RESEARCH ARTICLE

Novel HPLC Method Validation for Quantification of Dapagliflozin in Bulk Drug and Formulation

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ABSTRACT

A new, steady, easy-to-use, and straightforward high-performance liquid chromatography (HPCL) technique was also tested to determine dapagliflozin concentration in medicinal and API forms. For the chromatographic extraction study, a Phenomenex Gemini-NX C18 Column was used. This column was filled with a mobile phase that was 70:30 v/v methanol to sodium 1-octanesulphonate. This experiment used a 20-liter syringe amount and a flow rate of 1-mL per minute. A bunch of factors were used to make sure the new method worked. The drug dapagliflozin was released at a wavelength of 203 nm after 6.5 ± 0.5 minutes. A correlation coefficient (r^2) of 0.9994 between 15 to 55 µg/mL shows that the planned process is linear. The theoretical plates and peak tailing were found to be 4129 and 1.03, respectively. In terms of healing, the percent accuracy that was reached was within the acceptable range of 98 to 100. New suggestions say that the regular testing of dapagliflozin in large amounts must be done using a proven method that is unique and easy to repeat. Forced breakdown tests with drugs were used to show that the proposed method is stable. The product was tested according to the steps laid out in the ICH Q2R1 Guidelines. The test looked at many chemical processes, such as oxidation, photolysis, heat stress, and acids and alkaline hydrolysis.

Keywords: Stability Indicating, HPLC, Dapagliflozin.

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INTRODUCTION

The compound known as phenyl (2-chloro-3-(4-ethoxybenzyl)) is under consideration. The IUPAC officially recognizes dapagliflozin as 6-(hydroxyl-methyl)tetra-hydro-2H-pyran-3,4,5-triol (Figure 1).

Dapagliflozin is crystalline compound with a color spectrum ranging from white to off-white. The compound's molecular weight is 408.875 grams per mole, while its chemical formula is $C_{21}H_{25}ClO_6$. Dapagliflozin exhibits solubility with DMAP (dimethyl formamide), ethanol, and dimethyl sulfoxide (DMSO). Dapagliflozin is taken by oral route for the treatment of diabetes. The compound belongs to the newly formed gliflozin class. A chronic disease that is characterized by inadequate insulin release from the pancreas or impaired insulin use throughout the body is known as diabetes.[1]

In contrast to type-2 diabetes, which is characterized by impaired use of endogenously generated insulin due to metabolic dysfunction, type-1 diabetes is an autoimmune condition that hinders the generation of insulin by the pancreas. The kidneys reabsorb glucose *via* an enzymatic mechanism called glucose transporter 2. Dapagliflozin blocks this process by inhibiting sodium-glucose co-transporter 2, the enzyme responsible for at least 90% of this process. Inhibiting the transporter system causes an increase in glucose excretion in urine because it promotes the removal of glucose from the bloodstream *via* the urinary system. A study was conducted to investigate the analytical methods used in the estimate of gliflozin. A limited selection of analytical techniques was used for the analysis of dapagliflozin. These techniques included high-performance liquid chromatography (HPCL) with a photo diode array (PDA) detector. The mobile phase employed for this estimation consisted of a combination of phosphate buffers, methyl alcohol, and acetonitrile. The current work used ion pair buffers as the mobile phase in order to estimate dapagliflozin.[2-4]

This work aims to make and validate a straightforward, economically compatible, and accurate HPLC method for measuring dapagliflozin concentration in API and prepared dosage forms. The aim of this research is to demonstrate the

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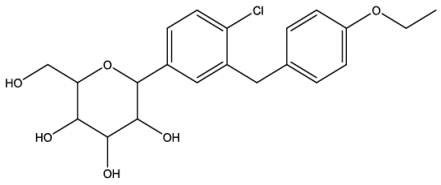

