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UV-Spectrophotometric Method Development and Validation for Quantifying Dapagliflozin in Bulk and Pharmaceutical Formulations

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ABSTRACT

This research focused on establishing a new and highly sensitive UV-spectrophotometric approach for quantifying dapagliflozin in tablet dosage form while ensuring comprehensive validation of analytical parameters following ICH guidelines. The maximum absorbance (λ_{max}) of dapagliflozin was identified at 220 nm using a UV-Vis spectrophotometer equipped with a 1 cm quartz cell. A stock solution of 1000 µg/ml was prepared using a solvent system consisting of methanol and water in a ratio of 15:85, and subsequent dilutions were performed using distilled water to obtain working solutions. The analytical method adhered to Beer's Lambert's law across a concentration range of 5–30 µg/ml, yielding a correlation coefficient of 0.999. Sensitivity parameters included a limit of detection (LOD) of 0.623 µg/ml and a limit of quantification (LOQ) of 1.889 µg/ml. The estimated drug content was approximately 103%, aligning well with the labeled claim of the marketed formulation (Udapa*10). Recovery studies were performed at three different levels, which showed satisfactory results. Furthermore, robustness and ruggedness evaluations showed that the method remained within acceptable limits. Given its simplicity, cost-effectiveness, and reliability, this method is well-suited for routine quantitative assessment of dapagliflozin in bulk and commercial pharmaceutical products.

Keywords: Validation, Dapagliflozin, Bulk and marketed dosage form, UV-Spectrophotometer

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Introduction

Dapagliflozin falls under category III of the Biopharmaceutics Classification System (BCS) as defined by the European Medicines Agency (EMA). This class of antidiabetic agents, known as flozins, exhibits high solubility but low permeability [1]. As a sodium-glucose co-transporter 2 (SGLT2) inhibitor, dapagliflozin primarily reduces glucose reabsorption in the kidneys, leading to increased glucose excretion through urine and subsequent blood sugar reduction in type 2 diabetes patients. Unlike insulin-dependent treatments, its mechanism relies solely on plasma glucose levels and kidney function. Taken orally, it is effective for type 2 diabetes mellitus (DM) management, either alone or alongside other hypoglycemic agents. Studies have reported a significant reduction in fasting plasma glucose within the first week of administration [2].

This compound appears as a crystalline white powder and dissolves readily [3] in solvents such as methanol, ethanol, dimethylformamide, and dimethylsulfoxide. Chemically, it is identified as (1S)-1, 5-anhydro-1-[4-chloro-3-(4-ethoxybenzyl) phenyl]-D-glucitol, with a molecular weight of 408.98 and the molecular formula C24H33ClO8. Its chemical structure is presented in **Figure 1**.

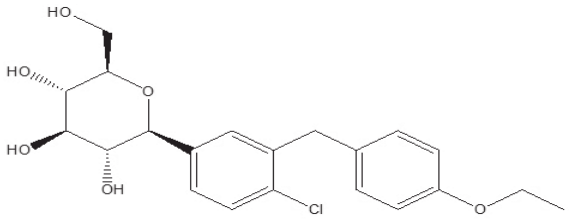

Figure 1. Chemical structure of dapagliflozin

A review of the literature [4] and existing studies [5] highlights the quantification of the drug from bulk and commercial formulations through UV spectroscopy [6-9], RP-HPLC [10-18], and UPLC [19, 20]. Limited methods have been reported for the UV spectrophotometric analysis of dapagliflozin, either alone or in combination with other pharmaceutical agents [21, 22], utilizing readily available solvents and buffering systems. This study was designed to introduce a novel and highly sensitive UV spectrophotometric technique for estimating dapagliflozin in tablet formulations, ensuring comprehensive validation of analytical parameters following ICH guidelines. Methanol was employed for solubilization, while distilled water served as the dilution medium. This approach represents the most cost-effective option for routine analysis and, based on a thorough literature review conducted before this research, has not been previously documented. Furthermore, the newly developed method underwent validation for key analytical attributes, including accuracy, precision, robustness, and linearity, following ICH Q2 (R1) guidelines [23]. The validation outcomes confirmed the method's reliability.

Materials and Methods

Instruments used

The study utilized a UV-1800 Shimadzu and UV-3200 Lab India UV-Vis spectrophotometer, both equipped with 1 cm quartz cells for analysis. Weighing of materials was performed using a Shimadzu-BL220H electronic balance, while sample sonication was carried out with a Sonica ultrasonic cleaner from Spincotech PVT LTD.

Chemicals and reagents

A pure dapagliflozin reference sample was generously provided by Dr. Reddy's Laboratories, Hyderabad. Marketed dapagliflozin tablets (Udapa*10), each containing 10 mg of the drug and manufactured by MSN Laboratories, were purchased from a local supplier. Analytical-grade methanol was obtained from Rankem, Maharashtra, India, for use in the study.

