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# DEVALOPMENT AND VALIDATION OF STABILITY INDICATING QUANTITATIVE ESTIMATION OF DAPAGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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**Abstract:**

*A new, simple, fast, selective, precise and accurate RP-HPLC method was developed and validated for the estimation of Dapagliflozin from bulk and marketed formulations. The proposed method was developed by HPLC Waters ODS $C_{18}$ column, 5µm, 25cmx4.6mm i.d, Separation Module with UV detector connected to D Elite 2000 software with an injection volume of 20 µl was injected and eluted with a mobile phase composition of Buffer (Potassium hydrogen orthophosphate & pH adjusted to 4.2 with orthophosphoric acid) and Methanol in a ratio of 65:35. Mobile phase is pumped at a flow rate of 1.0 ml/min and detected by UV detector at 225nm. Ambient column temperature has maintained. The retention time of Dapagliflozin was found to be 2.93 min. Linearity was observed in the concentration range of 20-100 µg/ml for Dapagliflozin with correlation coefficient 0.999. The proposed method was found to be precise and reproducible with %RSD of 0.42 for dapagliflozin. Percent recovery obtained in the range of 99.25 to 101.16% w/w for dapagliflozin. The LOD & LOQ were found to be 0.003 & 0.009 µg/ml respectively.The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The results of the stress studies indicated the specificity of the method that has been developed. Dapagliflozin was stable in both oxidation & acidic stress conditions. The result shows the developed method is yet another suitable method for assay & stability which can help in the analysis of Dapagliflozin in different formulations.*

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## INTRODUCTION:

Dapagliflozin is indicated for the management of diabetes mellitus type 2, and functions to improve glycemic control in adults when combined with diet and exercise. Dapagliflozin is a sodium-glucose cotransporter 2 inhibitor, which prevents glucose reabsorption in the kidney. Using dapagliflozin leads to heavy glycosuria (glucose excretion in the urine), which can lead to weight loss and tiredness. Dapagliflozin was approved by the FDA on Jan 08, 2014. Dapagliflozin is not recommended for patients with type 1 diabetes mellitus or for the treatment of diabetic ketoacidosis.

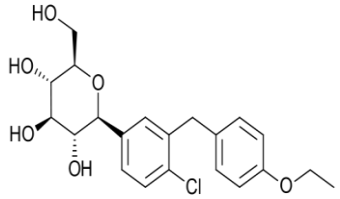


**Fig.1: Dapagliflozin**

A competitive inhibitor of the sodium-glucose transport subtype 2 protein, dapagliflozin blocks glucose reabsorption into the kidney, resulting in the elimination of blood glucose through the urine. Dapagliflozin is used with a proper diet and exercise program to control high blood sugar in people with type 2 diabetes. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems. Proper control of diabetes may also lessen your risk of a heart attack or stroke. Dapagliflozin works by increasing the removal of sugar by your kidneys.

## MATERIALS AND METHODS:

**Chemicals and Reagents:** Doubled distilled water, Methanol, Dipotassium hydrogen orthophosphate, Potassium dihydrogen orthophosphate, Orthophosphoric acid and Glacial acetic acid**.**

**Instruments:**

**HITACHI L2130** with D Elite 2000 Software with Isocratic with UV-Visible Detector (L-2400), **ELICO SL-159** UV–Vis spectrophotometer, Electronic Balance **(SHIMADZU ATY224),** Ultra Sonicator **(Wensar wuc-2L),** Thermal Oven and $P^H$ Analyzer **(ELICO)**

**Reagents and Solutions**

**Mobile phase preparation**

The mobile phase used in this analysis consists of a mixture of Buffer (Potassium hydrogen orthophosphate & pH adjusted to 4.2 with orthophosphoric acid) and Methanol in a ratio of 65:35. 650 ml of this buffer solution was added and properly mixed with 350 ml of Methanol and a homogenous solution is achieved. This mobile phase was filled and sonicated for 15 minutes before using in the experiment.