Figure 1: Dapagliflozin composition

effectiveness of a newly developed approach for assessing the target product response in the existence of its degradation products. The method's validation process ensured compliance with the requirements set out by the ICH for maintaining the quality of dapagliflozin in both bulk and formulation.[5,6]

MATERIALS AND METHODS

Instruments Used

Throughout the technique development process, researchers and validation specialists used a Shimadzu LC-10AT HPLC systems, which Shimadzu Corporation manufactures. This system featured a variable wavelength detector (VWD) capable of UV-vis light detection, with data analysis and control conducted through Lab Solutions programming. To ensure that our findings were not influenced by any one particular system, we carried out robustness tests. A VWD, the Agilent 1100 series HPLC platform, and the Chemstation software were the instruments that we used. A Metrohm model 780 computerized pH meter with a reproducibility of ± 0.01 pH was employed for pH adjustments.

Chemicals and Reagents

The receipt of a gift sample from Aurobindo Pharma Limited, an Indian pharmaceutical company facilitated the acquisition of dapagliflozin (active pharmaceutical ingredient). The sodium 1-octansulphonate used in the chromatographic analysis was obtained from SD-Fines Chemicals Ltd., a company based in India. Phosphoric acid of analytical reagent (A.R.) quality, deionized water, and methanol of chromatographic grade were obtained from Rankem Limited, a supplier based in India. Astra Zeneca India generously provided trial packs of Forxiga 10 mg for the dapagliflozin market segment.

Chromatographic Conditions

The mobile phase, which contained 30% 0.005 M sodium octane sulphonate buffer with 3 pH and seventy percent methanol (v/v), was cycled at room temperature. The elution of the analyte of interest was carried out using a Phenomenex Gemini-NX C18 Column by specifications: 250 mm in length, 4.6 mm in diameter, and a particle size of 5 µm. Vacuum filtration using a 0.45 µ millipore filter paper was employed prior to cycling the produced mobile phase. A variable wavelength (UV) sensorestablished at a wavelength of 203 nm in addition a flow amount of 1.0 ml/min facilitated the circulation.

Chromatographic parameters[7-9]

Parametric chromatography was conducted using a Phenomenex Gemini-NX C18 LC-Column, featuring dimensions of 250 mm in length, 4.6 mm in diameter, and a particle size of 5 µm. Using a Shimadzu LC series HPLC system with VWD, the flow rate was set at 1.0 mL per minute and the wavelength at which it was measured was 203 nm. Throughout the procedure, an injection volume of 20 µL was used, with the column oven's temperature kept constant with ambient air. A total of 10 minutes was determined as the duration of the event.

Preparation of samples

- *Buffer solution*

To make a preparation of octane sulphonate by a concentration of 0.005 M, sodium 1-octane sulphonate in 5.88 g quantity dissolve in 500 mL of water of HPLC quality. Phosphoric acid, by a concentration of 1.0 N, is recommended for adjusting the pH of the solution to 3.0.

- *Mobile phase preparation*

A volumetric proportion of 30:70 (v/v) was maintained between octane sulphonate buffer and HPLC-grade methanol to prepare the mobile phase. Following filtration through a 0.45 µ millipore film paper, the mixture underwent degassing through sonication[10].

- *Preparation of diluents*

Methanol is used as a diluent in a direct manner.

Standard stock solution preparation (1-mg/mL)

In a 50 mL volumetric flask containing 20 mL of methanol, 50 mg dapagliflozin was added. A period of eight minutes was devoted to sonicating the solution in order to promote dissolution. It was then diluted to its final volume using methanol and then filtered using a 0.45 µ millipore film paper. This process was carried out with the objective of obtaining a standard stock preparation by a concentration of 1-mg/mL.

