International Journal of Drug Development and Research

Debata et al., Int J Drug Dev & Res 2017, 9:2

Research Article

# A New RP-HPLC Method Development and Validation of Dapagliflozin in Bulk and Tablet Dosage Form

**Jitendra Debata[1]*, Sundeep Kumar[2], Sajal Kumar Jha[1] and Amjad Khan[1]**

[1]*School of Pharmacy, Guru Nanak Institutions Technical Campus, Hyderabad, Telangana, India*
[2]*Institute of Pharmacy and Technology, Hyderabad, Telangana, India*

***Corresponding author:** Jitendra Debata, School of Pharmacy, Guru Nanak Institutions Technical Campus, India, Tel: +918414202120; E-mail: Jitendra_debata@rediffmail.com

**Received** May 22, 2017; **Accepted** June 24, 2017; **Published** June 26, 2017

## Abstract

A new, simple, selective, accurate, rapid and precise reversed-phase high-performance liquid chromatographic technique of Dapagliflozin was established as per ICH Guidelines. RP-HPLC was performed on a Waters C18, 5 µm particle size, 25 cm × 4.6 mm i.d., with phosphate buffer and acetonitrile in the ratio of 60:40 v/v as a mobile phase and a flow rate of 1.0 ml min-1. UV detection was performed at 237 nm. Total run time was 6.0 min. The retention time of Dapagliflozin was found to be 3.461 minutes. Validation of the developed method was done as per USP and ICH guidelines. Method validation revealed that the method is rapid, accurate, precise, reliable, and reproducible. Linear calibration plots were obtained in the concentration range of 10-60 µg/ml for Dapagliflozin. Limit of detection were 0.02 µg/ml and limit of quantification were 0.06 µg/ml for Dapagliflozin. The high recovery and low coefficients of variation confirm the effectiveness of process in the dosage form. The validated method was successfully used for quantitative analysis of commercial available tablets.

**Keywords:** Dapagliflozin; RP-HPLC; Method development; Method validation; Accuracy; Precision

## Introduction

Dapagliflozin is a drug of the gliflozin class and it can be used to treat type 2 diabetes [1-4]. Dapagliflozin inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2) which are responsible for at least 90% of the glucose reabsorption in the kidney. Blocking this transporter mechanism causes blood glucose to be eliminated through the urine [5-7].

Dapagliflozin is chemically (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl}-6-(hydroxymethyl) oxane-3,4,5-triol. The molecular formula is $C_{21}H_{25}ClO_6$. The molecular weight is 408.873 g/mol. Dapagliflozin [8] is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, which should be purged with an inert gas. Soluble in Methanol and Dichloromethane.

The objective of the research work is to develop and validate a simple and accurate reverse phase chromatographic method to estimate amount of drug in dosage form.

The developed method successfully can be applied to estimate the amount of Dapagliflozin in tablet dosage form. The structure of Dapagliflozin was shown in Figure 1.

## Materials and Methods

### Materials

HPLC grade water, Acetonitrile, Methanol, Potassium dihydrogen orthophosphate, ortho phosphoric acid obtained from Sd fine-Chem ltd, Mumbai. Dapagliflozin was provided as a gift sample by SUN Pharmaceuticals limited [Mumbai, India].

### Instruments

The HPLC system employed was WATERS with Empower2 Software with Isocratic with UV-Visible Detector. The lambda max can be determined by ELICO SL-159 UV-Vis spectrophotometer.

### Preparation of phosphate buffer

6.8 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC grade water. pH was adjusted to 2.9 with Orthophosphoric acid.

### Preparation of mobile phase

Mix a mixture of above buffer 600 mL (60%) and 400 mL of acetonitrile HPLC grade (40%) and de gas in ultrasonic water bath for 15 minutes. Filter through 0.45 µ filter under vacuum filtration.

### Standard solution preparation

Twenty tablets were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well [9,10]. A quantity of powder of Dapagliflozin equivalent to 100 mg were transferred to clean and dry 100 ml volumetric flask and 70 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. One ml (1 ml) of the prepared stock solution diluted to 100 ml and was filtered through membrane filter (0.45 µm) and finally sonicated to degas.