**Preparation of Standard Solutions**

25 mg of Dapagliflozin standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

**Preparation of Sample Solutions**

Take average weight and crush in a mortar by using pestle and weight powder 25 mg equivalent weight of Dapagliflozin sample into a 25ml clean dry volumetric flask, dissolve and make up to volume with mobile phase. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

**Determination of wavelength of maximum absorbance for Dapagliflozin**

The absorbance of the final solution scanned in the UV spectrum in the range of 200 to 400nm against solvent mixture as blank.

**Optimization of Rp-HPLC method**

The chromatographic conditions were optimized by different ways using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc. The Optimum conditions obtained from Methanol: Phosphate buffer in a ratio of 35:65, Waters ODS $C_{18}$ column, 5µm, 25cmx4.6mm i.d, with flow rate 1ml/min and at a wavelength of 225 nm.

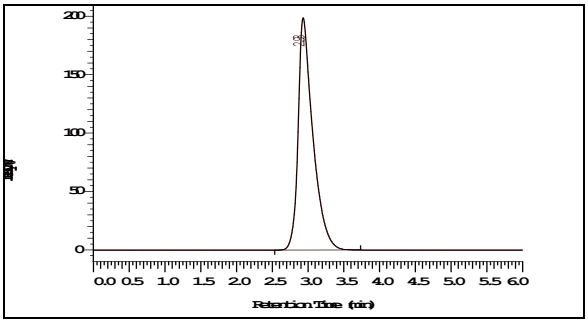


**Fig-2: Optimized of Dapagliflozin (10ppm)**

**Method Validation**

**1. Accuracy:** ***Recovery study:*** To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Dapagliflozin were

taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in Table-2.

**2. Precision:**

***Repeatability***

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Dapagliflozin (API) the percent relative standard deviations were calculated for Dapagliflozin is presented in the Table-3.

***Intermediate Precision:***

***Intra-assay & inter-assay:***

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Dapagliflozin revealed that the proposed method is precise. The results were shown in Table-4.

**3. Linearity & Range:**

The calibration curve showed good linearity in the range of 0-14µg/ml, for Dapagliflozin (API) with correlation coefficient ($r^2$) of 0.999 (Fig-28). A typical calibration curve has the regression equation of y = 72394x + 10725 for Dapagliflozin.

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 6, 8, 10, 12 and 14µg/ml. The prepared solutions were filtered through whatmann filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed[28] under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). The results which are given in Table-5 below were within acceptable limits..

**4. Method Robustness:**

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±$2^0$C), Wavelength of detection (±2nm) & Acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-6, % RSD < 2%) the developed RP-HPLC method for the analysis of Dapagliflozin (API).

**5. LOD & LOQ:**

The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding calibration plot.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.003 & 0.009 µg/ml respectively.

**6. ASSAY OF DAPAGLIFLOZIN IN DOSAGE FORM:**

DAPAGLIFLOZIN 5mg

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of HPLC grade methanol was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (3.5 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The amount of drug present and percentage purity was calculated by comparing the peak areas of the standards with that of test samples. The data are shown in Table-7.

**6. Stability studies:**

Following protocol was strictly adhered to for forced degradation of Dapagliflozin Active Pharmaceutical Ingredient (API). The API (Dapagliflozin) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. The results were shown in Table-1.

