STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN IN BULK AND TABLET DOSAGE FORM BY UV SPECTROPHOTOMETRY

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ABSTRACT

Dapagliflozin is an anti-diabetic drug that works on the kidneys of reabsorption of glucose in kidneys by the sodium-glucose co-transporter2 (SGLT2). It is used in patients with type 2 diabetes. It is administered as tablets. The objective of the present work is to develop a novel simple and economic method for the quantification of dapagliflozin in bulk drug and in tablet formulation. Further this study is designed to validate the developed methods as per ICH guidelines. The quantification process was performed on UV-spectrophotometer. Different analytical performance parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) repeatability, stability studies were determined according to ICH guidelines. The solutions of standard and sample were prepared in methanol after suitable dilutions. 100µg/ml of the Dapagliflozin was prepared and scanned in the UV visible range 400 to 200nm. In the quantitative determination of the drug was carried at selected wavelength 224nm and the linearity range was formed to be 1 to 10µg/ml and r^2 (0.9995). LOD and LOQ for dapagliflozin was found to be 0.0262µg/ml and 0.079µg/ml. The proposed method can be adopted for routine quality control for estimation of drug in formulation.

Key words: Dapagliflozin, spectrophotometric method, validation, linearity, precision.

INTRODUCTION

Validation and quality assurance will go hand in hand, ensuring the through quality for the products. Validation is the most widely used word in the areas of drug development, manufacturing and specification of finished products. The consistency and reliability of a validated process to produce a quality product is the very important for an industry.

Dapagliflozin is chemically - (2S,3R,4R,5S,6R)-2-[4-Chloro-3-(4-ethoxybenzyl) phenyl]-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol was illustrated in Fig. 1. Dapagliflozin, an oral anti-diabetes drug which is sold under the brand name of Farxiga. It is mainly used to treat type 2 diabetes with certain limitations, type 1 diabetes. It is also used to treat patients with heart diseases like heart failure and turning down their need to be in the hospital. Dapagliflozin is highly sensitive, orally active and comes under the class of SGLT2 (Sodium-glucose co-transporter-2) inhibitor. The analysis of dapagliflozin was achieved by using double beam UV-Visible spectrophotometer. Method validation parameters like specificity, linearity and range, precision, accuracy, solution stability, limit of detection, limit of quantification, robustness, ruggedness and drug stability studies such as acid hydrolysis, alkaline hydrolysis, neutral hydrolysis, photolytic degradation, degradation under dry heat, oxidative hydrolysis were performed. Assay of dapagliflozin was performed by using Dapasmart 5mg commercially available tablet dosage form. Stability and validation procedures are performed as per ICH guidelines.

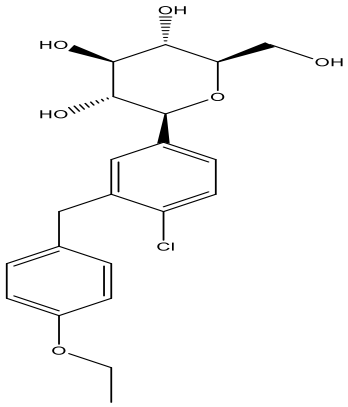

Fig. 1: Chemical structure of dapagliflozin

EXPERIMENTAL CONDITIONS

Reagents and chemicals

API of dapagliflozin was given as a gift sample from Hetero Labs Pvt. Ltd., Hyderabad. Farxiga (Dapasmart) marketed formulation has been purchased from local pharmacy, Hyderabad. Methanol and water were purchased from Rezon Scientific and UV Scientifics, Hyderabad.

Instrumental conditions

The method that has been carried out is UV spectrophotometric method. The instruments used were Ultra Sonicator purchased from Life care Equipment's Pvt Ltd., model 2k811056, Digital Electronic Balance and UV-Visible Spectrophotometer from Shimadzu UV-1800.

Preparation of Standard Solution

Standard stock solution of dapagliflozin was prepared by dissolving 1mg of drug in 10ml of methanol and was sonicated for proper dilution of drug in ultra sonicator for about 15 min. The concentration of above prepared solution of dapagliflozin is 100µ/ml.

