Estimation Method for Dapagliflozin in Bulk and Marketed Dosage Form: Development and Validation by UV-Spectroscopy

Saipranavi Vadla[1], Vaishnavi Putta[1], Saipriya Nadipudi[1], Sowmaya Bilakanti[1], Neelima Kudumula[1]*

[1]Department of Pharmaceutical Analysis, Sarojini Naidu Vanita Pharmacy Mahavidyalaya, Affiliated to Osmania University, Tarnaka, Hyderabad-500017, Telangana, India.

ABSTRACT

The present study aimed to develop a novel and sensitive method for spectrophotometric estimation in the UV region for the determination of dapagliflozin in its tablet formulation and to validate all analysis parameters according to ICH guidelines. Dapagliflozin was found to show its λ max at 220 nm using a UV-Vis spectrophotometer with a 1 cm quartz cell and methanol: water in the ratio of 15:85 for the preparation of stock solution (1000 µg/ml) and distilled water was used for further dilutions, for the preparation of working solutions. The technique used followed Beer's Lambert's law in the concentration range of 5–30 µg/ml, with a correlation value of 0.999. The limits of detection (LOD) and quantification (LOQ) were 0.623 µg/ml and 1.889 g/ml, respectively. The estimated percentage of the drug was nearly 103%, in good agreement with the marketed dosage form label (Udapa*10). Recovery experiments were carried out at three distinct levels, and the results were determined to be good. Furthermore, the findings of the methodologies devised for robustness and roughness are within their limitations. The suggested method is inexpensive, simple to use, and appropriate for regular analysis of dapagliflozin in bulk and commercial dose forms.

Key Words: *Dapagliflozin, UV-Spectrophotometer, Bulk and marketed dosage form, Validation*

eIJPPR 2023; 13(4):1-6

HOW TO CITE THIS ARTICLE: Vadla S, Putta V, Nadipudi S, Bilakanti S, Kudumula N. Estimation Method for Dapagliflozin in Bulk and Marketed Dosage Form: Development and Validation by UV-Spectroscopy. Int J Pharm Phytopharmacol Res. 2023;13(4):1-6. https://doi.org/10.51847/eyofcLxcp9

INTRODUCTION

Dapagliflozin is a Category III antidiabetic drug under the Biologics Classification System (BCS) of the European Medicines Agency (EMA). These inhibitors are a new class of antidiabetic drugs called flozins, which are more soluble and nearly impermeable [1]. It is a sodium-glucose co-transporter 2 (SGLT2) inhibitor that works largely by inhibiting glucose reabsorption from the liver, resulting in higher urine glucose excretion and, as a result, decreased blood sugar levels in type 2 diabetes patients. The medication has demonstrated an enhanced mode of action that is independent of insulin and only depends on plasma glucose and renal function. Dapagliflozin is a pill that is taken orally. It is particularly effective in the treatment of type 2 diabetes mellitus (DM) patients, both as a single agent and in combination with other anti-diabetic medications. Recent studies have shown that the fast action of dapagliflozin decreased the fasting plasma glucose levels within one week of treatment [2].

This is a crystalline white powder that is readily soluble [3] in methanol, ethanol, dimethylformamide, and dimethylsulfoxide. Chemically, it is (1S)-1, 5-Anhydro-1-[4-chloro-3-(4-ethoxybenzyl) phenyl]-D-glucitol with a molecular weight of 408.98 and a molecular formula of $C_24H_{33}ClO_8$. **Figure 1** depicts the chemical structure of dapagliflozin.

Corresponding author: Neelima Kudumula
Address: Department of Pharmaceutical Analysis, Sarojini Naidu Vanita Pharmacy Mahavidyalaya, Affiliated to Osmania University, Tarnaka, Hyderabad-500017, Telangana, India.
E-mail: ✉ Neelima_kudumula@yahoo.com
Received: 27 May 2023; **Revised:** 28 July 2023; **Accepted:** 08 August 2023