Preparation of standard solution

Given that methanol effectively dissolves the drug, a stock solution was formulated by dissolving 10 mg of dapagliflozin in 1.5 ml of methanol and then diluting the solution to a final volume of 10 ml with distilled water, resulting in a concentration of 1000 g/mL. A working standard solution with a concentration of 10 g/ml was prepared by further diluting the stock solution using distilled water.

Identification of maximum absorption wavelength

A UV spectrophotometric scan of a 10 g/ml dapagliflozin solution over a wavelength range of 190–300 nm revealed that the drug exhibited its peak absorbance (λ_{max}) at 220 nm, as depicted in **Figure 2**.

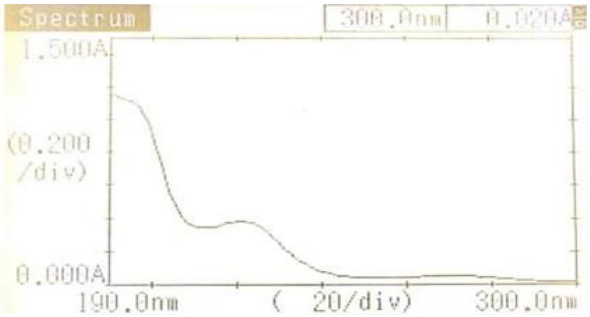

Figure 2. λ_{max} of dapagliflozin

Assay procedure

An amount of tablet powder corresponding to 10 mg of dapagliflozin was carefully weighed and placed into a 100-ml volumetric flask. The sample was dissolved in 15 mL of methanol and sonicated for 10 minutes. Afterward, the solution was diluted with distilled water up to the 100 ml mark to achieve a concentration of 100 g/ml. The solution was filtered through the Whatman filter paper. From this filtered solution, 1 ml was transferred into a 10 ml volumetric flask, and distilled water was added to bring the volume up to 10 ml, resulting in a final concentration of 10 g/ml. The absorption values obtained at the chosen wavelength are presented in **Table 1**.

Weight of 10 tablets = 2830 mg

10 tablets average weight = 2830/10 = 283 mg

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

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

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

Method validation refers to the process of establishing documented evidence that a system, procedure, or process has been implemented, tested, and consistently maintained across all stages. Validated scientific methods are crucial for enhancing diagnostic procedures and have been rigorously tested for parameters such as specificity, accuracy, linearity, precision, range, detection limits, and quantification thresholds. Ultimately, the development and approval of a structured approach ensure that the potency estimation of pharmaceutical products is both precise and dependable.

Validation parameters

Validation parameters are employed to provide evidence that a method's performance meets the requirements of the intended analytical purpose. Validation aims to confirm that the analytical results generated using a particular method are appropriate for the intended application, as outlined below.

Method validation

The objective of this study was to introduce a straightforward, cost-effective, and novel spectroscopic method for quantifying dapagliflozin. In line with ICH guidelines, the method's linearity, precision, accuracy, and reliability were evaluated.

Linearity

Various analytical techniques must be used to assess linearity. To confirm linearity, at least five different concentrations are recommended. The method's linearity is established by directly correlating the standard stock solution to the active pharmaceutical ingredient (API) via dilution. Key parameters, including the correlation coefficient, y-intercept, and slope of the regression line, should be recorded.

Accuracy

To verify the method's validity, recovery studies were performed by adding known amounts of the standard drug to formulation samples. Recovery tests were conducted at three different levels: 50%, 100%, and 150%.

Precision

Precision is evaluated by the degree of consistency between repeated measurements of the same sample. For validation, both intraday and interday precision must be determined by analyzing the performance of the method and conducting statistical analysis of the results. Tolerance for variation in the analytical data is established by calculating variance, standard deviation, or coefficient of variation.

Reproducibility

Reproducibility, or intra-assay precision, refers to the consistency of results under identical conditions over short periods. It assesses the method's reliability under repeated tests.

Robustness

The robustness of a method is evaluated by intentionally altering certain method parameters. The robustness test ensures the method's reliability despite these deliberate changes in conditions.

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

LOD and LOQ are determined based on the standard deviation of the response and the slope of the calibration curve. The formulas for LOD and LOQ are as follows:

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

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

Where σ is the standard deviation of the response and S is the slope of the calibration curve. The slope is derived from the analyte's calibration curve.

Results and Discussion

The validation process adhered to the ICH Q2 R (1) guidelines.

Linearity and range

The linearity test was conducted using five different concentrations (5, 10, 15, 20, and 25 g/mL) of the reference solution. Measurements were taken at a wavelength of 220 nm. Additionally, the limit of detection (LOD), limit of quantification (LOQ), and standard curve were calculated, with the results summarized 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.