### Sample solution preparation

25 mg of Dapagliflozin working standard was accurately weighed and transferred into a 25 ml clean dry volumetric flask. Add about 20 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution) [11]. Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Dapagliflozin working standard solution. The solution was mixed well and filtered through 0.45 µm filter.

## Results and Discussion

### Method development

Waters $C_{18}$, 5 µm, 25 cm × 4.6 mm i.d. used as a stationary phase, whereas phosphate buffer and acetonitrile in the ratio of 60:40 used a mobile phase. pH can be adjusted to 2.9 with orthophosphoric

acid and a flow rate of 1.0 ml/min with detection wavelength of 237 nm achieved the best separation of Dapagliflozin [12,13]. Standard solution of Dapagliflozin was prepared by using above procedure and the chromatograms were recorded (Figure 2). The retention time of Dapagliflozin was found to be 3.461 minutes.

## Assay

Average weight of twenty (20) tablets was taken as per I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Dapagliflozin equivalent to 100 mg were transferred to clean and dry 100 ml volumetric flask and 70 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. One ml (1 ml) of the prepared stock solution diluted to 100 ml and was filtered through membrane filter (0.45 µm) and finally sonicated to degas. From this prepared stock solution 10 ml was transferred to clean and dry 10 ml volumetric flask and make up the volume up to the mark with same solvent [14,15].

An aliquot of this solution was injected into HPLC system and the observation was recorded. Same like only a duplicate injection of the standard solution was also injected into the HPLC system and the peak area was recorded. By comparing the peak area from the standard, amount of Dapagliflozin present in the tablet dosage form can be estimated (Table 1).

## Method Validation

### Linearity

The standard graph was constructed covering a concentration range 10.0-60.0 µg/mL (three independent determinations were performed at each concentration). Linear relationships between the peak area signal of Dapagliflozin and the corresponding drug concentration were observed which was evaluated by linear regression analysis [16,17] the results were represented in Table 2. The standard deviation of the slope and intercept were low shown in Figure 3. A typical calibration curve has the regression equation of y=47789x+14111 and the regression coefficient of 0.995 (Figure 3) for Dapagliflozin.

### Accuracy

The accuracy study was performed for 80%, 100% and 120% for Dapagliflozin. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery [18]. Results demonstrates that the recovery of Dapagliflozin found to be in between 98.0% and 102% with less relative standard deviation (≤ 2%) (Table 3).

### Precision

**Repeatability:** The precision of this method was checked by repeatability of injection, repeatability intermediate precision and reproducibility. Peak areas and retention times obtained by determining six replicates of fixed amount of drug [19]. The %RSD of Dapagliflozin was calculated and presented in Table 4.

**Intermediate precision:** The high values of mean assay and low standard deviation of the drug revealed that the proposed method is precise [20]. The results of intermediate precision were shown in Table 5.

### Limit of detection and limit of quantification

Limits of detection (LOD) and limits of quantification (LOQ) represent the concentration of the analyte that would yield signal to noise ratios of 3 for LOD and 10 for LOQ respectively [21].