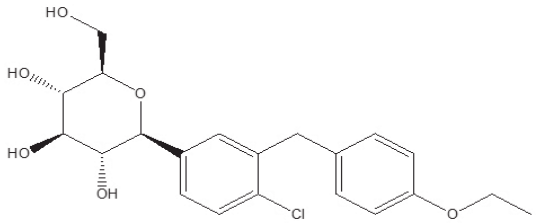

Figure 1. Chemical Structure of dapagliflozin

Literature review [4] and overview [5] deduce the drug from bulk and marketed formulations by UV spectroscopy [6-9] and RP-HPLC [10-18], UPLC [19, 20]. There have been only a couple of techniques published for UV spectrophotometric measurement of dapagliflozin alone and in combination with other drugs [21, 22], utilizing commercially available solvents and buffers. The present study aimed to develop a novel and sensitive method for spectrophotometric estimation in the UV region for the determination of dapagliflozin in its tablet formulation and to validate all analysis parameters according to ICH guidelines. This method was developed using methanol for solubilization and distilled water as the solvent for dilution. This is the most economical method of routine analysis and has not been previously reported based on a literature search performed before the work began. In addition, the newly proposed technique was validated for accuracy, precision, robustness, and linearity following ICH Q2 (R1) [23]. The results showed the reliability of the method.

MATERIALS AND METHODS

Equipment

The suggested study was conducted using a UV-1800 SHIMADZU and UV-3200 LAB INDIA UV-visible spectrophotometer with 1 cm quartz-matched cells. Weighing was done on an electronic balance (Shimadzu-BL220H), sonicated with Sonica Ultrasonic Cleaner, Spincotech PVT LTD.

Chemicals and reagents

Dapagliflozin-standard was acquired as a gift sample from Dr.Reddy Laboratories, Hyderabad, dapagliflozin-tablets (Udapa*10) label claim 10 mg produced by MSN Laboratories was purchased from the local market, and Analytical grade solvent-methanol was obtained from Rankem, Maharashtra, India.

Standard preparation

Considering that methanol was discovered to make the medicines soluble, the standard stock solution was produced by dissolving 10 mg of dapagliflozin in 1.5 ml of methanol and then increasing the volume to 10 ml with distilled water to reach a concentration of 1000 g/mL. A suitable dilution of the standard stock solution using distilled water was used to create the working standard solution, which contained 10 g/ml.

Determination of wavelength of maximum Absorption

The drug's maximum absorbance (max) was discovered to be 220 nm, as shown in **Figure 2** when 10 g/ml of standard dapagliflozin was scanned in a UV spectrophotometer between 190 and 300 nm.

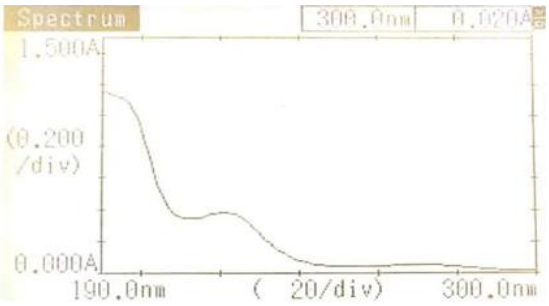

Figure 2. λ_{max} of dapagliflozin

Assay

The quantity of tablet powder equal to 10 mg of dapagliflozin was precisely weighed, transferred to a 100-ml volumetric flask, and solubilized by dissolving in 15 mL of methanol, sonicating for 10 minutes, and then diluting with distilled water to the mark to obtain a concentration of 100 g/ml, followed by filtering through a Whatman filter paper. A 10 ml volumetric flask containing 1 ml of the filtrate was filled with the mixture, which was then diluted with distilled water to achieve the final concentration of 10 g/ml. The results of measuring the absorption using a selected wavelength are displayed in **Table 1**.