Preparation of sample solution

Appropriate known volumes of aliquots from first dilution were transferred to separate 10ml volumetric flasks. The volume was adjusted to the mark with methanol to a series of concentration in the range of 1-10µg/ml. The solution was scanned in the UV range 200-400nm. Absorbance of these solutions were recorded at 224nm and calibration curve was plotted, absorbance vs concentration as shown in Fig. 2.

Method Development

Selection of solvent: Dapagliflozin is tested for their solubility in different solvents. The solution of dapagliflozin was prepared in methanol, ethanol and water. UV spectra of each was recorded and scanned between 200-400nm. Among these solvents methanol gave good response. Hence, methanol was selected as solvent for further studies.

Selection of wavelength (λ_{max}): The stock solution was prepared for dapagliflozin and were scanned in UV region 200-400nm. The peak was obtained at a wavelength of 224nm against methanol as blank. The wavelength of 224nm was selected for dapagliflozin for further studies.

Method Validation

Linearity: Standard solutions of Dapagliflozin were prepared in the concentration range of 1-10µg/ml. The volume in each volumetric flask were made with methanol and mixed. Calibration curves were plotted by taking concentration on X-axis and absorbance on Y-axis as shown in Fig. 3. The correlation coefficient was found to be 0.9979 at 224nm. The slope

was found to be 0.0693 and intercept was found to be 0.0102 at 224nm as shown in Fig. 4 and Table 1.

Range: The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of the analyte in the sample (including these concentrations) for which it has been shown that the analytical procedure has a suitable level of precision, accuracy and linearity.

Accuracy: The accuracy studies were carried out at three different levels i.e., at 80%, 100% and 120% levels. To ensure the reliability of the above method recover studies were carried out by mixing a known quantity of the standard drug with the pre analysed sample formulation and the contents were reanalysed by the proposed method and the results were shown in Table 2.

Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions.

Intraday precision: Intraday precision was found by carrying out the analysis of the intermediate concentration for 10 times on the same day. Mean and standard deviation were then calculated. The results are shown in Table 3.

Interday precision: Intraday precision was found by carrying out the analysis of the intermediate concentration for 10 times for 5 different days. Mean and standard deviation were then calculated. The results were shown in Table 4.

Repeatability: Repeatability was found by carrying out the analysis of the intermediate concentration for 10 times on the same day for every 5 min. Mean and standard deviation were then calculated. The results were shown in Table 5.

Limit of Detection (LOD): The LOD of dapagliflozin was determined by using standard deviation of response and slope. The experimental LOD was found to be 0.026208696.

Limit of Quantification (LOQ): The LOQ of dapagliflozin was determined by using standard deviation of response and slope. The experimental LOQ was found to be 0.7942029.

Ruggedness: Ruggedness of the method was determined on carrying out the method by two different analysts, labs and instruments. The results are shown in Table 6.

Robustness: The robustness of developed method is its capacity to remain unaffected by small changes in condition. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ±2nm. The results are shown in Table 7.

Specificity: Specificity is the ability to access unequivocally the analyte in the presence of components that maybe expected to be present. Specificity is determined by analysing 10µg/ml concentration repeatedly and measuring the absorbance at 224nm wavelength. The specificity of the method for determination of dapagliflozin in tablet dosage form was determined by comparing the spectrum of tablet solution with that of standard solution. The sample spectrum was checked for any interference from the excipients. The results are shown in Table 8.

Assay of formulation: Twenty Dapagliflozin tablets (Dapasmart) each containing 5mg of dapagliflozin were weighed, average weight was calculated and powdered. A quantity equivalent to 5mg of dapagliflozin is weighed and transferred into 50ml volumetric flask and is dissolved in methanol to obtain final concentration of 1000µg/ml. The solution was further diluted with methanol to get a solution having concentration of 10µg/ml of dapagliflozin.

This procedure was repeated at different concentrations of 1ppm and 5ppm. The absorbance was measured at wavelength 224nm. A graph has been plotted with Absorbance on X-axis and Concentration on Y-axis. Then the amount of drug present in the formulation was calculated and results were reported in Table 9. The slope was found to be 0.0483 and intercept was found to be 0.0247 at 224nm.

