

SIMPLE QUANTIFIED AND VALIDATED STABILITY INDICATING STRESS DEGRADATION STUDIES OF ORAL ANTI-DIABETIC AGENT DAPAGLIFLOZIN BY RP-HPLC METHOD

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ABSTRACT

Objective: This method is focused on developing a precisely simplified and more accurate Reverse Phase-High Pressure Liquid Chromatography (RP-HPLC) method for the determination of Dapagliflozin in bulk and pharmaceutical dosage form as per guidelines of International Council for Harmonization (ICH).

Methods: Evaluation and validation carried out using the RP-HPLC ZORBAX (C18) column (250 x 4.6 mm, 5 µm particle size) with a mobile phase consisting of Phosphate Buffer: Acetonitrile: Methanol in a ratio of 55:40:05 (v/v/v) at a flow rate of 1 ml/min with an injection volume of 10 µl.

Results: Dapagliflozin was eluted at 2.12±0.05 min and detected at 225 nm. The regression equation $y = 55762x - 29679$ found to be linear with correlation coefficient r^2 value of 0.9997. The developed RP-HPLC method was conveniently validated as per the ICH guidelines and found method was robust, sensitive, accurate, selective, specific, precise and linear.

Conclusion: The proposed method was found to be accurate, precise, and robust for API and pharmaceutical dosage form as per experimentation analysis. The above developed method was found to be satisfied for Active Pharmaceutical Ingredient (API) and pharmaceutical formulation of Dapagliflozin to study its degradation products.

Keywords: Dapagliflozin, Forced stability, ICH, SGLT2, Stress degradation, RP-HPLC, Validation

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INTRODUCTION

Dapagliflozin (C₂₁H₂₅ClO₆) is chemically named as (1S)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol, physical appearance of it is a white to off white solid with melting range of 74-78 °C. The molecular weight is 408.873 g/mol. It has a good solubility profile in organic solvents such as methanol, and dimethyl sulfoxide but is poorly soluble in aqueous media.

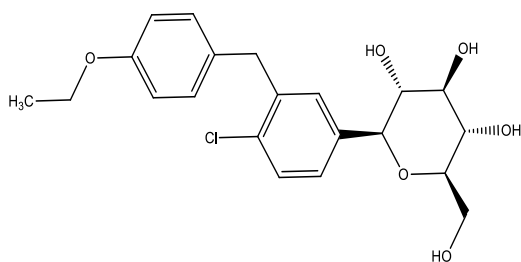


Fig. 1: Chemical structure of dapagliflozin

Dapagliflozin is an oral anti-diabetic agent which belongs to Selective Sodium-glucose co-transporter-2 (SGLT2) Inhibitor. Sodium-glucose co-transporter-2 (SGLT2) inhibitors are expressed in proximal renal tubules and are responsible for 90% reabsorption of glucose filtered by kidneys. Sodium-glucose co-transporter 2 (SGLT2) lowers reabsorption of filtered glucose into the body by decreasing the renal threshold for glucose (RTG) which leads to the production of a supplement to urinary glucose excretion in diabetic patients [1, 2]. Dapagliflozin is a C-glycosyl comprising beta-D-glucose in which the anomeric hydroxyl group is replaced by a 4-chloro-3-(4-ethoxybenzyl) phenyl group to improve glycemic control in adults with type 2 diabetes along with improved lifestyle. Adults with proper diet and exercise showed improved glycemic

control by Gliflozin derivatives by inhibiting glucose resorption in the proximal tubule of the nephron and causing glycosuria [2, 3].

Various studies performed and reported for estimation of gliflozin derivatives and Dapagliflozin using Ultraviolet-Visible (UV-Vis) spectroscopy as single dosage form [4-6] as well as in its combination form [7-10].

Few researchers studied its pharmacokinetics in biological fluids by selecting Bioanalytical methods [11-13] and there are some other methods reported like HPTLC, Fluorescence and capillary electrophoresis for estimation of Dapagliflozin, in combination with other anti-diabetic agents like Saxagliptin/Metformin [14-16].

Literature review shows minimal methods has been developed and reported for Dapagliflozin estimation by HPLC as an individual drug [3, 17-20] and in combination with Saxagliptin and Metformin [21-24] where the reported methods has vary retention time, high run time and more consumption of organic solvents for the estimation. This study focused on developing an RP-HPLC method of Dapagliflozin which is more economical, new, rapid, precise and accurate with a good amount of separation, consuming less organic solvents for separation as compared to the reported research work mentioned above.