Weight of 10 tablets = 2830 mg
10 tablets average weight = 2830/10 = 283 mg

$$Weight\ to\ be\ taken = \frac{Weights\ to\ be\ taken \times Equivalent\ weight}{Label\ claim} = \frac{283 \times 10}{10} = 283\ mg \quad (1)$$

$$Assay = 100 \times \frac{Absorbance\ sample}{Absorbance\ standard} \times \frac{Concentration\ standard}{Concentration\ sample} = 100 \times \frac{0.455 \times 10\ \mu g/ml}{0.441 \times 10\ \mu g/ml} = 103\% \quad (2)$$

Table 1. Percentage assay of dapagliflozin

S. No	Brand name	Available form	Label claim	Standard absorbance (10 µg/ml)	Sample absorbance (10 µg/ml)	Assay (%)
1	UDAPA*10	Tablets	10 mg	0.441	0.455	103

Method validation

The process of creating a narrative proof that a system, procedure, or movement has been put into use or tested and maintained the necessary level of consistency across all phases is known as methodology validation. Approved scientific strategies are essential for improving diagnostic techniques and have been time and again tested for specificity, linearity, accuracy, precision, range, limits of detection, and quantization cutoffs. In summary, the development and approval of a systematic strategy confirm that accurate and reliable potency estimation of medicinal products has been performed.

Validation parameters

To prove in writing that a method's performance complies with the demands of the intended analytical application, validation parameters are utilized. The purpose of the verification is to demonstrate that the analytical results obtained using a particular method are suitable for that purpose and are as specified below.

Method validation

The goal of this work was to provide a novel, simple, and affordable method for measuring dapagliflozin spectroscopically. Following the ICH recommendations, the method's linearity, accuracy, precision, and dependability were evaluated.

Linearity

The Linear relationships must be evaluated using a variety of analysis methods. For linearity to be established, at least five concentrations are advised. By diluting the standard stock solution using the suggested technique, it may be directly identified with the API. The correlation coefficient, the point of intersection with the ordinate, and the slope of the regression line should be provided.

Accuracy

Recovery tests were carried out by combining known quantities of the standard medication with formulation samples to verify the validity of the aforementioned procedure. Three distinct levels—50%, 100%, and 150%—were used for recovery tests.

Precision

The accuracy of the process is determined by the degree of agreement between the individual test results when it is employed for several samples of a homogeneous sample. The performance of feature parameters and the statistical processing of analytical data for both intraday and Interday are both necessary for the validation of analytical procedures. These procedures establish the analytical data's tolerance for variance. Typically, the variance, standard deviation, or coefficient of variation of a collection of measurements is used to describe the analytical procedure's accuracy.

Reproducibility

Reproducibility is also referred to as intra-assay precision. Reproducibility is defined as short-term accuracy under identical conditions of use.

Robustness

Robustness evaluation relies on the type of approach being researched and should be taken into account throughout the design process. The reliability of the assay concerning intentional changes to method parameters should be demonstrated.

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

Response standard deviation and determined by the linearity slope

The detection limit (DL) can be written as follows: DL = 3.3 σ/ S, where σ is the response's standard deviation and S is the calibration curve's slope. The limit of quantification (LOQ) can be written as LOQ = 10 σ/S, where σ is the response's standard deviation and S is the calibration curve's slope. The slope can be calculated using the analyte calibration curve.

RESULTS AND DISCUSSION

All validation parameters were carried out under the conditions mentioned in the ICH Q2 R (1) guidelines.

Linearity and range

The results from the linearity research were produced using five distinct aliquots of the reference solution and a chosen wavelength of 220 nm to test various concentrations (5, 10, 15, 20, and 25 g/mL). The limit of detection (LOD), limit of quantification (LOQ), and standard curve plot of the assay were also computed. The findings are presented in **Table 2**.

Table 2. Linearity of dapagliflozin in working standards.

S. No	Concentration in µg/ml	Absorbance
1	5	0.215
2	10	0.455

3	15	0.692
4	20	0.918
5	25	1.13
6	30	1.365
Standard deviation		0.4277
Correlation coefficient		0.999
Slope		0.045

Acceptance criteria: correlation coefficient (r^2) -0.999.

Dapagliflozin concentration was plotted on the X-axis, and absorbance was plotted on the Y-axis to create a calibration curve. **Figures 3 and 4** of the r^2 correlation reveal that a linear association was seen in the concentration range of 5-30 g/ml (r^2-0.999).