Drug stability studies:

Acid hydrolysis: To 10mg of drug add 5ml of methanol and make up the volume to 10ml with 0.01N HCl. The above solution was kept for reflux for 2hrs. After the exposure, pipette out 1ml from the above solution to 10ml of volumetric flask and make up to 10ml with methanol to get the final concentration 10µg/ml. Now, observe under UV at wavelength 224nm. The result is shown in Fig. 5.

Alkaline hydrolysis: To 10mg of drug add 5ml of methanol and make the volume to 10ml with NaOH. The above solution was kept for reflux for 2hrs. After exposure, pipette out 1ml from the above solution to 10ml volumetric flask and make up the volume to 10 ml with methanol to get the final concentration 10µg/ml. Now, observe under UV at wavelength 224nm. The result is shown in Fig. 6.

Neutral hydrolysis: To 10mg of drug add 5ml of methanol and make the volume to 10ml with water. The above solution was kept for reflux for 2hrs. After exposure, pipette out 1ml from the above solution to 10ml volumetric flask and make up the volume to 10ml with methanol to get the final concentration 10µg/ml. Now, observe under UV at wavelength 224nm. The result is shown in Fig. 7.

Photolytic degradation: The photo degradation study of the drug was carried out by exposing the drug to sunlight by keeping 10mg of drug in petri dish for 2hrs. After exposure, 10mg of drug was transferred to volumetric flask and further diluted to make a concentration of 10µg/ml with methanol. Now, observe under UV at wavelength 224nm. The result is shown in Fig. 8.

Degradation under dry heat: Dry heat studies were performed by keeping 10mg of drug sample in hot air oven (80°C) for a period of 2hrs. A sample was withdrawn and transferred to volumetric flask and further diluted to make a concentration of 10µg/ml with methanol. Now, observe under UV at wavelength 224nm. The result is shown in Fig. 9.

Oxidative hydrolysis: To 10mg of drug add 10ml of H_2O_2 solution (1000µg/ml). The above solution was kept for 2hrs at dark place. After exposure the volume was diluted to make a concentration of 10µg/ml with methanol. Now, observe under UV at wavelength 224nm. The result is shown in Fig. 10. The results of complete degradation studies of dapagliflozin were shown in Table 10.

RESULTS AND DISCUSSION

The analysis of dapagliflozin was achieved by using double beam UV-Visible spectrophotometer. The linearity was checked in different concentrations and Beer's law was obeyed in the concentration range of 1-10µg/ml for dapagliflozin. The slope, intercept and correlation coefficient values of dapagliflozin are 0.0693, 0.0102 and 0.9979 respectively.

The precision studies were carried in terms of intraday, interday and repeatability. The percentage relative standard deviation (%RSD) values were found to be less than 2, which indicate that the method is precise.

The recovery studies were carried out to ensure that reproducibility and reliability of the method by adding known amount of standard drugs and analysis was carried out as per formulation procedure. The recovery values were within the limits indicating that the method is accurate.

The robustness and ruggedness were carried out and was found to be within the limits. LOD and LOQ were carried out according to ICH guidelines and were found to be 0.026 and 0.79.

In order to test the appropriateness of the developed method to the pharmaceutical formulation, an assay of dapagliflozin tablets was performed at working concentration.

Acid hydrolysis, alkaline hydrolysis, neutral hydrolysis, photolytic degradation, degradation under dry heat and oxidative hydrolysis were performed for dapagliflozin and the drug stability was found to be 100%.