MATERIALS AND METHODS

Chemicals and reagent

For this study, HPLC grade Acetonitrile (ACN), HPLC grade Methanol (MeOH), HPLC grade O-Phosphoric acid (OPA) from Merck (Mumbai, India) and HPLC grade water of SD fine-Chem Ltd; Mumbai were utilized. Market samples of FORXIGA 10 mg tablets of Dapagliflozin were purchased from a local chemist shop.

Instrumentation and optimized chromatographic conditions

Shimadzu 2010CHT HPLC system supplied with a gradient pump connected to a UV detector set at 225 nm used. Lab solution software (5.5.2 Version) was used for data acquisition and for system suitability

calculations. A 0.001 gm sensitive electronic analytical weighing balance Shimadzu and an Ultra-sonicator (Equibtron) were used in this entire study. Thermal stability studies were carried out in an I-Therm dry air oven. Optimized chromatographic conditions for the developed method summarized in (table 1).

Table 1: Optimized conditions for the validated developed method

Parameter	Chromatographic conditions
Column	ZORBAX C ₁₈ (250×4.6 mm, 5 µm)
Mobile phase	Phosphate buffer: Acetonitrile: Methanol in a ratio of 55:40:05 (v/v/v)
Solvent	Dilute the solute in Mobile phase
Flow rate	1.0 ml/min
Column oven temperature	28 °C
Detection Wavelength	225 nm
Injection volume	10 µl
Retention time	2.12±0.5 min.

Preparation of 0.025 M phosphate buffer

3.4 g of potassium dihydrogen orthophosphate weighed and transferred into a 1000 ml volumetric flask and made the volume with Distilled water. Phosphate buffer pH was adjusted to 4 with OPA and filtered the solution using 0.45 µm membrane filters, sonicated it before use.

Preparation of mobile phase

550 ml of phosphate buffer solution, 400 ml of Acetonitrile and 50 ml of methanol were mixed in the correct proportion to prepare the mobile phase. The prepared mobile phase was degassed using the ultrasonicator and filtered using 0.45 µm vacuum filters. The mobile phase prepared for this experiment was also used as diluent for the entire sample and standard preparation.

Preparation of stock solution

10 mg Dapagliflozin weighed and transferred into a 10 ml volumetric flask, dissolved and diluted up to the mark with Mobile phase prepared. The solution was ultrasonicated for 10 min to get 1 mg/ml solution of Dapagliflozin (1000 µg/ml), further dilutions were made as per the requirement by mixing it in diluent and filtered through 0.45 µm and degassed before injection.

Preparation of sample solution

Twenty Dapagliflozin tablets were accurately weighed and triturated to get a fine powder. A 10 mg equivalent weight tablet powder was transferred into a 100 ml volumetric flask and dissolved in methanol. The solution was ultra-sonicated for 10 min and made the volume with methanol. The tablet sample solution was then filtered through whatman filter paper (No. 41) and utilized for preparing sample solution for the assay.

Method validation

The developed method was validated for different prescribed parameters like accuracy, specificity, robustness, precision and linearity as per guidelines of ICH Q2A and Q2B [25, 26].

Linearity

To perform the calibration method, a series of solutions over the range of 10, 20, 30, 40, 50, 60, 80, 100, 120 and 180 µg/ml of Dapagliflozin were prepared and 10 µl of each solution was injected into the HPLC system to record peak area of the chromatogram. Calibration curve plotted against the concentration of the solutions on the x-axis and its corresponding peak area on the y-axis.

Precision

Intra-day and Inter-day Precision variation was analyzed by selecting concentrations 5 µg/ml (lower concentration) 10 µg/ml (middle concentration) and 15 µg/ml (higher concentration) Dapagliflozin and injecting that sample into HPLC system at different schedule time to measure the RT and peak area. The respective peak

areas for different concentrations of Intra-day and Inter-day Precision were calculated and reported.

Accuracy

Recovery studies carried out by preparing different concentration solution of Dapagliflozin (80 %, 100 % and 120 %) by adding 18 µg/ml, 20 µg/ml and 22 µg/ml into 10 µg/ml (100 %) solution of three replicate drug samples (n=3).

Robustness

Deliberate variation method conducted by changing the method parameters such as flow rate (±0.2 ml/min), temperature (±2 °C), mobile phase (±3 ml) and wavelength (±2 nm) determines the robustness of the selected method. A 10 µg/ml solution of Dapagliflozin utilized for conducting robustness study.