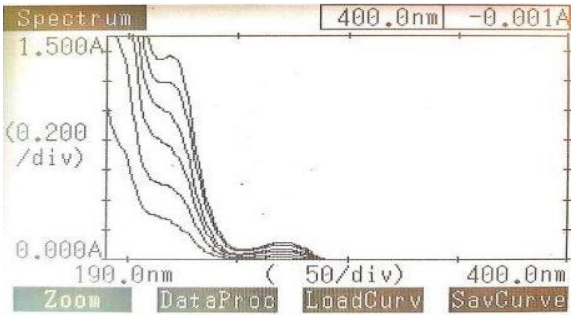

Figure 3. Overlay spectra of dapagliflozin of the concentrations used for linearity.

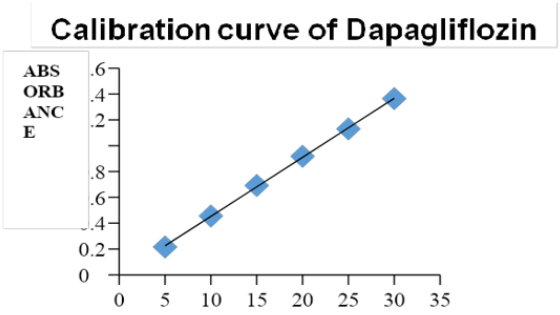

Figure 4. Linearity plot of dapagliflozin

Accuracy

Recovery studies evaluated the proposed accuracy. A known amount of the drug substance dapagliflozin (100 µg/ml) diluted from a stock solution was added to the pre-analytical tablet formulation (100 µg/ml) into a 10 ml volumetric flask and volume made up to 10 ml with distilled water. Analysis of dapagliflozin was performed at 50%, 100%, and 150% concentrations. The suggested procedure's recoveries were estimated. The results are displayed in **Table 3**.

Table 3. Accuracy readings of dapagliflozin

S. No	Concentration (µg/mL)		Final concentration (µg/ml)	Absorbance	Recovery (%)	Standard deviation	RSD (%)
	Sample volume (ml Taken)	Standard volume (ml Spiked)					
50%	0.4	0.1	5	0.224	98.46153846	0.001527525	**0.67%**
	0.4	0.1	5	0.226	99.34065934		
	0.4	0.1	5	0.227	99.78021978		
100%	0.4	0.2	6	0.271	99.26739927	0.001527525	**0.56%**
	0.4	0.2	6	0.272	99.63369963		
	0.4	0.2	6	0.274	100.3663004		
150%	0.4	0.3	7	0.317	99.52904239	0.00057735	**0.18%**
	0.4	0.3	7	0.317	99.52904239		
	0.4	0.3	7	0.318	99.84301413		

Precision

The accuracy of an analytical procedure is defined as the degree of agreement between a series of measurements obtained using the same homogeneous sample many times under particular circumstances. The findings are shown in **Table 4**.

Table 4. Precision studies data of dapagliflozin

Intraday precision		Interday precision	
Sample No	Absorbance	Day	Absorbance
1	0.439	Day 1	0.452
2	0.446	Day 2	0.449
3	0.454	Day 3	0.44
4	0.445	Day 4	0.449
5	0.441	Day 5	0.444
6	0.441		
Mean	0.4443333	**Mean**	0.4468
SD	0.0054283	**SD**	0.004764452
RSD (%)	**1.2216776**	**RSD**	**1.066349978**

	(%)

Acceptance criteria: less than 2
Equation-1: Standard deviation = $\sqrt{\Sigma(x-\bar{x})2/n}$
Equation-2: %RSD =$\sigma/\bar{x}$ *100

Robustness

Robustness is an indicator of dependability under normal circumstances since it quantifies the capacity to be unaffected by small but intentional adjustments in procedure parameters.

By adjusting the λmax and monitoring the generated drug concentrations' absorbance, this method was carried out. Equations 1 and 2 were used to obtain the standard deviation and percent RSD. The findings are displayed in **Table 5**.

Table 5. Robustness of dapagliflozin

Concentration (10 µg/mL)	Wavelength 220 NM	Wavelength 223 NM
	Absorbance	Absorbance
1	0.441	0.399
2	0.439	0.397
3	0.444	0.4
4	0.445	0.398
5	0.441	0.41
6	0.446	0.399
Mean	0.442666667	0.4005
SD	0.00273252	0.004764452
RSD (%)	**0.617286191**	**1.189625893**

Ruggedness

It determined the intraday and Interday accuracy of the approach. A repeatability study (intra-day) was performed by repeatedly evaluating the dapagliflozin solution (10 µg/ml) throughout the day. Dapagliflozin solution (10 µg/ml) was frequently examined on multiple days to get inter-day precision. Results are displayed in **Table 6**.