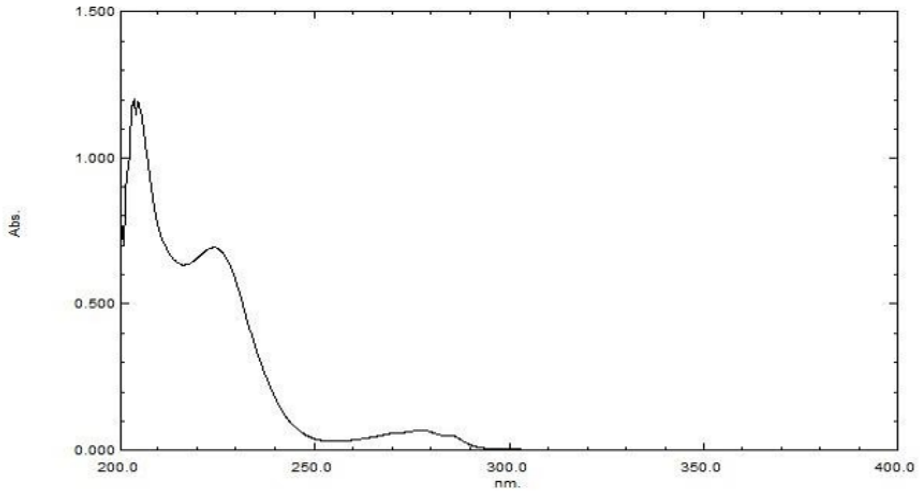

Fig. 2: Overlain normal spectra of dapagliflozin in methanol

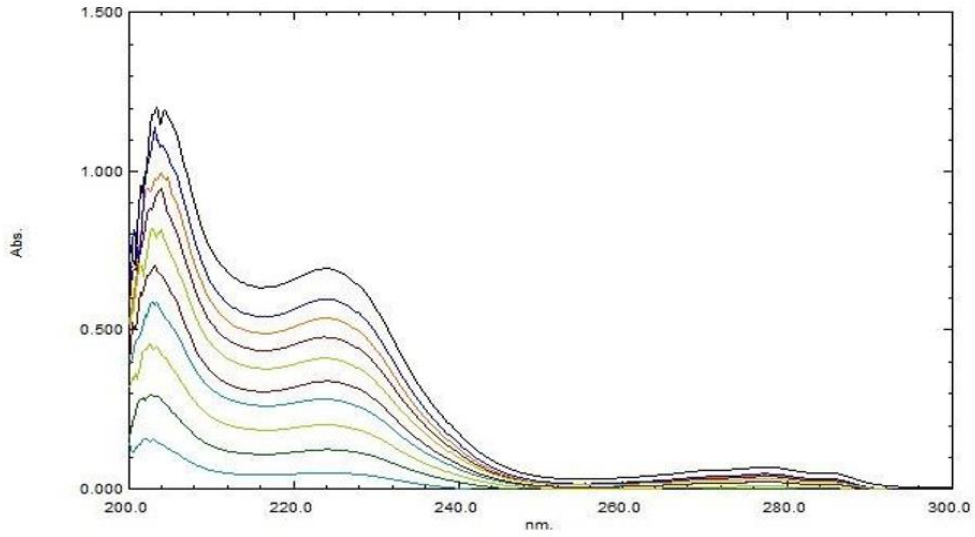

Fig. 3: Overlain linearity spectra of dapagliflozin

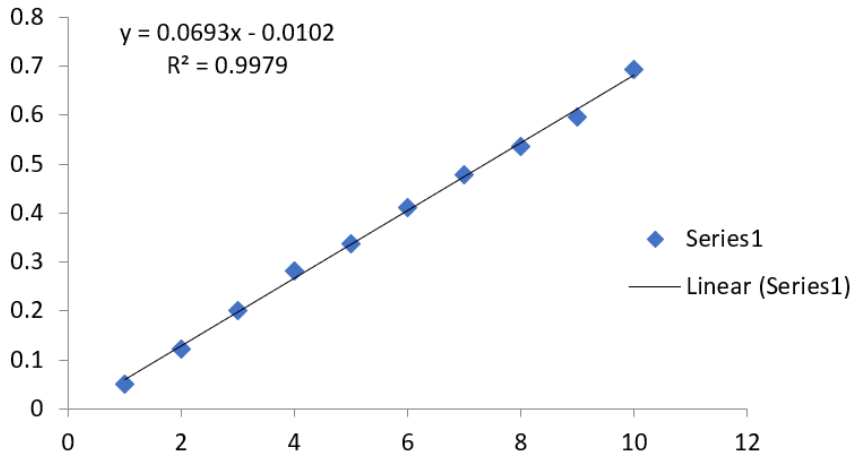

Fig. 4: Calibration graph of marketed product at 224nm

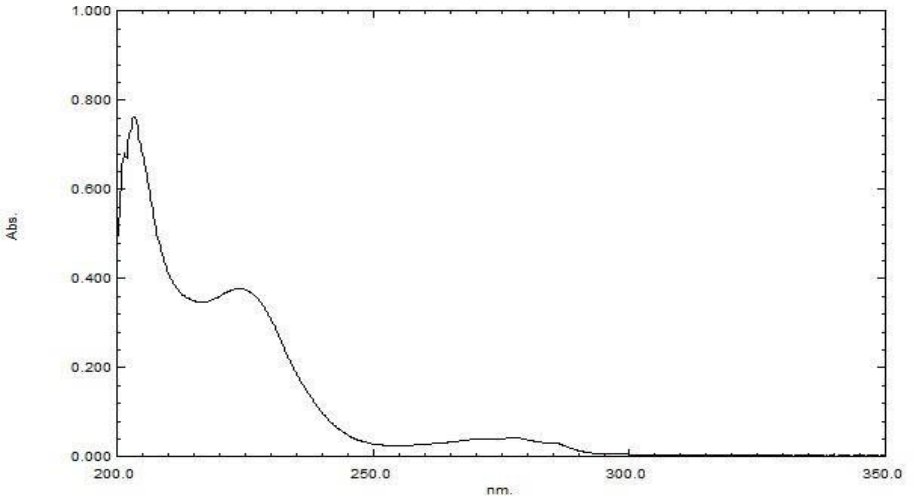

Fig. 5: Spectrum of acid degradation of dapagliflozin at 224nm

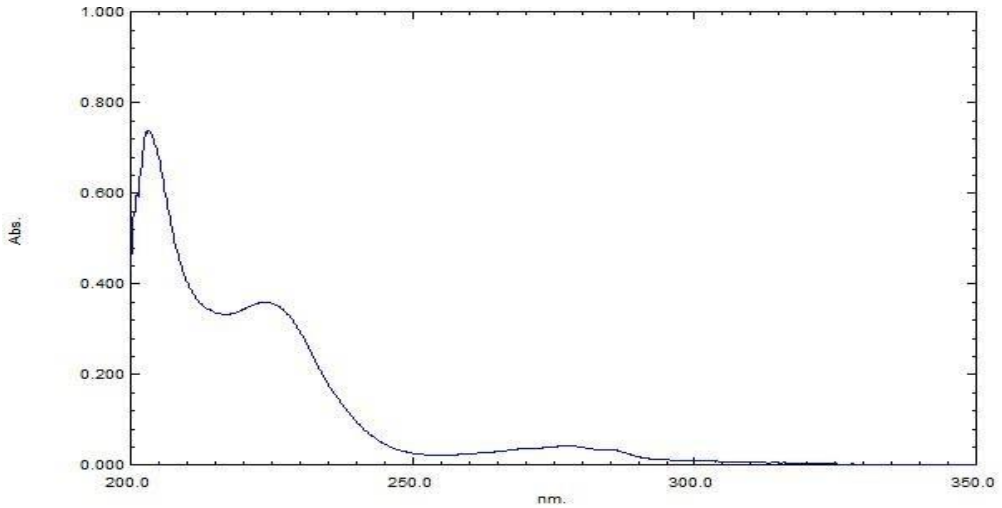

Fig. 6: Spectrum of alkaline degradation of dapagliflozin at 224nm

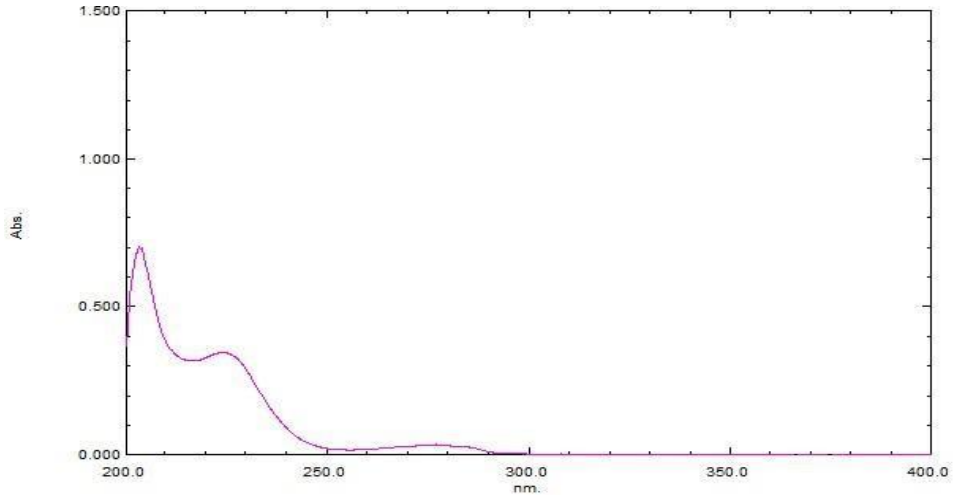

Fig. 7: Spectrum of neutral degradation of dapagliflozin at 224nm

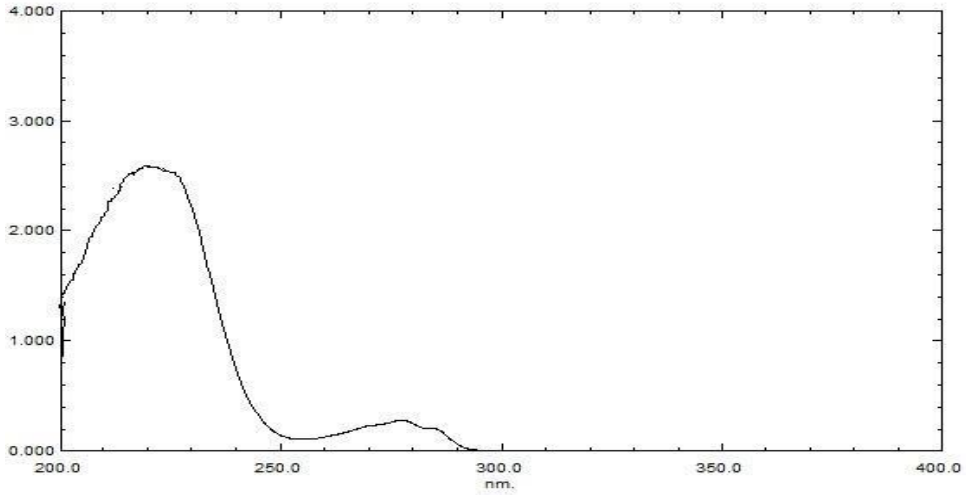