Forced degradation studies

The stability indicating property of the developed HPLC method carried out by Force degradation studies as per ICH recommended guidelines. Dapagliflozin was subjected to acidic, alkaline, oxidative, photolytic and thermal conditions to study its degradation property. All solutions utilized for this study were diluted from the Sample stock solution prepared and kept with concentration of 1000 µg/ml.

Acidic degradation performed by taking 1 ml of stock solution and treating it with 9 ml of 0.1 (N) HCl for 1 h in a thermostat maintained at 80 °C in laboratory condition. Alkali degradation was performed by taking a known 1 ml of stock solution and treating it with 9 ml of 0.1 (N) NaOH for 1 h in a thermostat condition maintained at 80 °C. For oxidative degradation, the 10 ml of the stock solution was pipette out and transferred into a 250 ml round bottom flask. The contents were then mixed with oxidative agent 30 % H₂O₂ (90 ml). A volume of 10 µl was injected into the HPLC system to measure peak height, peak area and retention time at multiple points and diluted to 10 ml using diluents.

A crushed fine powder of Dapagliflozin (100 mg) was exposed to UV light (254 nm) for 2 d on a neat and clean surface. After the exposure period, a known amount of drug was transferred into a 10 ml volumetric flask, diluted with diluent and the triplicate solution was injected into the HPLC system.

A crushed fine powder of Dapagliflozin (100 mg) was exposed to thermal energy in a hot air oven at a temperature of 80 °C for 48 h. After the exposure period, a known amount of drug was transferred into 10 ml volumetric flask, diluted with diluent and the triplicate solution has been injected into the HPLC system to measure peak height, area and retention time.

Table 2: Optimized conditions for stress degradation studies

Stress applied	Stress conditions
Acidic	9 ml of 0.1 (N) HCl for 1 h at 80 °C
	9 ml of 01 (N) HCl for 1 h at 80 °C
Alkaline	9 ml of 0.1 (N) NaOH for 1 h at 80 °C
	9 ml of 01 (N) NaOH for 1 h at 80 °C
Oxidative	10 % oxidative reagent H ₂ O ₂ (90 ml)
	30 % oxidative reagent H ₂ O ₂ (90 ml)
Thermolytic	100 mg powdered sample kept at 80 °C for 48 h
	100 mg powdered sample kept at 80 °C for 48 h
Photolytic (UV light)	100 mg powdered sample kept under UV light for 24 h
	100 mg powdered sample kept UV light for 48 h

RESULTS

The developed method quantified and validated as per the ICH guidelines prescribed for evaluating pharmaceutical dosage formulations [25, 26].

System suitability

The chromatogram of the blank, Standard Dapagliflozin and sample Dapagliflozin provided in (fig. 2) with a summary of its optimized conditions reported in (table 3).

Table 3: System suitability parameter report in optimized conditions

S. No.	Injection no	RT	Area	USP plate	USP tailing factor
1	Injection 1	2.12	552 631	4948	1.02
2	Injection 2	2.13	552 024	4997	1
3	Injection 3	2.12	551 926	4947	1.02
4	Injection 4	2.12	551 749	4956	1.01
5	Injection 5	2.13	552 772	4986	1.005
6	Injection 6	2.12	552 038	4998	0.96
Mean			552 190		
SD			411.84		
%RSD			0.07		

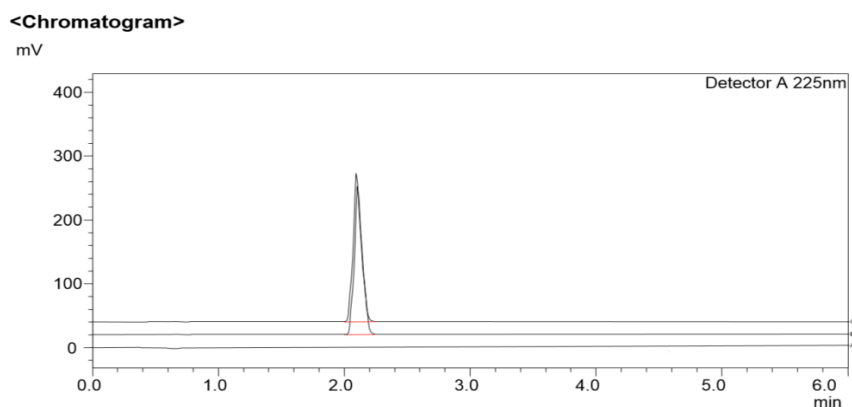


Fig. 2: Typical chromatogram of dapagliflozin in optimized condition A-Blank, B-standard and C-DAPA sample

Linearity

The correlation coefficient of regression value and intercept value

were calculated using the formula $y = 55\,762x - 29\,679$ ($r^2 = 0.9997$) (fig. 3) respectively, and obtained values are summarized in (table 4).