Table 6. Ruggedness of dapagliflozin

Concentration (10 µg/ml)	Day-1 Analyst-1	Day-1 Analyst-2	Day-2 Analyst-1	Day-2 analyst-2
	Absorbance	Absorbance	Absorbance	Absorbance
1	0.449	0.439	0.439	0.44
2	0.455	0.441	0.441	0.439
3	0.454	0.439	0.451	0.45
4	0.445	0.447	0.442	0.44
5	0.446	0.45	0.447	0.441
6	0.435	0.448	0.439	0.436
Mean	0.4473	0.444	0.443	0.441
SD	0.0072	0.00489	0.00483	0.0047
RSD (%)	1.628	1.1033	1.09	1.073

The limit of detection and limit of quantification

The formulas were used to calculate the limits of detection and quantification that are listed in **Table 7**.

$$LOD = (3.3\ X\ \sigma)\ /S \quad (3)$$

$$LOQ = (10\ X\ \sigma)\ /S \quad (4)$$

σ = standard deviation,
S = slope of the calibration curve.

Table 7. LOD and LOQ value for dapagliflozin

Name of the Drug	LOD (ppm)	LOQ (ppm)
Dapagliflozin	0.62362	1.88976

CONCLUSION

Dapagliflozin dose composition in tablet and bulk form was both examined. It was determined that the formulation's medication content was within acceptable limits. All validation parameters were tested following ICHQ2 (R1) criteria, and it was found that every parameter was within allowable ranges. As a result, the recommended method may be used to determine dapagliflozin concentration using a UV-visible spectrophotometer in both bulk and commercial dosage forms.

Acknowledgments: The authors are grateful to the authorities of Sarojini Naidu Vanita Pharmacy Mahavidyalaya, Hyderabad for providing the necessary facilities to carry out this research work.

Conflict of interest: None

Financial support: None

Ethics statement: None

REFERENCES

[1] Aswini R, Eswarudu MM, Srinivasa BP. A review of analytical methods for estimation of dapagliflozin and saxagliptin in bulk and in pharmaceutical dosage forms. Int J Res Pharm Chem. 2018;8(3):460-8. doi:10.13040/IJPSR.0975-8232

[2] Fioretto P, Giaccari A, Sesti G. Efficacy and safety of dapagliflozin, a sodium-glucose cotransporter 2 (SGLT2) inhibitor, in diabetes mellitus. Cardiovasc Diabetol. 2015;14(1):142. doi:10.1186/s12933-015-0297-x

