ABSTRACT

Keywords: Pharmaceutical dosage form.

Conclusion: The proposed method was found to be specific, accurate, precise and robust can be used for estimation of dapagliflozin in API and Pharmaceutical dosage form.

INTRODUCTION

Dapagliflozin is chemically a (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)tetrahydro 2H-pyran-3,4,5-triol) with molecular weight of 408.873 g/mol. Dapagliflozin is a sodium glucose co transporter inhibitor (antidiabetic drug), which prevents glucose reabsorption in the kidney. Thus leads to the elimination of glucose through urine [1-2].

Various analytical methods have been reported for dapagliflozin alone and in combination with metformin hydrochloride. Methods such as UV spectroscopy for estimation of dapagliflozin alone or in combination with metformin hydrochloride [3-7, 9]. HPLC method for estimation of dapagliflozin in API [10]. LC MS/MS for dapagliflozin [11] has been reported.

However, an extensive literature search didn’t reveal any estimation method for dapagliflozin in API and Pharmaceutical dosage form. Therefore an attempt has been made to develop and validate simple, precise, accurate HPLC method for estimation of dapagliflozin in API and Pharmaceutical dosage form.

MATERIALS AND METHODS

Drugs, chemicals and solvents: Dapagliflozin in API was kindly given by advanced analytical research and training institute, gujarat. All the chemicals and solvents used were of analytical grade.

Objective: To develop precise, accurate and reproducible stability assay method by RP-HPLC for estimation of dapagliflozin in API and Pharmaceutical dosage form.

Methods: The adequate separation was carried using agilent C18 (4.6 ml (millimeter)*150.5 µm (micrometer), mixture of acetonitrile: di-potassium hydrogen phosphate with pH 6.5 adjusted with OPA (40:60 %v/v) as a mobile phase with the flow rate of 1 ml/min (milliliter/minute) and the effluent was monitored at 222 nm (nanometer) using photo diode array detector. The retention time of dapagliflozin API and dapagliflozin tablet were 3.160 min (minute) and 3.067 min (minute) respectively.

Results: Linearity for dapagliflozin was found in the range of 50-150µg/ml (microgram/milliliter) (R² = 0.99) respectively. The accuracy of the present method was evaluated at 50 %, 100% and 150%. The % recoveries of dapagliflozin API and tablet were found to be in the range of 99.00-99.99 % and 98.50-99.99 % respectively. Precision studies were carried out and the relative standard deviation values were less than two. The method was found to be robust.

Conclusion: The present method was found to be specific, accurate, precise and robust can be used for estimation of dapagliflozin in API and Pharmaceutical dosage form.

Keywords: HPLC, Dapagliflozin, API, Pharmaceutical dosage form, OPA
Acid degradation

Procedure for API
Transfer 1 ml of standard solution to 10 ml of volumetric flask. Add 1 ml of 3% H₂O₂ keep the volumetric flask in a water bath at 60 °C for 2 h. After time period cool the contents to ambient temperature. Dilute the volume with diluent. After inject the peroxide degradation sample into HPLC, peak area and peak shape were observed fig. 6.

Procedure for tablet
The average of 10 tablet was determined and grounded in mortar. An accurately weigh the amount of powder equivalent to 10 mg of dapagliflozin was taken. It was transferred to 10 ml of volumetric flask. Add 5 ml of diluents and sonicated for 5 min to ensure complete solubilization of drug. After sonication, volume was made up to the mark with diluents (1000 µg/ml of dapagliflozin stock solution). Pipette out 5 ml from above solution and dilute up to 10 ml with diluents (500 µg/ml of dapagliflozin). Pipette out 1 ml from the sample solution and add 1 ml of 3% H₂O₂ keep the volumetric flask in water bath at 60 °C for 2 h. After time period cool the contents to ambient temperature. Dilute the volume with diluent. After inject the peroxide degradation sample into HPLC, peak area and peak shape were observed fig. 7.

Thermal degradation

Procedure for API
Transfer 1 ml of standard solution to 10 ml of volumetric flask. Keep the volumetric flask in water bath at 60 °C for 2 h. After time period cool the contents to ambient temperature. Make up the volume with diluent. After injecting the thermal degradation sample into HPLC, peak area and peak shape were observed fig. 8.