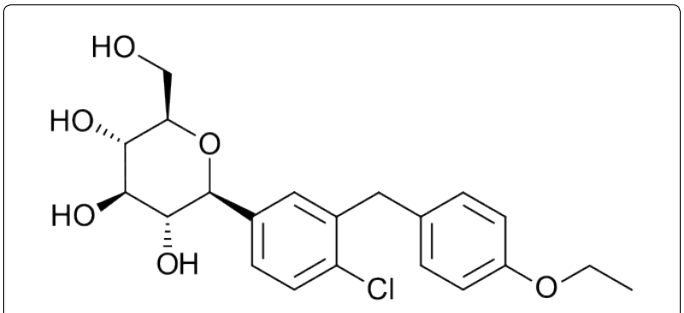


**Figure 1:** Structure of Dapagliflozin.

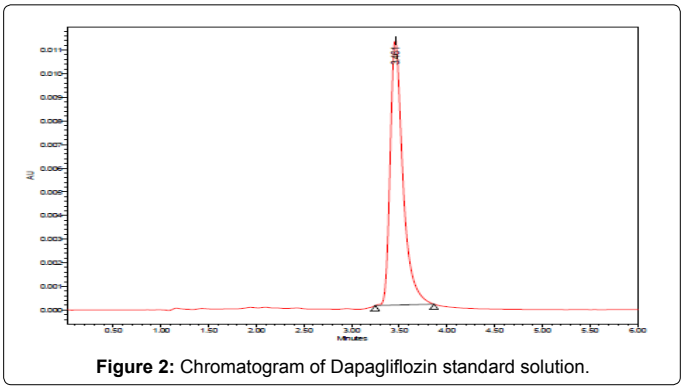

**Figure 2:** Chromatogram of Dapagliflozin standard solution.

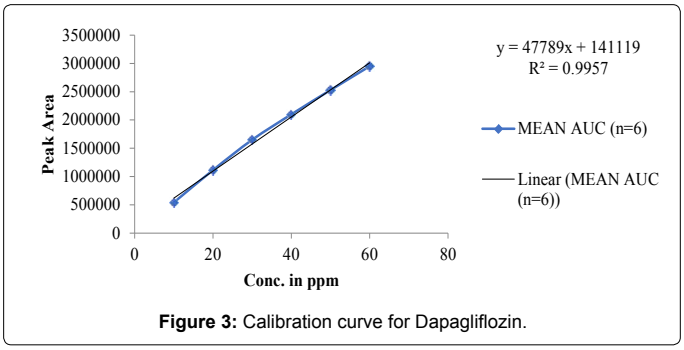


**Figure 3:** Calibration curve for Dapagliflozin.

To determine the limit of detection (LOD) and limit of quantification (LOQ) serial dilutions of mixed standard solution of Dapagliflozin was made from the standard stock solution and prepared in replicates of three. The samples were injected in HPLC system and measured signal from the samples was compared with those of blank samples.

### Robustness

Repeatability of sample application and measurement of peak area were carried out using three replicates of same concentration of standard and sample, respectively [22]. The results were shown in the Table 6.

## Conclusion

A new, simple, selective, accurate, rapid and precise reversed-phase high-performance liquid chromatographic technique of Dapagliflozin was established as per ICH guidelines Finally the developed RP-HPLC

| Brand name of tablets | Labelled amount of Drug (mg) | Mean (± SD) amount (mg) found by the proposed method (n=6) | % Purity |
|---|---|---|---|
| Forxiga tab (Astra Zeneca Pharmaceuticals LP.) | 5 | 5.16 (± 0.09) | 100.16% (± 0.48) |

**Table 1:** Quantitative estimation of Dapagliflozin in tablet dosage form.

| Conc. (µg/ml) | Mean AUC (n=6) |
|---|---|
| 10 | 542085 |
| 20 | 1114541 |
| 30 | 1649433 |
| 40 | 2099084 |
| 50 | 2527943 |
| 60 | 2949356 |

**Table 2:** Linearity data of Dapagliflozin.

| Accuracy level | Concentration (µg/ml) | | Area | %Recovery of Pure drug | Statistical Analysis |
|---|---|---|---|---|---|
| | Amount added | Amount found | | | |
| 80% | 8 | 7.89 | 391355 | 98.625 | Mean= 99.4167% SD=0.970932 %RSD=0.976629 |
| 80% | 8 | 8.12 | 402314 | 100.5 | |
| 80% | 8 | 7.93 | 393121 | 99.125 | |
| 100% | 10 | 10.19 | 501102 | 101.9 | Mean=100.90% SD=0.888819 %RSD=0.880891 |
| 100% | 10 | 10.06 | 495216 | 100.6 | |
| 100% | 10 | 10.12 | 498213 | 100.2 | |
| 120% | 12 | 12.05 | 590213 | 100.49 | Mean= 100.2167% SD=0.553203 %RSD=0.552007 |
| 120% | 12 | 11.95 | 585231 | 99.58 | |
| 120% | 12 | 12.07 | 591245 | 100.58 | |

**Table 3:** Accuracy data of Dapagliflozin.

| HPLC Injection Replicates of Dapagliflozin | Retention Time | Area |
|---|---|---|
| Replicate-1 | 3.464 | 1015245 |
| Replicate-2 | 3.463 | 1032541 |
| Replicate-3 | 3.464 | 1065223 |
| Replicate-4 | 3.463 | 1021345 |
| Replicate-5 | 3.462 | 1045872 |
| Replicate-6 | 3.461 | 1021745 |
| Average | 3.46283 | 1033662 |
| Standard Deviation | 0.001169 | 18864.4 |
| %RSD | 0.033756 | 1.825017 |

