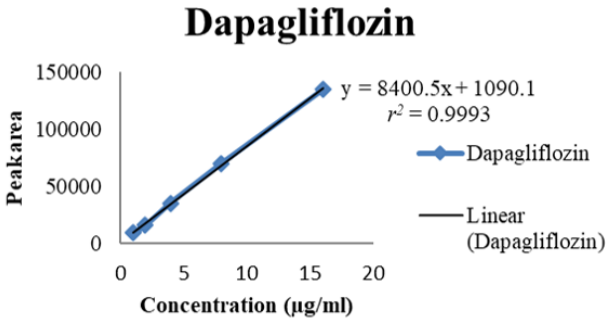

Figure 5: Linearity graph of Dapagliflozin

was depicted in Figure 4.

The precision of the method has been measured for variable timings, days with accepted repeatability, and the results were reported in Table 3. With a coefficient of variance value below 2.0, the method was proved to be precise.

Linearity

Linearity was plotted utilizing five-level calibration concentrations and was found to be within limits (1-16μg/ml) for dapagliflozin with a regression coefficient (*r2*) value of $\geq$0.999, as depicted in Figure 5. The slope and the intercept were observed to be 8400.5 and 1090.1, respectively, through the regression equation (Table 4).

Limits of Detection and Quantification

The detection limit and quantification limit of dapagliflozin were observed to be 0.049μg/ml and 0.1485μg/ml (Table 4), respectively, thereby confirming the sensitivity of the method.

System suitability

Suitability of the method for the regular analytical usage and validation shall be confirmed through parameters that shall give information of probable elution of analyte in regularity with the system, and the values for the respective parameters are reported in Table 4.

Robustness

For testing the robustness, variations in the experimental conditions like the composition of the mobile phase, detection wavelength, and flow rate showed no significant changes, and the results were reported in Table 5.

CONCLUSION

The developed and validated RP-HPLC method for dapagliflozin is believed to be compatible with further analysis using hyphenated techniques like LC-MS/MS. With reference to the ICH Q2R1 guidelines, values for the validation parameters were found to be within the acceptable limits confirming the method validity for analyzing dapagliflozin. Also, the present method was found to be accurate, precise, rapid, simple and sensitive. With a 98-102% recovery ability of the present method for dapagliflozin from pharmaceutical formulations, this method can make its way for its application in the pharmaceutical industries for frequent analysis of dapagliflozin, which is available in the form of bulk and pharmaceutical dosage forms.

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