Procedure for tablet
The average of 10 tablet was determined and grounded in mortar. An accurately weigh the amount of powder equivalent to 10 mg of dapagliflozin was taken. It was transferred to 10 ml of volumetric flask. Add 5 ml of diluents and sonicated for 5 min to ensure complete solubilization of drug. After sonication, volume was made up to the mark with diluents (1000 µg/ml of dapagliflozin stock solution). Pipette out 5 ml from above solution and dilute up to 10 ml with diluent (500 µg/ml of dapagliflozin). Pipette out 1 ml of the sample solution to 10 ml of volumetric flask. Keep the volumetric flask in water bath at 60 °C for 2 h. After time period cool the contents to ambient temperature. Make up the volume with diluent. After inject the thermal degradation sample into HPLC, peak area and peak shape were observed fig. 9.

Photolytic degradation

Procedure for API
Transfer 1 ml of standard solution to 10 ml of volumetric flask. It was exposed to direct sunlight for 1 h, make up the volume with diluent. After inject the photolytic degradation sample into HPLC, peak area and peak shape were observed fig. 10.

Procedure for tablet
The average of 10 tablet was determined and grounded in mortar. An accurately weigh the amount of powder equivalent to 10 mg of dapagliflozin was taken. It was transferred to 10 ml of volumetric flask. Add 5 ml of diluents and sonicated for 5 min to ensure complete solubilization of drug. After sonication, volume was made up to the mark with diluents (1000 µg/ml of dapagliflozin stock solution). Pipette out 5 ml from above solution and dilute up to 10 ml with diluents (500 µg/ml of dapagliflozin). Pipette out 1 ml of the sample solution to 10 ml of volumetric flask. It was exposed to direct sunlight for 1 h, make up the volume with diluent. After inject the photolytic degradation sample into HPLC, peak area and peak shape were observed fig. 11.

Method validation
System suitability was carried out by injecting standard solutions of API and tablet 5 times into the chromatographic system. The system
suitability parameters were then evaluated for tailing factor, retention time and theoretical plates of standard chromatograms.

**Accuracy**
The accuracy of the test method was demonstrated by % recovery across its range by making three different concentrations at 50%, 100% and 150% level using standard addition method.

**Intrarady precision**
Intrarady precision was performed by injecting standard preparations three times on the day by maintaining the optimized chromatographic conditions and calculate % relative standard deviation of retention time and peak areas for dapagliflozin.

**Inter-day precision**
Inter-day precision was performed by injecting standard preparations three times into chromatographic system on 2 different days by maintaining the optimized chromatographic conditions and calculate % relative standard deviation of retention time and peak areas for dapagliflozin.

**Repeatability**
Method precision of experiment was performed by preparing the standard solutions of Dapagliflozin (500 µg/ml) for six times and analysed as per proposed method and % RSD was calculated.

**Linearity**
Transfer an accurately weighed quantity about 100 mg of dapagliflozin in 100 ml volumetric flask, dissolve and dilute the volume with diluents. Prepare different linearity concentration solutions in the range of 250–750 µg/ml.

**Robustness**
The robustness was studied by analyzing the sample of dapagliflozin by deliberate variation in method parameters. The change in response of dapagliflozin was noted. Robustness of the method was studied by changing flow rate±0.2 ml, mobile phase composition and column temperature. The change in the response of dapagliflozin was noted and compared with the original one.

**Limit of detection and limit of quantification**
LOD and LOQ were determined by using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations:

\[
\text{LOD} = 3.3 \sigma / S
\]
\[
\text{LOQ} = 10 \sigma / S
\]

Where, \( \sigma \) = Standard deviation of response
\( S \) = Slope of calibration curve

**RESULTS AND DISCUSSION**
The detection wavelength was carried out in the UV range of 222 nm. Chromatographic separation was carried out using mobile phase composed 1 molar dipotassium hydrogen phosphate and acetonitrile (60:40 % v/v) and pH was adjusted to 6.5 with orthophosphoric acid on agilent C18 (4.6 mm 150,5 µm) at a flow rate of 1 ml/min using PDA detector.