**Table-1: Results of forced degradation studies of Dapagliflozin API.**

| Stress condition | Time | Assay of active substance | Assay of degraded products | Mass Balance (%) |
|---|---|---|---|---|
| Acid Hydrolysis (0.1 M HCl) | 24Hrs. | 89.99 | 9.71 | 99.70 |
| Basic Hydrolysis (0.I M NaOH) | 24Hrs. | 99.54 | --------- | 99.54 |
| Thermal Degradation (50 $^0$C) | 24Hrs. | 98.03 | 0.61 | 98.64 |
| UV (254nm) | 24Hrs. | 99.51 | ---------- | 99.51 |
| 3 % Hydrogen peroxide | 24Hrs. | 95.23 | 3.81 | 99.04 |

## RESULTS AND DISCUSSION:

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Dapagliflozin different chromatographic conditions were applied & the results observed are given in tables. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here waters $C_{18}$, 5µm, 50 x 4.6 mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). The drug was found to be Dapagliflozin is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, which should be purged with an inert gas. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Dapagliflozin it is evident that most of the HPLC work can be accomplished in the wavelength range of 240-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis. Concentration range of 20-100µg/ml for Dapagliflozin was found to be linear with correlation coefficients 0.999 for Dapagliflozin. The proposed method was found to be precise and reproducible with %RSD of 0.42 for dapagliflozin. Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be 99.25 to 101.16% w/w for dapagliflozin. The LOD & LOQ were found to be 0.003 & 0.009 µg/ml respectively. The results of the stress studies indicated the specificity of the method that has been developed. Dapagliflozin was stable in both oxidation & acidic stress conditions.

The result shows the developed method is yet another suitable method for assay & stability which can help in the analysis of Dapagliflozin in different formulations.

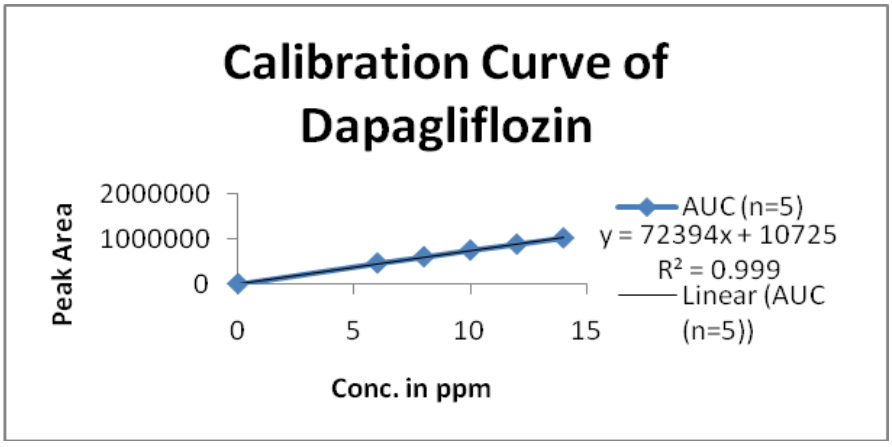


**Fig-3: Calibration curve of Dapagliflozin (API)**

**Table-2: Results of accuracy**

| Sample ID | Concentration (µg/ml) | | Peak Area | % Recovery of Pure drug | Statistical Analysis |
|---|---|---|---|---|---|
| | Amount Added | Amount Found | | | |
| $S_1$ : 80 % | 8 | 8.079 | 595625 | 100.987 | Mean= 100.7453% S.D. = 0.670976 % R.S.D.= 0.66601 |
| $S_2$ : 80 % | 8 | 8.021 | 591457 | 101.262 | |
| $S_3$ : 80 % | 8 | 7.999 | 589875 | 99.987 | |
| $S_4$ : 100 % | 10 | 9.998 | 734587 | 99.98 | Mean= 99.82667% S.D. = 0.517333 % R.S.D.= 0.51823 |
| $S_5$ : 100 % | 10 | 9.925 | 729268 | 99.25 | |
| $S_6$ : 100 % | 10 | 10.025 | 736524 | 100.25 | |
| $S_7$ : 120 % | 12 | 11.910 | 872949 | 99.25 | Mean= 100.0357% S.D. = 0.837025 % R.S.D.= 0.83672 |
| $S_8$ : 120 % | 12 | 12.110 | 887456 | 100.916 | |
| $S_9$ : 120 % | 12 | 11.993 | 878975 | 99.941 | |