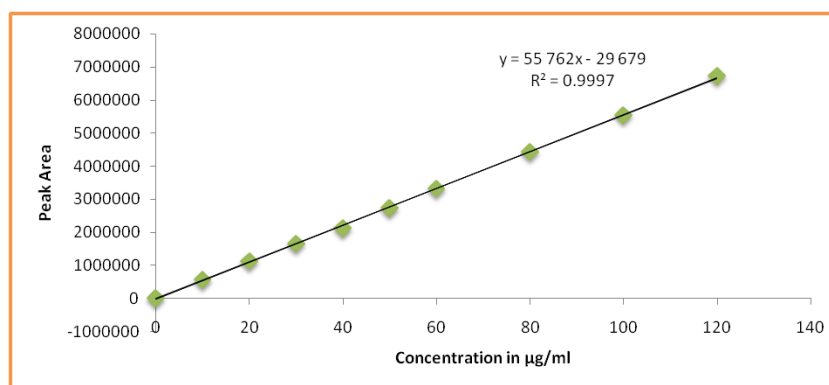


Fig. 3: Calibration curve of dapagliflozin at 225 nm wavelength

Table 4: Linearity results

S. No.	Concentration (µg/ml)	*Mean peak area±SD (% RSD)
1	0	0
2	10	552 084±1024.80 (0.19)
3	20	1 122 300±2525.75 (0.23)
4	30	1 646 080±3366.04 (0.20)
5	40	2 112 737±4986.14 (0.24)
6	50	2 722 510±5967.67 (0.22)
7	60	3 315 573±6682.89 (0.20)
8	80	4 425 123±5053.81 (0.11)
9	100	5 530 461±6060.31 (0.11)
10	120	6 708 924±10 104.11 (0.15)
Slope		55 762
Intercept		-29 679.29
Coefficient correlation value r^2		0.9998

mean±SD (n=3)

Accuracy

The % Mean recovery for Dapagliflozin was found to be 98.90, 99.10 and 98.51 for 80 %, 100 % and 120 %, respectively and these results are promising and within acceptable limits of 98-102.

The % RSD for Dapagliflozin was found to be 0.14, 0.30 and 0.23, which is within limit of ≤ 2 and its high value of recoveries at different concentrations indicates the proposed method is accurate. The accuracy data of the proposed method is summarized in (table 5A).

Table 5A: Accuracy studies of dapagliflozin

Spiked concentration ($\mu\text{g/ml}$)	Total concentration ($\mu\text{g/ml}$)	*mean peak area \pm SD (% RSD)	Drug found ($\mu\text{g/ml}$)	Recovery (%)
8 (80 %)	18	982 495 \pm 1353.52 (0.14)	17.80	98.90
10 (100 %)	20	1 093 955 \pm 3300.26 (0.30)	19.82	99.10
12 (120%)	22	1 196 119 \pm 2747.26 (0.23)	21.67	98.51

mean \pm SD (n=3)

Table 5B: Precision studies of dapagliflozin

Concentration ($\mu\text{g/ml}$)	Intra-day	Inter-day		
	*mean peak area \pm SD	% RSD	*mean peak area \pm SD	% RSD
5	269 594 \pm 600.93	0.22	269 803 \pm 607.02	0.22
10	549 317 \pm 549.62	0.10	548 323 \pm 1769.78	0.32
15	823 856 \pm 2026.11	0.25	825 106 \pm 2186.40	0.26

mean \pm SD (n=3)

Precision

Precision method performed by injecting three replicates of standard solutions (5, 10 and 15 $\mu\text{g/ml}$ concentration) within a day at different time intervals. The Intra-day and Inter-day precision studies were carried out and the result is summarized in terms of % RSD. The % RSD values of Intra-day and Inter-day for Dapagliflozin are less than 2% (Intra-day 0.34, 0.10, 0.25 and Inter-day 0.25, 0.32, 0.26) which reveal that the proposed method is precise (table 5B).