Fig. 8: Spectrum of photolytic degradation of dapagliflozin at 224nm

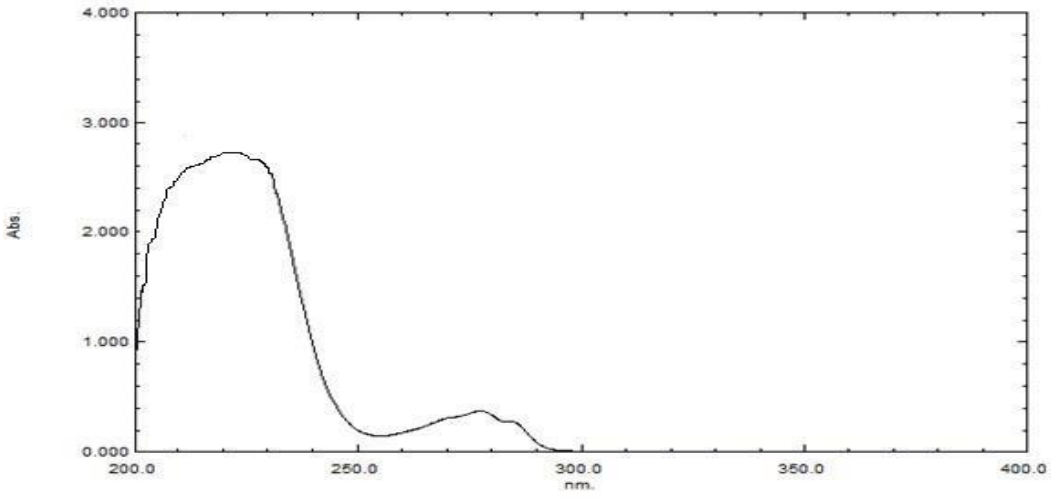

Fig. 9: Spectrum of degradation under dry heat of dapagliflozin at 224nm

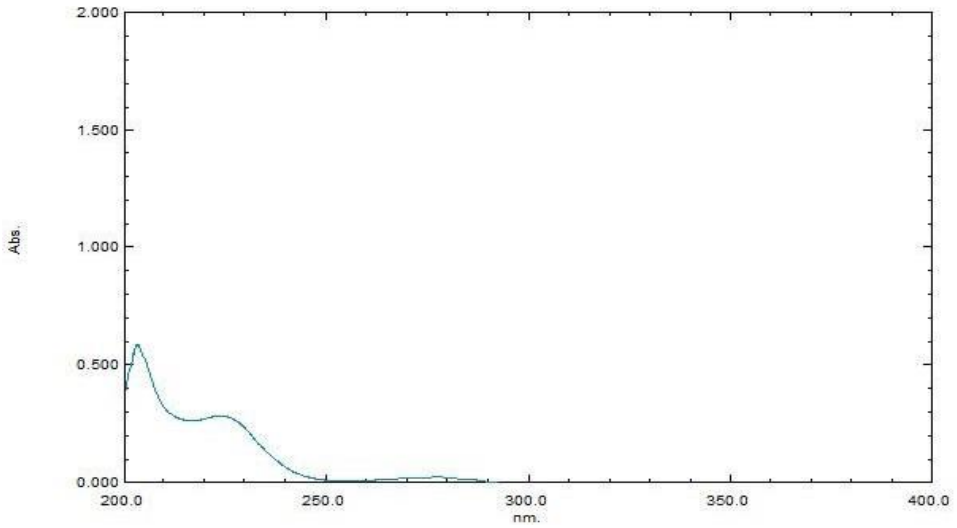

Fig. 10: Spectrum of oxidative degradation of dapagliflozin at 224nm

Table 1: Linearity data of dapagliflozin

S. No.	Concentration (µg/ml)	Absorbance
1	1	0.05
2	2	0.123
3	3	0.201
4	4	0.282
5	5	0.338
6	6	0.411
7	7	0.477
8	8	0.537
9	9	0.596
10	10	0.692

Table 2: Accuracy results for dapagliflozin

S. No.	% Taken	Concentration	Absorbance	Concentration
1	80%	6 µg/ml	0.110	100%
2	100%	5 µg/ml	0.179	100%
3	120%	4 µg/ml	0.191	100%