A calibration curve was established by plotting dapagliflozin concentration against absorbance. As illustrated in **Figures 3 and 4**, a strong linear relationship was observed across the concentration range of 5-30 g/ml, with a correlation coefficient (r^2) of 0.999.

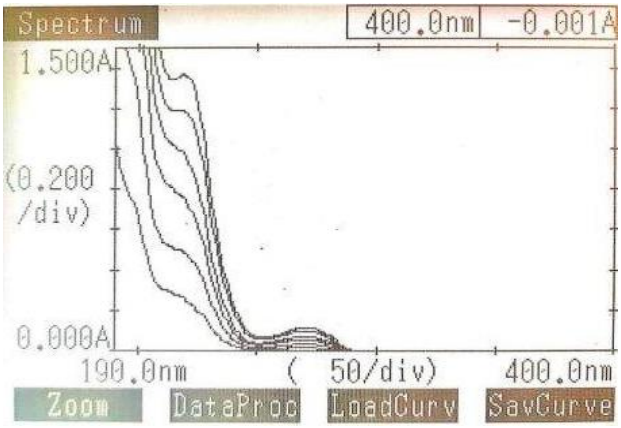

Figure 3. Overlay spectra of dapagliflozin of the concentrations used for linearity

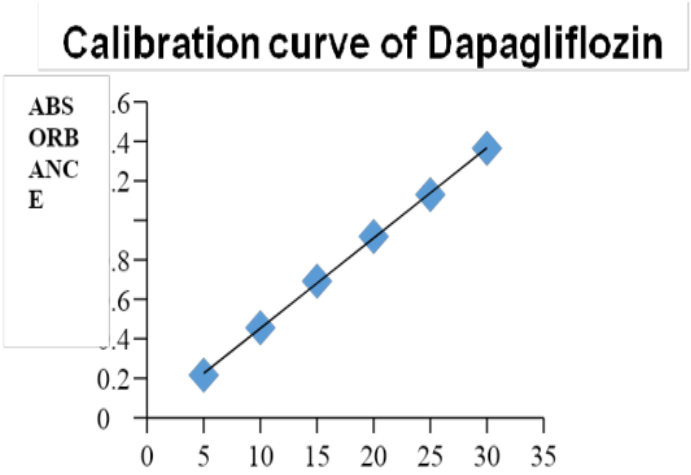

Figure 4. Linearity plot of dapagliflozin

Accuracy

The accuracy of the proposed method was assessed through recovery studies. A known quantity of dapagliflozin (100 µg/ml) was taken from a stock solution and mixed with the pre-analytical tablet formulation (100 µg/ml) in a ten ml volumetric flask, with the volume adjusted to 10 ml using distilled water. Dapagliflozin was analyzed at concentrations of 50, 100, and 150%. The recoveries obtained using the outlined procedure were calculated, and the results are presented 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

Precision is the measure of how closely multiple results align when the same sample is analyzed repeatedly under the same conditions. The corresponding findings are provided 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: < 2

Equation (1): Standard deviation = $\sqrt{\Sigma(x-\bar{x})2/n}$

Equation (2): % RSD = $\sigma/\bar{x} * 100$

Robustness

Robustness reflects the reliability of a method under typical conditions, assessing its ability to remain unaffected by small, intentional changes in procedural parameters. The method was executed by varying the λ_{max} and measuring the absorbance of the resulting drug concentrations. The standard deviation and percent relative standard deviation (RSD) were calculated using Equations (1) and (2). The results are shown 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

The ruggedness of the method was assessed by evaluating both intraday and interday precision. To test intraday repeatability, the dapagliflozin solution (ten µg/ml) was analyzed multiple times within a single day. For interday precision, the same solution was tested on different days. The findings are provided in **Table 6**.

Table 6. Ruggedness of dapagliflozin

	Day-1		**Day-2**	
Concentration (10 µg/ml)	**Analyst-1 Absorbance**	**Analyst-2 Absorbance**	**Analyst-1 Absorbance**	**Analyst-2 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

Detection and quantification thresholds

The detection and quantification limits were calculated using the specified formulas, and the outcomes are summarized in **Table 7**.

$$LOD = (3.3 \times \sigma) / S \quad (5)$$

$$LOQ = (10 \times \sigma) / S \quad (6)$$

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

The dapagliflozin content in both tablet and bulk forms was analyzed, and it was confirmed that the medication content of the formulation met the required standards. All validation criteria were evaluated according to ICHQ2 (R1) guidelines, and the results showed that each parameter fell within acceptable limits. Therefore, the proposed method is suitable for determining the concentration of dapagliflozin using a UV-visible spectrophotometer in both bulk and commercial preparations.

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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).