**Acceptance criteria**: correlation coefficient should not be less than 0.990.

**2. Precision:**

**Repeatability:**

**Table-3: Results of Repeatability**

| S.No. | Repeatability | Drug Name | Rt | Peak Area | Theoretical Plates | Tailing Factor |
|---|---|---|---|---|---|---|
| **1** | Repeatability-1 | Dapagliflozin | 2.93 | 652542 | 3298 | 1.08 |
| **2** | Repeatability-2 | Dapagliflozin | 2.93 | 653345 | 3286 | 1.09 |
| **3** | Repeatability-3 | Dapagliflozin | 2.93 | 652841 | 3276 | 1.04 |
| **4** | Repeatability-4 | Dapagliflozin | 2.93 | 653687 | 3286 | 1.07 |
| **5** | Repeatability-5 | Dapagliflozin | 2.93 | 653874 | 3253 | 1.06 |
| **6** | Repeatability-6 | Dapagliflozin | 2.93 | 653427 | 3236 | 1.06 |
| **7** | **Average** | | **2.93** | **653286** | - | - |
| **8** | **Standard Deviation** | | **0** | **506.2545** | - | - |
| **9** | **% RSD** | | **0** | **0.077494** | - | - |

**Table-4: Results of intra-assay & inter-assay**

| Conc. Of Dapagliflozin (API) (µg/ml) | Observed Conc. Of Dapagliflozin (µg/ml) by the proposed method | | | |
|---|---|---|---|---|
| | Intra day | | Inter day | |
| | Mean (n=6) | % RSD | Mean (n=6) | % RSD |
| 8 | 8.06 | 1.08 | 7.86 | 1.05 |
| 10 | 10.26 | 0.97 | 10.13 | 0.94 |
| 12 | 12.51 | 0.92 | 11.09 | 0.96 |

**Table-5: Results of Linearity**

| S.No. | **Linearity** | Drug Name | Rt | Peak Area | Theoretical Plates | Tailing Factor |
|---|---|---|---|---|---|---|
| 1 | Linearity-1 | Dapagliflozin | 2.91 | 425874 | 2325 | 1.01 |
| 2 | Linearity-2 | Dapagliflozin | 2.94 | 565872 | 2456 | 1.03 |
| 3 | Linearity-3 | Dapagliflozin | 2.93 | 714542 | 2865 | 1.05 |
| 4 | Linearity-4 | Dapagliflozin | 2.93 | 865632 | 3236 | 1.06 |
| 5 | Linearity-5 | Dapagliflozin | 2.94 | 1013121 | 3236 | 1.06 |

**Acceptance criteria**: correlation coefficient should not be less than 0.990

**Table-6: Result of Method Robustness Test**

| Change in parameter | % RSD |
|---|---|
| Flow (1.1 ml/min) | 0.09 |
| Flow (0.9 ml/min) | 0.56 |
| Temperature ($27^0$C) | 0.12 |
| Temperature ($23^0$C) | 0.17 |
| Wavelength of Detection (227 nm) | 0.42 |
| Wavelength of detection (223 nm) | 0.46 |

**Table-7: Assay of Dapagliflozin Tablets**

| Brand name of tablets | Labelled amount of Drug (mg) | Mean (±SD) amount (mg) found by the proposed method (n=6) | % Purity |
|---|---|---|---|
| Forxiga tab (Astra Zeneca Pharmaceuticals LP.) | 5 | 5.16 (±0.09) | 100.16% (±0.48) |

The amount of drug in Forxiga tablets was found to be 5.16 (±0.09) mg/tab for Dapagliflozin and 100.16 (±0.48) mg/tab for Dapagliflozin.

## CONCLUSION:

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Dapagliflozin. Further, the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The results of the stress studies indicated that Dapagliflozin was stable in both oxidation & acidic stress conditions. The result shows the developed method is yet another suitable method for assay, purity & stability which can help in the analysis of Dapagliflozin in different formulations.

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