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

Gouru Santhosh Reddy¹, Animesh Bera¹, Madhurima Basak¹, Krishnaveni Nagappan*¹, Ramalingam Peraman²

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

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 C18 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 7.029 minutes. 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 0.049 μg/ml and 0.1485 μg/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.

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 x 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 acetonitrile & made up the volume to 10ml to achieve a final concentration of 1.0mg/ml. From the
above stock solution, serial dilutions viz., 100 μg/ml, 10 μg/ml, and 1.0 μg/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 5 mg and 10 mg of formulation powder is taken into a 100 ml volumetric flask separately. The powdered formulation was dissolved in 75 ml of mobile phase and sonicated for 5 minutes using an Ultrasonicator to obtain a homogeneous solution, which was then made up to 100 ml with the mobile phase.

Then the above solution was filtered using a 0.45 μm nylon filter and diluted appropriately to obtain solutions with a concentration of 5 μg/ml and 10 μg/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 Q2(R1) 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 200-400 nm and obtained an absorption maximum at 277 nm, as depicted in Figure 2. The mobile phase has also been screened at 277 nm 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 ± 0.2 min with ideal run time at a flow rate of 1.0 ml/min using a C18 column as a stationary phase, acetonitrile and water (40:60%v/v) as a mobile phase, and 277 nm 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 0.45 μm 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.
Table 1: Accuracy studies of Dapagliftin

| S.No | Actual concentration ($\mu$g/ml) | Recovered concentration ($\mu$g/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 Dapagliftin

| S.No | Concentration ($\mu$g/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          | Dapagliftin                  |
|------|---------------------|------------------------------|
| 1.   | Retention Time (min)| 7.029 min                   |
| 2.   | Theoretical plates (N)| 3162.03                    |
| 3.   | Tailing Factor (T)  | 0.83                         |
| 4.   | Asymmetry Factor (As)| 1                            |
| 5.   | Regression Coefficient (r^2) | 0.9993                 |
| 6.   | Regression Equation | Y=8400.5x +1090.1            |
| 7.   | Linearity and Range | 1-16 $\mu$g/ml              |
| 8.   | Detection Limit (LOD)| 0.049 $\mu$g/ml             |
| 9.   | Quantification Limit (LOQ) | 0.1485 $\mu$g/ml       |

Table 5: Robustness studies

| Parameters          | Retention Time |
|---------------------|----------------|
| Mobile phase ratio (% v/v) | 7.031±0.2 |
| 58:42               | 7.029±0.2     |
| 60:40               | 7.025±0.2     |
| 62:48               | 7.022±0.2     |
| Wavelength (nm)     |                |
| 272                 | 7.022±0.2     |
| 277                 | 7.022±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     |
**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 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 (1-16 μg/ml) for dapagliflozin with a regression coefficient ($r^2$) value of ≥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 0.049 μg/ml and 0.1485 μg/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.

**REFERENCES**

Deepan, T., Dhanaraju, M. D. 2018. Stability indicating HPLC method for the simultaneous determination of dapagliflozin and saxagliptin in bulk and tablet dosage form. *Current Issues in Pharmacy and Medical Sciences*, 31(1):39–43.

Game, M. D., Naglaxmi, B. 2018. Development and Validation of Stability Indicating HPLC Method for Estimation of Dapagliflozin in Marketed Formulation. *International Journal of Pharmacy & Pharmaceutical Research*, 12(3):123–144.

Ghadir, A. K., Ismail, S., Mohammed, S. G., ., M. A. 2018. Validated RP-HPLC Method for Simultaneous Determination of Canagliptin, Dapagliflozin, Empagliflozin and Metformin. *International Journal of Pharmaceutical, Chemical and Biological Sciences*, 8(1):1–13.

ICH 2005. Validation of analytical procedures: text and methodology Q2 (R1). *International conference on harmonization*, pages 11–12.

Manasa, S., Dhanalakshmi, K., Reddy, N. G., Sreenivas, S. 2014a. Development and validation of stability-Indicating RP-HPLC method for determination of Dapagliflozin. *Journal of Advanced Pharmacy Education & Research*, 4(3):350–353.

Manasa, S., Dhanalakshmi, K., Reddy, N. G., Sreenivas, S. 2014b. Method Development and Validation of Dapagliflozin in API by RP-HPLC and UV-Spectroscopy. *International Journal of Pharmaceutical Sciences and Drug Research*, 6(3):250–252.

Mante, G. V., Gupta, K. R., Hemke, A. T. 2017. Estimation of Dapagliflozin from its Tablet Formulation by UV-Spectrophotometry. *Pharmaceutical Methods*, 8(2):102–107.

Nachiket, S. D., Priyanka, R. V., Ganesh, S. S., Priya,
S. R. 2019. Quantitative Estimation and Validation of Dapagliflozin and Metformin Hydrochloride in Pharmaceutical Dosage form by RP-HPLC. *Asian Journal of Research in Chemistry*, 12(3):2019–2031.

Olokoba, A. B., Obateru, O. A., Olokoba, L. B. 2012. Type 2 Diabetes Mellitus: A Review of Current Trends. *Oman Medical Journal*, 27(4):269–273.

Sano, M. 2018. A new class of drugs for heart failure: SGLT2 inhibitors reduce sympathetic overactivity. *Journal of Cardiology*, 71(5):471–476.

Sayali, More, S., Sandeep, Sonawane, S., Santhosh, Chhajed, S., Sanjay, J. 2018. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Saxagliptin and Dapagliflozin in Tablets. *Asian Journal of Pharmacy and Technology*, 8(3):2018–2026.

Verma, M. V., Patel, C. J., Patel, M. M. 2017. Development and stability-indicating HPLC method for dapagliflozin in API and pharmaceutical dosage form. *International Journal of Applied Pharmaceutics*, 9(5):33–41.