Standard working solution preparation (100 µg/mL)

A solution with a concentration of 100 µg/mL was created by diluting 1.0 mL of the original 1-mg/mL standard stock solution with methanol, which served as the solvent. This process was repeated until the total volume reached 10 mL. As a further step, more dilutions were carried out to support the development of techniques and validation studies. In order to conduct the studies, a solution that contained 100 µg/mL of dapagliflozin was used as the working solution. We used this particular technique in order to get the desired concentrations of 10, 20, 30, 40, and 50 µg/mL.

Sample solutions

A 100 mL volumetric vial, which had been well dried and cleaned, was used to contain a mixture of 40 mL of diluent and finely powdered tablets, corresponding to a quantity of 100 mg of dapagliflozin. After subjecting the flask to an 8-minute sonication process, methanol was added to the flask in order to achieve the desired volume. The methanol was introduced into a volumetric vial that contained a 400 µL aliquot of the dapagliflozin sample stock solution. This was done in order to get the desired concentration of 40 µg/mL in the sample solution that was produced.

Procedure

For each specimen, encompassing the blank, standard, and sample solution, a volume of 20 microliters was injected into the chromatography setup. The evaluation of dapagliflozin %assay entailed examining peak magnitudes, accomplished by juxtaposing the primary peak magnitudes derived from standard and specimen chromatograms.

$$Assay\ \% = \frac{AT}{AS} \times \frac{WS}{WT} \times \frac{DT}{DS} \times \frac{P}{100} \times \frac{Avg.\,WT}{Label\ Claim} \times 100$$

Within this context, AS represents the mean peak area of the leading peak acquired from the reference chromatogram, while AT indicates the mean peak area of the primary peak from the examined or specimen chromatogram. WS denotes the weight of the reference material in milligrams, and WT signifies the weight of the examined or specimen material in milligrams. P denotes the purity percentage of the reference material. Additionally, DS indicates the dilution factor of the reference preparation, while DT indicates the dilution factor of the specimen preparation.

Force Degradation Studies

Blessy *et al*. and Bakshi *et al*. did a comprehensive literature study on the topic of "Development of validated stability-indicating assay methods." Stress tests assessed the stability-demonstrating capacity of the suggested HPLC approach. In order to induce a significant deterioration in the API sample, dapagliflozin was subjected to several degradation settings. The primary aim of this research effort is to determine the efficacy of the suggested approach in detecting the response of the analyte in the existence of its degradation products.[11-13]

Acid and alkali hydrolysis

A round-bottom flask was used to mix a one-millilitre aliquot solution of dapagliflozin (1000 µg/mL) with one millilitre of 0.1 N hydrochloric acid/sodium hydroxide. The aforementioned solutions were kept at a 35°C for the period of 48 hours. In order to achieve neutralization, an equal amount of acid or base was added after the solutions were cooled to a 25°C. To achieve further dilution, methanol was used, and filtered using membrane filter a 0.45 µm pore size.[14]

Oxidation

After transferring 9.0 mL of a H_2O_2 solution with a strength of 30% into a flask with a rounded bottom containing 1.0 mL of dapagliflozin solution with a concentration of 1000 micrograms per mL, the resultant mixture underwent intermittent agitation at a temperature of 35°C for 48 hours. The samples underwent a cooling process, resulting in a temperature of 25°C. In order to further dilute the solution, methanol was employed, and then filtering was performed using a membrane filter that had a pore size of 0.45 micrometers.[15]

UV light

The dapagliflozin subjected to UV radiation with 365 nm wavelength for forty-eight hours. The material that underwent treatment was solubilized in methanol and then diluted to a final amount of 10 milliliters. After suitable dilution with methanol, the filter is used as a membrane filter.[16]

Thermal degradation

The dapagliflozin specimen underwent processing for a period of forty hours at a temperature of 70°C, employing a reflux condenser. Following the treatment, the processed specimen was thinned with methanol as per the specified procedure, and subsequently filtered using a membrane filter with a pore size of 0.45 micrometers.[17,18]