(1) Acid degradation for API and tablet

![Fig. 2: Acid degradation for dapagliflozin in API](image)

![Fig. 3: Acid degradation for dapagliflozin in tablet](image)
(2) Base degradation for API and tablet

Fig. 4: Base degradation for dapagliflozin in API

Fig. 5: Base degradation for dapagliflozin tablet

(3) Peroxide degradation

Fig. 6: Peroxide degradation for dapagliflozin API

Fig. 7: Peroxide degradation for dapagliflozin tablet
(4) Thermal degradation

Fig. 8: Thermal degradation for dapagliflozin API

Fig. 9: Thermal degradation for dapagliflozin tablet

(5) Photolytic degradation

Fig. 10: Photolytic degradation for dapagliflozin API

Fig. 11: Photolytic degradation for dapagliflozin
Table 1: Degradation summary for API

| Type               | Solution     | Area        | %Degradation |
|--------------------|--------------|-------------|--------------|
| As such            | Dapagliflozin| 5231398     | -            |
| Acid               | Dapagliflozin| 3270712     | 6.25%        |
| 0.1 N' HCL* at 60 °C for 2 h in water bath | Dapagliflozin | 27787835 | 5.31% |
| Base               | Dapagliflozin| 5059869     | 9.66%        |
| 0.1 N NaOH* at 60 °C for 2 h in water bath | Dapagliflozin | 6116184 | 11.68% |
| Peroxide           | Dapagliflozin| 7849709     | 9.23%        |
| 3% H$_2$O$_2$* at Room Temperature for 3 h | Dapagliflozin | 81920313 | - |
| Thermal            | Dapagliflozin| 18920313    | -            |
| Photolytic in sun light for 1 h | Dapagliflozin | 1500380 | 7.93% |

N*-normal, HCl*-hydrochloric acid, NaOH*-sodium hydroxide, H$_2$O$_2$*-hydrogen hydroxide

Table 2: Degradation summary for tablet

| Type               | Solution     | Area          | % Degradation |
|--------------------|--------------|---------------|--------------|
| As such            | Dapagliflozin| 18920313      | -            |
| Acid               | Dapagliflozin| 1500380       | 7.93%        |
| 0.1 N' HCL* at 60 °C for 2 h in water bath | Dapagliflozin | 1738776 | 9.19% |
| Base               | Dapagliflozin| 1920411       | 10.15%       |
| 0.1 N NaOH* at 60 °C for 2 h in water bath | Dapagliflozin | 1651743 | 8.73% |
| Peroxide           | Dapagliflozin| 2104050       | 11.12%       |
| 3% H$_2$O$_2$* at Room Temperature for 3 h | Dapagliflozin | 1902411 | 10.15% |
| Thermal            | Dapagliflozin| 1651743       | 8.73%        |
| Photolytic in sun light for 1 h | Dapagliflozin | 2104050 | 11.12% |

N*-normal, HCl*-hydrochloric acid, NaOH*-sodium hydroxide, H$_2$O$_2$*-hydrogen hydroxide

Validation data

Fig. 12: Chromatogram of API

Table 3: System suitability results (API)

| S. No. | System suitability parameter | Results  |
|--------|------------------------------|----------|
| 1      | Tailing                      | 1.28     |
| 2      | Retention Time               | 3.160 min|
| 3      | Plate count                  | 2350     |
| 4      | Area                         | 5165316  |
| 5      | Correlation coefficient       | 0.99     |
| 6      | LOD*                         | 5.14 µg/ml|
| 7      | LOQ*                         | 15.6 µg/ml|

LOD*-Limit of detection, LOQ*-Limit of quantification

Table 4: System suitability results (tablet)

| S. No. | System suitability parameters | Results  |
|--------|------------------------------|----------|
| 1      | Tailing                      | 1.20     |
| 2      | Retention time               | 3.067    |
| 3      | Plate count                  | 2030     |
| 4      | Area                         | 18920313 |
Table 5: System suitability data

| Parameters          | Observation | Specification |
|---------------------|-------------|---------------|
| % RSD of Area       | 0.26        | Tablet        |
| Resolution(Rs)      | 0.00        | Rs>2%         |
| Tailing Factor(T)   | 1.28±0.04   | T ≤ 2         |
| Theoretical plates(N)| 2350±185.02| ≥2000         |

Table 6: Linearity data for dapagliflozin

| Conc* (ng) | Peak area±SD* (n=5) | %RSD* |
|------------|---------------------|-------|
| 250        | 527484.2±11182.6    | 0.21  |
| 400        | 895864.53±10236.58  | 0.12  |
| 500        | 1119055.8±30236.23  | 0.27  |
| 600        | 1343826.54±50923.2  | 0.38  |
| 750        | 1679783.69±99983.5  | 0.59  |