[3] Mante GV, Hemke AT, Umekar MT. RP HPLC method for estimation of dapagliflozin from its tablets. Int J Chem Tech Res. 2018;11(01):242-8.
[4] Pathak S, Mishra P. A review on analytical methods of dapagliflozin: an update. Int J Pharm Qual Assur. 2020;11(3):355-60. doi:10.25258/IJPQA.11.3.9
[5] Suma BV, Deveswaran R. An overview on analytical methods for dapagliflozin - an antidiabetic drug. Int J Pharm Sci Res. 2019;10:2688-92. doi:10.13040/IJPSR.0975-8232.10(6).2688-92
[6] Ahmad S, Usman MR, Shaikh T, Imran M, Akhtar R. Development and validation of UV-spectrophotometric method for estimation of saxagliptin and dapagliflozin in bulk and dosage form. Int J Pharm Sci Res. 2021;12(4):2185-92. doi:10.13040/IJPSR.0975-8232
[7] Bhavyasri K, Navyasri V, Sumakanth M, Swethasri R. Method development and validation for the estimation of dapagliflozin in bulk and tablet dosage forms by UV visible spectroscopy. Int J Biol Pharm Allied Sci. 2020;10(3):010. doi:10.24327/ijrsr.2019.1008.3892
[8] Zameeruddin M, Bundel SS, Bharkad VB, Khan HN, Thoke S. Development and validation of UV spectroscopic method for simultaneous estimation of dapagliflozin and saxagliptin in synthetic mixture. Int J Pharm Anal Res. 2019;8:59-66.
[9] Bhadauria RS, Agarwal V. Development and validation of UV spectroscopic method for simultaneous estimation of dapagliflozin and saxagliptin in marketed formulation. J Drug Deliv Ther. 2019;9(4-s):1160-4. doi:10.22270/jddt.v9i4-s.3828
[10] Gaikwad AV, Gawade AS, Hupparage Vrushabh B, Mantry S, Kale A, Kale J. Method development and validation of dapagliflozin by RP-HPLC. J Pharm Negat Res. 2022;13(6):4316-35. doi:10.47750/pnr.2022.13.S06.571
[11] Vankalapati KR, Alegete P, Boodida S. Stability-indicating HPLC method development and validation for simultaneous estimation of metformin, dapagliflozin, and saxagliptin in bulk drug and pharmaceutical dosage form. Biomed Chromatogr. 2022;36(7):e5384. doi:10.1002/bmc.5384
[12] Verma MV, Patel CJ, Patel MM. Development and stability indicating HPLC method for dapagliflozin in API and pharmaceutical dosage form. Int J Appl Pharm. 2017;9(5):33-41.
[13] Game MD, Bopudi M. Development and validation of stability indicating HPLC method for estimation of dapagliflozin in marketed formulation. Int J Pharm Sci. 2018;12:123-44.
[14] Vinutha K, Chowdary KPR, Prasad SVUM. Development of new stability indicating RP-HPLC method for simultaneous estimation of saxagliptin and dapagliflozin and its validation as per ICH guideline. Indo Am J Pharm Sci. 2017;4(09):2920-32.
[15] Patel KJ, Chaudhary AB, Bhadani SM, Raval RJ. Stability indicating RP-HPLC method development and validation for estimation of dapagliflozin and metformin HCL. World J Pharm Pharm Sci. 2017;6(9):796-809. doi:10.20959/wjpps20179-9638
[16] Manasa M, Aanandhi VM. Stability indicating simultaneous method development and validation of dapagliflozin and saxagliptin by RP-HPLC. Res J Pharm Technol. 2021;14(2):1045-9. doi:10.5958/0974-360X.2021.00187.6
[17] Bhavyasri K, Surekha T, Begum S, Sumakanth M. RP-HPLC method for dapagliflozin and metformin HCL in bulk and combined formulation. Arch Pharm Pract. 2021;12(4):107. doi:10.51847/Czx10wYrYr
[18] Debata J, Kumar S, Jha SK, Khan A. A new RP-HPLC method development and validation of dapagliflozin in bulk and tablet dosage form. Int J Drug Dev Res. 2017;9(2):48-51.
[19] Madhavi S, Rani AP. Development and validation of a method for simultaneous determination of dapagliflozin and saxagliptin in a formulation by RP-UPLC. World J Pharm Res. 2017;6(12):904-16.
[20] Patel AB, Vaghasiya EH, Dudhatra AR, Vyas AJ, Patel AI, Patel NK, et al. Stability indicating photodiode array detector based estimation of dapagliflozin propanediol monohydrate in API and tablet dosage form by RP-UPLC. Res J Pharm Technol. 2021;14(9):4635-9. doi:10.52711/0974-360X.2021.00805
[21] Ukibayev J, Datkhayev U, Myrzakozha D, Frantsev A, Karlova E, Nechepurenko Y, et al. Rectal methods of delivery of medical drugs with a protein nature in the therapies of tumor disease. J Adv Pharm Educ Res. 2021;11(1):18-22.
[22] Sinuraya RK, Rianti A, Suwantika AA. Cost minimization of cardiovascular disease (CVD) drugs in primary healthcare centers in Bandung, Indonesia. J Adv Pharm Educ Res. 2021;11(1):63-9.
[23] ICH I. Q2 (R1): Validation of analytical procedures: text and methodology. International conference on harmonization, Geneva 2005 Nov (pp. 1-13).