RESULTS AND DISCUSSION

Analytical Method Development

Various methanol-to-water ratios were evaluated as mobile phases to establish a fundamental reverse phase fluid chromatography technique. Manipulating the pH of the mobile phase notably reduced the tailing effect and significantly improved peak shape. Enhanced peak shape and elution were observed when utilizing a sodium octane sulphanate buffer with a pH of 3.0. After that, the sodium octane sulphanate buffer and methanol were added to the mixture at a steady flow rate of 1.0 milliliters per minute, maintaining a volume/volume ratio of 30:70 throughout the analysis. To enhance elution efficiency and decrease peak tailing, the stationary phase was selected as the Phenomenex Gemini-NX C18 Column, with dimensions of 250 mm length, 4.6 mm diameter, and 5 µ particle size. Dapagliflozin's ultraviolet spectra exhibited the highest absorption at a wavelength of 203 nanometers. Consequently, the investigation proceeded with a fixed detection wavelength of 203 nm. The main peak retention time (RT) was established at 5.5 ± 0.5 minutes, accompanied by a tailing factor of 1.03 and a plate count of 4229. Figures 2-4 correspondingly illustrate the chromatograms for the test sample, dapagliflozin standard solution, and blank.

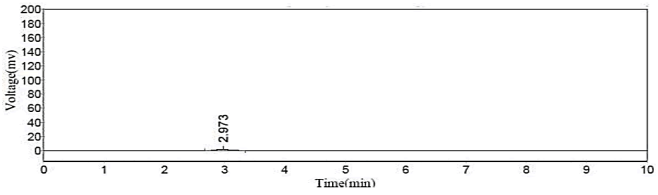

Figure 2: Blank solution chromatogram acquired at 203 nm

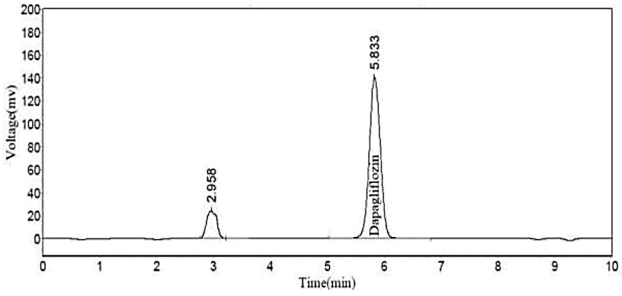

Figure 3: Standard solution chromatogram acquired at 203 nm

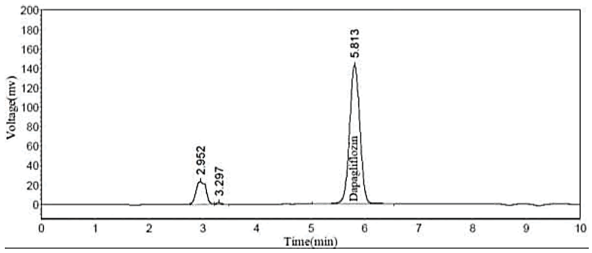

Figure 4: Dapagliflozin solution chromatogram acquired at 203 nm

Validation of Analytical Method

In accordance with the recommendations that are given in the ICHQ2A and Q2B guidelines, the validation of the analytical technique was evaluated using a variety of standards. These criteria included the appropriateness of the system, precision, accuracy, robustness, limit of detection (LoD), and limit of quantification (LoQ).

System suitability

Strict adherence to chromatographic parameters was upheld to ensure optimal effectiveness of the HPLC system. Following the establishment of a stable baseline, the adequacy of the system was tested by introducing a single 40 micrograms per milliliter standard solution, representing the 100% concentration of dapagliflozin under examination. In order to assess whether or not the system is compatible with the approach that was wanted, many parameters were analyzed. These parameters included the retention time (Rt), capacity factor, theoretical plates, and peak asymmetry. A compilation of the results of this examination is shown in Table 1, which may be seen here.