Table 3: Intraday precision of dapagliflozin

S. No.	Conc. (µg/ml)	Absorbance					Mean	SD	%RSD
		1 h	2 h	3 h	4 h	5 h			
1	1	0.05	0.051	0.051	0.05	0.052	0.0508	0.000837	1.645
2	2	0.123	0.124	0.123	0.124	0.125	0.1238	0.000837	0.676
3	3	0.201	0.202	0.201	0.203	0.201	0.2016	0.000894	0.4434
4	4	0.272	0.272	0.271	0.272	0.271	0.2716	0.000548	0.20176
5	5	0.338	0.337	0.338	0.337	0.337	0.3374	0.000548	0.16241
6	6	0.411	0.412	0.411	0.412	0.413	0.4118	0.00037	0.20325
7	7	0.474	0.477	0.475	0.478	0.476	0.476	0.001581	0.33217
8	8	0.536	0.537	0.538	0.537	0.539	0.5374	0.00114	0.21216
9	9	0.596	0.599	0.595	0.597	0.598	0.597	0.001581	0.26484
10	10	0.691	0.692	0.694	0.69	0.693	0.692	0.001581	0.22848

Table 4: Interday precision of dapagliflozin

Conc. (µg/ml)	Absorbance (Days)					Mean	SD	%RSD
	1	2	3	4	5			
1	0.05	0.049	0.048	0.05	0.049	0.0492	0.000784	1.520325
2	0.123	0.122	0.124	0.125	0.126	0.124	0.001581	1.275
3	0.201	0.202	0.204	0.205	0.207	0.2038	0.002387	1.17124
4	0.272	0.273	0.275	0.276	0.278	0.2748	0.002387	0.86863
5	0.338	0.339	0.341	0.343	0.345	0.3412	0.002864	0.83939
6	0.411	0.412	0.412	0.413	0.414	0.4124	0.00114	0.27669
7	0.476	0.478	0.479	0.477	0.474	0.4768	0.001924	0.403427
8	0.535	0.536	0.538	0.537	0.534	0.536	0.001581	0.294989
9	0.598	0.597	0.594	0.598	0.593	0.596	0.002345	0.393491
10	0.696	0.691	0.693	0.694	0.695	0.6938	0.001924	0.277247