Robustness

The deliberate variation for this method was performed by robustness parameters like varying the flow rate, detector wavelength, Column temperature, and change in mobile phase composition. No palpable change in mean retention time (R_t), mean % assay and % RSD and also assay results are within the acceptable

limit of ≤ 2 . The tailing factor and plate count are within acceptable limits i.e., >2500 and <2.0 , respectively. The % RSD obtained was 1.26–1.79 for Dapagliflozin which is less than 2.0 % indicating the proposed method is Robust. Summary of robustness reports under different conditions (change in flow rate, temperature and wavelength) are summarized in (table 6).

Analysis of marketed formulation

The Content of Dapagliflozin in the FORXIGA 10 mg tablets was determined by the proposed analytical method. The assay value calculated by assaying 6 samples of the Dapagliflozin, the percentage value found to be 99.05 with standard deviation 0.3 and % relative standard deviation of 0.273. The mean concentration of Dapagliflozin in the given sample FORXIGA was found to be 9.91 mg (99.05 % w/v). The obtained assay values were within the acceptable limit (98-102 %) against the amount claimed in the label (table 7).

Table 6: Result of robustness method

S. No.	Parameters	Condition	*Mean peak area \pm SD (% RSD)
1	Flow rate	(0.8 ml/min)	541 023 \pm 8843 (1.63)
2	Flow rate	(1.0 ml/min)	
3	Flow rate	(1.2 ml/min)	
4	Wavelength	223 nm	530 816 \pm 9506 (1.79)
5	Wavelength	225 nm	
6	Wavelength	227 nm	
7	Temperature	26 °C	539 553 \pm 6824 (1.26)
8	Temperature	28 °C	
9	Temperature	30 °C	
10	Mobile phase	(43: 57)	534 576 \pm 8888 (1.65)
11	Mobile phase	(45: 55)	
12	Mobile phase	(47: 53)	

mean \pm SD (n=3)

Table 7: Assay estimation of dapagliflozin tablets

Formulation	Labeled claim (mg)	Amount found* (mg)	Recovery* (%)
Brand 1	10	9.91	99.05

Stress degradation studies

Physiochemical variations of Dapagliflozin were studied by implementing stress degradation studies as per ICH guidelines from the stressed stability studies, it was clear that the drug withstands

chemical parameters such as acid hydrolysis alkaline hydrolysis and oxidation.

At the same time a high amount of degradation was observed in the sample kept on irradiation with UV light (fig. 4). Obtained

chromatogram for the assay of stress sample is shown in (fig. 4). The concentration of the obtained degradation products analogous to the standard Dapagliflozin was calculated and reported to be acidic 3.64 %, alkaline (0.68 %), Oxidative (1.69 %), Thermal (4.17 %),

Photolytic (2.99 %) and acidic (11.02 %), alkaline (6.80 %), Oxidative (9.01 %), Thermal (11.66 %) and Photolytic (8.18 %) for acid hydrolysis, alkaline hydrolysis, oxidation, thermal and photolytic stability at 0.1 N and 01 N conditions respectively (table 8).

Table 8: Data of stress degradation studies dapagliflozin

Stress condition at 0.1 (N)	*mean peak area	*Drug recovered (%)	*Drug decomposed (%)
Standard drug (untreated)	551 911	100	-
Acidic degradation	531 807	96.36	3.64
Alkaline degradation	548 162	99.32	0.68
Oxidative degradation	542 556	98.31	1.69
Thermal degradation	528 879	95.83	4.17
Photolytic degradation	535 409	97.01	2.99
Stress Condition at 01 (N)	*mean peak area	*Drug recovered (%)	*Drug decomposed (%)
Standard drug (untreated)	551 911	100	-
Acidic degradation	491 085	88.98	11.02
Alkaline degradation	514 384	93.20	6.80
Oxidative degradation	502 181	90.99	9.01
Thermal degradation	487 571	88.34	11.66
Photolytic degradation	506 762	91.82	8.18