**Table 4:** Repeatability data of Dapagliflozin.

| 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 |
| 8 | 8.28 | 0.75 | 8.17 | 0.65 |
| 10 | 10.03 | 0.10 | 10.02 | 0.98 |
| 12 | 11.95 | 0.46 | 12.08 | 0.77 |

**Table 5:** Intermediate precision of Dapagliflozin.

| Change in parameter | %RSD |
|---|---|
| Flow (1.1 ml/min) | 0.57 |
| Flow (0.9 ml/min) | 0.59 |
| Temperature (27°C) | 0.24 |
| Temperature (23°C) | 0.27 |
| Wavelength of Detection (240 nm) | 0.91 |
| Wavelength of detection (229 nm) | 0.95 |

**Table 6:** Robustness data of Dapagliflozin.

method has excellent accuracy, precision and reproducibility.

The results suggested that developed method is suitable method for assay, purity which can help in the analysis of Dapagliflozin in different formulations.

**References**


1. Beckett AH, Stenlake JB (1988) Practical Pharmaceutical Chemistry: Part II. 4th edn. A&C Black 2: 275-298.
2. Quality Assurance (2003) Worth the effort. Inforum 7: 4.
3. Shethi PD (1997) Quantitative Analysis of Drugs in Pharmaceutical Formulations. 3rd edn., pp: 51-56.
4. Kasture K, Shethi PD (2001) Hand book of Pharmaceutical Analysis. High Performance Liquid Chromatography 1: 11.
5. Validation of Analytical Procedures, Methodology (1996) ICH Harmonized Tripartite Guidelines.
6. Text on Validation of Analytical Procedures (1994) ICH Harmonized Tripartite Guidelines.
7. Ravi S (2015) A Text book of Pharmaceutical Analysis. 3rd edn. p: 22.
8. Charles F, Lora L, Morton P, Leonard L (2004) Lexi-Comp's Drug Information Handbook. 12th edn.
9. Momin MY, Yeole PG, Puranik MP (2006) Institute of pharmaceutical Education and Research, Borgan (Meghe), India.
10. Snyder LR, Kirkland JJ, Glajch JL (2012) Practical HPLC method development. John Wiley & Sons.
11. Bioanalytical Method Validation (2001) US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM).
12. Center for Drug Evaluation and Research (1994) Reviewer Guidance; Validation of Chromatographic Methods ICH, Q1A (R2) Stability Testing of New Drug Substances and Products, Geneva.
13. Nagogi KVG, Kirankumar M, Vijayasrinivas S (2004) Indian J Pharm Sci 51: 129.
14. Pharmacopeia of India (1996).
15. FDA (2000) Guidance for Industry on Analytical Procedures and methods Validation Chemistry, Manufacturing, and Controls Documentation.
16. ICH (1999) Q6A: Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances, Geneva.
17. Green JM (1996) A practical guide to analytical method validation. Anal. Chem. News & Features, pp: 305A-309A.
18. Winslow PA, Meyer RF (1997) Defining a master plan for the validation of analytical methods. J Validation Technology, pp: 361-367.
19. Manasa S, Dhanalakshmi K, Nagarjuna G, Sreenivasa S (2014) Development and validation of a RP-HPLC method for the estimation of dapagliflozin in active principle ingredient 5: 5394-5397.
20. Sanagapati M, Dhanalakshmi K, Nagarjuna G, Sreenivasa S (2014) Method Development and Validation of Dapagliflozin in API by RPHPLC and UV-Spectroscopy. International Journal of Pharmaceutical Sciences and Drug Research 6: 250-252.
21. Jani BR, Shah KV, Kapupara PP (2015) Development and validation of UV spectroscopic method for simultaneous estimation of dapagliflozin and metformin hydrochloride in synthetic mixture. International Journal of Research and Development in Pharmacy and Life Sciences 4: 1569-1576.
22. Manasa S, Dhanalakshmi K, Nagarjuna G, Kavitha B (2014) Method Development and Validation of Dapagliflozin API by UV Spectroscopy. Int J Pharm Sci Rev 27: 270-272.