Precision

Exactness refers to the level of confidence demonstrated by the outcomes obtained from a series of measurements conducted through repeated administrations of the same sample. The computation of the percentage standardized relative deviation from six repeated measurements of a sole standardized solution at a concentration of 40 µg/mL is required to evaluate the accuracy of the technique that was established. Accuracy besides intermediate exactness were assessed by watching sequential areas of the main peak on the similar day time and at exact solution concentrations on various days. This allowed for the determination of both accuracy and precision. Detailed information on the outcomes of the analysis can be found in Table 2, which is organized according to peak area, peak height, and the percentage of standardized relative deviation (%RSD) of retention period (RT).

Linearity

Linearity pertains to the capacity of an analytical methodology to provide an outcome that falls within a predetermined interval and is unswervinglycomparative to the sample's concentration. The total volume of the calibration standard solutions used was 20 µl. Chromatograms were acquired at a wavelength of 203 nanometers. Figure 5 exhibits the overlay correlation

Table 1: Findings from the characteristics of the system's suitability

S. No.	*Factor*	*Unit*	*Result*
1	Retention time	Rt	6.6 ± 0.6
2	Capacity factor	k	8.0
3	Theoretical plates	N	5075
4	Peak tailing factor	-	1.24

Table 2: Exactness statistics of intraday also inter-day for dapagliflozin

Sr. No.	*Suitability parameter*	*Unit*	*Intraday result*	*Interday result*
1	Capacity factor	k	8.04	8.04
2	Theoretical plates	N	4609	3076
3	Tailing	-	1.24	0.78
4	RSD of retention time	Rt	0.25%	0.98%
5	RSD of area under curve	-	1.09%	1.30%
6	RSD of height of peak	-	0.99%	1.05%

chromatograms of dapagliflozin at different concentrations. The calibration curve seen in Figure 6 was constructed using the highest response obtained from the average concentrations. It is postulated that a linear process takes place. The obtained straight line has a coefficient of determination (r2) of 0.9996. Figure 7 illustrates the residual plot obtained for dapagliflozin. Table 3 displays the consequences of the examination of modification (ANOVA) conducted to assess linearity.

Accuracy or (%Recovery)

Accuracy may be defined as the quantification of the discrepancy between the acquired outcomes and the true value. Approximations were made for the obtained concentration,

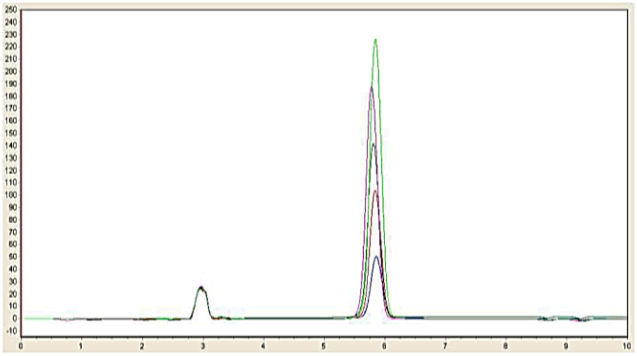

Figure 5: Chromatogram of dapagliflozin at 203 nm wavelength (Overlay)