Table 5: Repeatability of dapagliflozin

S. No.	Conc. (µg/ml)	Absorbance (Min)					Mean	SD	%RSD
		5	10	15	20	25			
1	1	0.05	0.049	0.049	0.051	0.05	0.0498	0.00037	1.68072
2	2	0.123	0.122	0.123	0.122	0.122	0.1224	0.000548	0.4477
3	3	0.201	0.2	0.201	0.201	0.202	0.201	0.000707	0.35174
4	4	0.274	0.271	0.271	0.272	0.272	0.2716	0.000548	0.35174
5	5	0.338	0.337	0.338	0.339	0.338	0.338	0.000707	0.20176
6	6	0.411	0.412	0.412	0.411	0.411	0.4114	0.000837	0.2034
7	7	0.477	0.476	0.475	0.476	0.477	0.4762	0.000837	0.175695
8	8	0.537	0.536	0.536	0.537	0.537	0.5366	0.000548	0.102073
9	9	0.596	0.596	0.597	0.596	0.597	0.5964	0.000548	0.091838
10	10	0.692	0.691	0.692	0.693	0.692	0.692	0.000707	0.102183

Table 6: Ruggedness of dapagliflozin

Parameter	Conc. (µg/ml)	Standard deviation	Relative standard deviation
Analyst 1	5µg/ml	0.000548	0.16%
Analyst 2	5µg/ml	0.000548	0.16%
Instrument 1	5µg/ml	0.000548	0.16%
Instrument 2	5µg/ml	0.000548	0.16%
Lab 1	5µg/ml	0.000548	0.16%
Lab 2	5µg/ml	0.000548	0.16%

Table 7: Robustness of dapagliflozin

Analyte	Wavelength	Standard deviation	Relative standard deviation
Dapagliflozin	222nm	0.000707	0.20%
Dapagliflozin	226nm	0.000548	0.35%

Table 8: Specificity of dapagliflozin

S. No.	Conc. (µg/ml)	Absorbance			SD	RSD
1	10µg/ml	0.691	0.692	0.692	0.000707	0.10%

Table 9: Assay of formulation of dapagliflozin

Sample	Label claim	% Label claim ± SD	%RSD
1	5mg	0.005 ± 0.01	0.80%

Table 10: Degradation studies of dapagliflozin at 224nm

S. No.	Degradation condition	Concentration	Absorbance	% Degraded
1	Acid	10µg/ml	0.345	49.8%
2	Alkaline	10µg/ml	0.349	50.4%
3	Neutral	10µg/ml	0.345	49.8%
4	Photolytic	10µg/ml	0.350	50.5%
5	Dry heat	10µg/ml	0.352	50.8%
6	Oxidative	10µg/ml	0.351	50.7%

CONCLUSION

The estimation of dapagliflozin was done by UV-method. In this method the methanol was selected as the solvent and dapagliflozin was quantified at 242nm. The linearity range of the drug was found to be from 1-10µg/ml. Linear regression coefficients were found to be within 0.9979-0.999. The values of %RSD are less than 2% indicating the precision of methods. The %recovery varies from 98-102% for Dapagliflozin indicating the method is accurate. LOD and LOQ were found to be within limits. The elevation of obtained values suggests that the proposed UV spectrophotometric method provide simple, precise and accurate quantitative analytical method for determination of dapagliflozin in dosage form. After validating the proposed method as per ICH guidelines and correlating the obtained values with the standard values, satisfactory results were obtained. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested no interference of formulation excipients in the estimation. The drug stability studies were performed according to ICH guidelines and were found to be stable. Hence, the method can be easily and

conveniently adopted for routine estimation of dapagliflozin in tablet dosage form. The method does not involve any tedious procedural steps, do not require any extra reagents or longer analysis time and a very simple instruments are required.

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CONFLICT OF INTEREST

None.

CONTRIBUTION OF AUTHORS

All authors contributed to experimental work, data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

ETHICAL APPROVAL

Not applicable.

SOURCE OF SUPPORT

Nil.

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