Number of experiment (n)-5, Conc*-concentration, SD*-standard deviation, %RSD*-relative standard deviation
Table 7: Accuracy for API and tablet

| Sample              | Level (%) | Amount recovered (µg/ml) | Mean % recovery±SD*          |
|---------------------|-----------|--------------------------|-----------------------------|
| Dapagliflozin API   | 50        | 402.96±0.507             | 99.72±0.12                  |
|                     | 100       | 503.28±0.55              | 99.65±0.109                 |
|                     | 150       | 601.12±0.24              | 99.19±0.03                  |
|                     | 50        | 398.65±0.55              | 98.67±0.13                  |
| Dapagliflozin tablet| 100       | 501.98±0.871             | 99.40±0.17                  |
|                     | 150       | 605.89±0.98              | 99.98±0.16                  |

Number of experiment (n)=3, SD*—standard deviation, precision study results

Table 8: Intraday precision

| Conc* (µg/ml) | Area±SD*     | % RSD* |
|---------------|--------------|--------|
| 400           | 11304882.67±45662.51 | 0.40   |
| 500           | 11205269±27555.81     | 0.26   |
| 600           | 11368903.33±23214.27  | 0.204  |

Conc*—concentration, Number of experiment (n)-3, SD*—standard deviation, RSD*—relative standard deviation

Table 9: Interday precision

| Conc* (µg/ml) | Area±SD*     | % RSD* |
|---------------|--------------|--------|
| 400           | 11386283±25806.57 | 0.23   |
| 500           | 11174585.67±46710.12 | 0.42   |
| 600           | 11192177±38642.96   | 0.34   |

Conc*—concentration, Number of experiment (n)-3, SD*—standard deviation, RSD*—relative standard deviation

Table 10: Repeatability data

| S. No. | Dapagliflozin (500 µg/ml) |
|--------|---------------------------|
| 1      | 11448945                  |
| 2      | 11367925                  |
| 3      | 11382354                  |
| 4      | 11283925                  |
| 5      | 11356486                  |
| 6      | 11345685                  |
| Mean   | 11364220                  |
| SD     | 11596.78                  |
| RSD*   | 0.102                     |

Number of experiment (n)-6, SD*—standard deviation, RSD*—relative standard deviation

Table 11: Robustness study

| Conc* (500 µg/ml) | Flow rate | Temperature (°C) | Mobile Phase |
|-------------------|-----------|------------------|--------------|
|                   | 0.8 ml    | 1.2 ml           | 25°          | 35°          | +5 ml | -5 ml |
| Avg. area         | 66176464  | 5234741          | 1456537.3    | 1856685      | 18478292.3 | 43198353 |
| SD*               | 78967.96  | 5998.1           | 12044.42     | 102585       | 132229.6   | 20341.1  |
| % RSD*            | 0.11      | 0.11             | 0.83         | 0.55         | 0.83     | 0.12     |

Conc*—concentration, Number of experiment (n)-3, SD*—standard deviation, RSD*—relative standard deviation

Table 12: LOD and LOQ

| Parameter       | Dapagliflozin |
|-----------------|--------------|
| LOD* (µg/ml)    | 5.14         |
| LOQ* (µg/ml)    | 15.6         |

LOD*—limit of detection, LOQ*—limit of quantification

DISCUSSION

A new stability indicating RP-HPLC method has been developed for estimation of Dapagliflozin in API and Tablet dosage form was rapid, accurate, precise, sensitive and robust.

From the above study, we can conclude that the dapagliflozin was subjected to acid, alkali hydrolysis, and oxidation, thermal and photolytic degradation. The degradation studies indicate that dapagliflozin is more susceptible to thermal degradation and Forxiga is more susceptible to photolytic degradation.
From the peak purity study, it was confirmed that the peak of degradation product and excipient was not interfering with the peak of the drug. Hence this method was used for the analysis of Dapagliflozin in API and tablet dosage form in quality control department for routine analysis.

Linearity of the developed method follows beer’s law and was near to 0.99. It found to be linear in the range 250–750 µg/ml. % RSD was found to be less than 2 for precision. The method is robust since by deliberate variation in method, % RSD was found to be less than 2. So the is found to be robust.

% Recoveries was found to be 99.65 %. Hence this method can be used for analysis of dapagliflozin API and Tablet dosage form in quality control department for routine analysis.

CONCLUSION

In the present study, we have developed a new, rapid RP-HPLC method and validated for different parameters (system suitability, linearity, accuracy, precision, LOD, LOQ robustness). By studying all these we have concluded that the method was linear, accurate, precise, robust and rapid for determination of dapagliflozin in API and Pharmaceutical dosage form. Hence the method was successfully applied for the estimation of dapagliflozin in API and Pharmaceutical dosage form.

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CONFLICT OF INTERESTS

Declare none

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