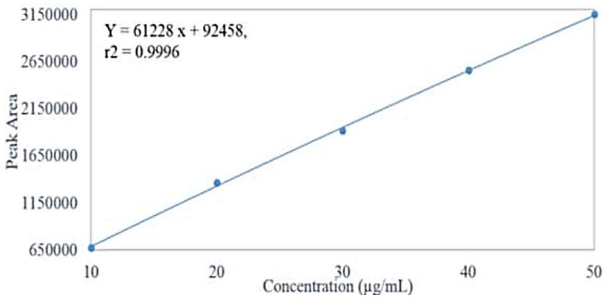

Figure 6: The measured calibration curve for dapagliflozin

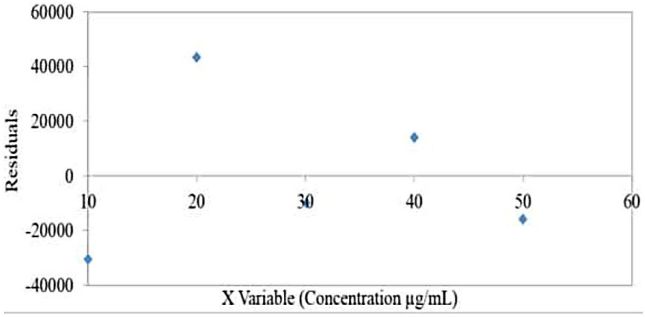

Figure 7: The residual design derived for dapagliflozin

Table 3: Findings of linearity for dapagliflozin using statistical data

S. No.	*Statistics factor*	*Result*
1	Slope	48982
2	Intercept	73966
3	r^2	0.9996
4	Sum of Squares Explained	2999052996235
5	Sum of Squares Residual	2695684299
6	Total	3001455680790

the anticipated concentration in the solution, and the mean of the three separate concentrations for the solutions created at proportions of 80, 100, and 120% (through a reference preparation amount of 40 µg/mL, respectively). The examination of the data provided in Table 4 substantiated that the mean recovery rate was 101.11%, with a range of individual recoveries spanning from 100.8 to 101.4%. These values are deemed to be adequate. The relative standard deviation for dapagliflozin was determined to be designate 0.22, a value that falls within the permissible threshold of ≤ 2. Consequently, the approach that has been established has a high grade of precision.

Robustness

The robustness investigations aimed to assess the proposed methodology's capacity to endure minor yet precisely controlled variations in method parameters. These adjustments encompassed changes in the mobile segment alignment, pH, temperature, then drift amount, all confined inside the acceptable parameters set by established chromatographical settings. Results indicate a negligible alteration in the average retention time (Rt), while the %RSD values remained within the acceptable range, with %RSD being ≤ 2. The predefined criteria for plate amount and peak tailing were observed to be above 2500 and below 2.5, respectively. Consequently, the proposed methodology demonstrates adaptability to diverse analytical settings, with specific findings related to dapagliflozin outlined in Table 5.

Stability studies of prepared solutions and mobile phase

The investigation focused on assessing the stability of several solutions, including the mobile phase, standard solution, and test solution, during a duration of 48 hours. The solutions described above were given and kept at 25°C for three separate

Table 4: Findings from recovery experiments conducted using typical addition techniques

Name of the sample	*Amount of drug (µg/ml)*		*% Recovered*
	Added	*Recovered*	
S1: 80%	32	31.86	99.50
S2: 80%	32	31.84	99.50
S3: 80%	32	31.84	99.18
S4: 100%	40	39.67	99.28
S5: 100%	40	39.71	99.20
S6: 100%	40	39.68	97.81
S7: 120%	48	46.95	97.91
S8: 120%	48	46.99	97.89
S9: 120%	48	46.98	99.50
Mean			98.97
Std. Deviation			0.23
%RSD			0.22

times after the experiment started: zero hours, 24 hours, and 48 hours. The solutions were produced per the prescribed methods and thereafter kept in securely sealed containers. After injecting the sample into the chromatographic system, the daily system suitability test parameters were determined. The resulting data was then compared and shown in Table 6. The findings of the %RSD fall within the permissible range of less than 2. Therefore, it can be seen that the mobile phase exhibits stability for 48 hours at a temperature of 25°C.

Results of limit of detection and limit of quantification studies

The following were the calculations made using the ICH-provided recommendations:

$$LOD = \frac{3.3 \times SD}{S}$$

$$LOQ = \frac{10 \times SD}{S}$$

For this specific case, "SD" is the answer's standard deviation, and "S" denotes the gradient received from the calibration curve. The "detection limit" (LoD) is the lowest concentration of a chemical needed to perform a certain experimental procedure's reaction measurement. The scientific community uses the term "quantification limit" (LoQ) to describe the lowest concentration of a medication that can be measured. Both the LoD and the LoQ were found to be 0.001 and 0.009 micrograms per mL, respectively. The methodology utilized in this investigation was shown to have very high sensitivity, as evidenced by the lowest values for the LoD and the LoQ.