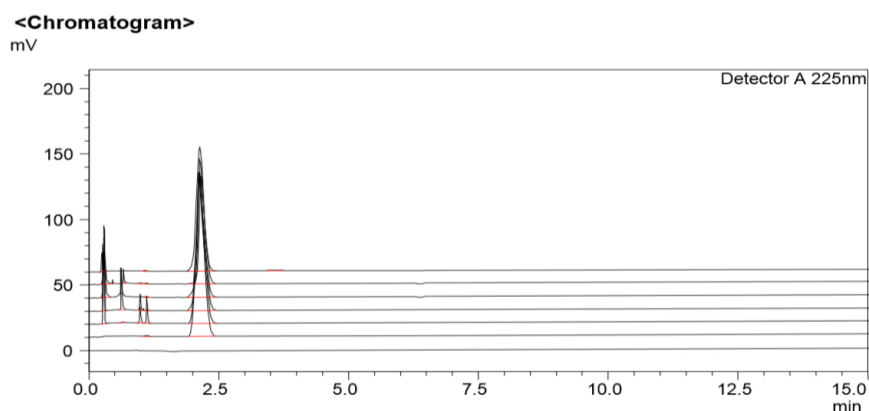


Fig. 4: Chromatogram of dapagliflozin in photolytic degradation A-Blank, B-Standard, C-Acidic, D-Alkaline, E-Oxidative, F-Thermal, G-Photolytic degradation

DISCUSSION

As of now, there is no RP-HPLC method reported on Dapagliflozin elution within specific chromatographic conditions, which is highly effective and more economical. Whatever was reported till date has its own drawbacks such as few experiments consumed more organic phases and at the same time, failed to integrate the degradants. Hence, there is a need of identifying the right analytical method which elutes the drug within a specific time limit when it is present alone in API or in the form of finished pharmaceutical formulation with other oral anti-hyperglycemic agents. Currently available analytical method mainly focuses on estimating the Dapagliflozin in combination with other oral antihyperglycemic agents [4-24].

To optimize the developed method various chromatographic conditions are implied such as different mobile phase, flow rate, different column, selected column with different temperature and different wavelengths during the chromatographic trial runs. Buffers with pH 4-6, column temperature 28 °C and flow rate 1.0 ml/min provided promising results with minimal errors such as increased peak width, moderate retention time and different Tailing factors compared with previous reported studies [3, 8, 9, 10].

Ideal parameters were decided based on chromatographic results obtained for Dapagliflozin at pH 4, column temperature 28 °C and Flow rate 1.0 ml/min; the developed method improved the tailing factor and robustness of the study during its trial runs. Phosphate buffer pH 4: Acetonitrile: Methanol ratio of 55:40:05 (v/v/v) was selected as mobile phase and diluents for this proposed method. Above mobile phase, composition eluted

the analyte within 3 min with improved tailing factor compared to the previous reports [8-10].

Analysis of the marketed formulation was carried out on the basis of peak area obtained for Dapagliflozin in UV detector detection wavelength 225 nm. The retention time obtained for Dapagliflozin was found at 2.12±0.5 min. The calibration curve response was recorded linear with the concentration range of 10-120 µg/ml. Good peak symmetry with exceptional separation observed for validation parameters such as Linearity, Accuracy, Precision and Robustness during evaluation.

The analytical method has been validated as per the guidelines provided by ICH [25, 26] and found more linear towards its range of concentration 10 µg/ml–120 µg/ml. Simple mobile composition of phosphate buffer: acetonitrile and methanol used in this study with the final pH less than 3.4 eluted the analyte at RT 2.12±0.5 min, Recovery percentage was found more accurate (98.90, 99.10, 98.51), no significant changes in deliberate variation method (1.63, 1.79, 1.26, 1.65), precision values fell less than 2 during intra and inter-day analysis, an acceptable limit as per ICH criteria compared to the reported methods [17-20].

Mild to moderate degradation occurred for the samples exposure to Thermal and oxidative conditions in forced degradation studies (11.02, 6.80, 9.01, 11.66, 8.18 %), and its % RSD and error fall less than 2 % that indicates the proposed method has good stability and also the degraded products not altered the peak area RT at any of its conditions; therefore it will be utilized to study degradation products of Dapagliflozin API and Pharmaceutical formulation.

Based on the Statistical data analysis report the developed method was found more reliable, cost-effective and highly reproducible.

CONCLUSION

The research work was promising, unique and less time-consuming, with a minimum amount of solvent utilization for estimating the Dapagliflozin. Hence, this method was found to be simple, rapid, economical, specific, accurate, precise and robust. Stress degradation studies revealed that Dapagliflozin can withstand the alkaline, oxidation conditions; meanwhile, in acidic, thermal and photolytic conditions mild degradation occurs over a period of time exposure. Based on the above experimentation results, our proposed method can be utilized as simple, rapid, economical and stability-indicating RP HPLC method to estimate and quantify degradation products of Dapagliflozin in API and pharmaceutical formulation.

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AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

The authors declared that there were no conflicts of interest for this study.

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