Forced Degradation Study

All of the statistical information on the retention time (Rt) and degradation percentage of dapagliflozin is shown in Table 7. Through the use of this technique, the objective was to successfully separate the indigestible byproducts of the breakdown of dapagliflozin from the active substance itself. The findings of the specificity experiments, on the other hand, indicate that the approach that was supplied had an

Table 5: Outcome robustness studies of dapagliflozin

Parameter	*Analytical condition*							
	Flow rate in mL/min		*Hydrogen ion concentration*		*Temperature of column (°C)*		*Mobile phase composition (%)*	
	1-0.02	1+0.02	3.5-0.1	3.5+0.1	22	33	-10%	+ 10%
Mean RT*	6.3396	5.4756	6.4152	6.372	6.3288	6.1884	6.3828	6.1884
Std. Dev*	0.918	1.7604	0.8532	0.4104	0	0	0.9612	0.5508
%RSD*	1.0368	2.052	0.9936	0.4752	0	0	1.0908	0.6372
Peak tailing	1.08	1.0584	1.1124	1.1124	1.1124	1.1016	1.1124	1.08
Plate count	4409.64	4651.56	3819.96	4599.72	4452.84	4386.96	4725	4301.64

Table 6: Results stability studies of prepared solutions and mobile phase

System suitability parameter	*Results*		
	0 hours	*24 hours*	*48 hours*
%RSD of area under curve	0.9072	1.1016	1.134
Pale count	4152.6	4773.6	4402.08
Peak tailing factor	1.0584	1.1232	1.1124

Table 7: The summary of results of forced degradation investigation

Factors	*Changed retention time (minutes)*	*Amount of degradant formed (%)*
Acidic route of hydrolysis	5.87	1.84
Basic route of hydrosyis	5.78	1.30
Effect of oxidising agents	5.85	19.54
Effect of irradiation by UV	5.78	19.34
Effect of heat	5.86	2.92

extraordinarily high level of specificity. The ability of this device to effectively isolate the degradation products from the principal peak of the analyte might be the reason for the occurrence of this phenomena.

CONCLUSION

We designed and validated a one-of-a-kind method for measuring dapagliflozin in line with the standards that the International Council established for Harmonization of Technical standards for Pharmaceuticals for Human Use (ICH). The Phenomenex Gemini-NX C18 Column, equipped with high-performance liquid chromatography (HPLC) and a UV detector, made it possible to carry out this operation more straightforwardly. The method that was presented had a good degree of linearity within the concentration range of 10 to 50 µg/mL, as shown by the correlation value of 0.9996. In situations where the percentage of relative standard deviation (RSD) that was achieved was lower than 2, it was considered to be within the acceptable range. According to the findings, the accuracy of the recovery percentage fell within the permitted range of 98 to 102%. Through the process of conducting an analysis of the system's resilience, it was possible to carry out an evaluation of the system's dependability under high usage situations. A more efficient assessment of dapagliflozin is possible to carry out due to the fact that it is not just short but also takes only ten minutes to complete. At a temperature of 25°C for a period of 48 hours, the mobile phase, the standard solution, and the sample solution are all put through a stability test to determine their level of stability. The method was regarded as exact, reliable, trustworthy, and selective because the existence of secondary peaks in the specificity trials did not impact the quantification of the principal peak. In addition, the chromatographic elution phase, which is a procedure that takes ten minutes to complete, is being carried out alongside the other processes. Having said that, the recently approved and developed procedure has the potential to be used for regular and stability examinations.

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