RESEARCH ARTICLE

Development and Validation of stability-Indicating RP-HPLC method for determination of Dapagliflozin

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ABSTRACT

The present study describes the development and subsequent validation of a stability indicating reverse phase HPLC (RP-HPLC) method for the analysis of Dapagliflozin in its API. The proposed method utilizes BDS column (maintained at ambient temperature), gradient run (using mixture of acetonitrile and ortho phosphoric acid as mobile phase), effluent flow rate (1ml/min) and detection at 245nm using PDA detector. The developed method was successfully validated for different validation parameters as per ICH guidelines. The stability of the drug was determined by studying the degradation of the drug under acidic, alkaline, peroxide, neutral, heat and UV conditions.

Keywords: Dapagliflozin (DAPA), RP-HPLC, Gradient run, Degradation.

INTRODUCTION

Dapagliflozin is a novel inhibitor of renal sodium-glucose co transporter 2, which allows an insulin-independent approach to improve type 2 diabetes hyperglycemia [1]. It is a C-aryl glucoside derivative and is chemically known as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol. The chemical structure of Dapagliflozin was given in Fig. 1.

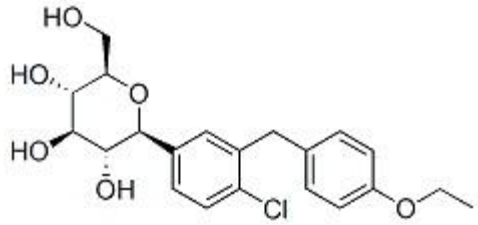

Fig. 1: Structure of Dapagliflozin

As per the literature review, DAPA was estimated by LC-MS/MS in biological fluids like human plasma and rat plasma. [2] Moreover several methods were there for the determination of its pharmacologic action. [3-9] But there was no stability indicating RP-HPLC method for the determination of DAPA in its API. So the aim of present work was to develop and validate a stability indicating RP-HPLC method for the determination of DAPA in its API.

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MATERIALS AND METHODS

Chemicals and Reagents:

Dapagliflozin was obtained as gift sample from Manus Aktteva Biopharma, Gujarat. Acetonitrile and water used were of HPLC grade.

Instrumentation:

A waters HPLC system with empower 2 software, fitted with a PDA detector and a BDS column was used for the analysis.

Chromatographic conditions:

An HPLC system (make: waters, model 2996) which is operated using a software, Empower 2, fitted with BDS column and PDA detector (at 245nm) was used for the analysis. Gradient run with a flow rate 1ml/min was preferred for resolving the drug.

Preparation of mobile phase:

A mixture (55:45) of Acetonitrile and Ortho phosphoric acid was used as mobile phase.

Diluents:

Initially, the drug was dissolved in methanol, and water HPLC grade was used as a diluent for further dilutions.

Preparation of stock solution:

Accurately weighed 10mg Dapagliflozin was dissolved in a few ml of methanol and then the volume was made up to 10ml with methanol to get a solution of 1000µg/ml concentration.

The chromatogram of standard Dapagliflozin solution was shown in Fig. 2. And the average retention time was found to be 2.873 min.

Validation:

System suitability:

It is an integral part of chromatographic technique. It is performed to ensure system performance before and during analysis, such as equipment, electronics, samples and analytical operations.

It was performed by injecting six replicate injections of standard solution of Dapagliflozin at 100% level (100µg/ml) and was expressed as %RSD of peak area. The results were tabulated in Table 1.

Linearity:

Linearity is the ability of the method to elicit test results that are proportional to concentration of the analyte in the sample. It was found to be in the range of 25-150 µg/ml.

Aliquots of standard stock solution were taken in a series of standard flasks and were diluted to obtain 25, 50, 75, 100, 125, and 150µg/ml of Dapagliflozin. The calibration graph was plotted and the drug was found to be linear with a correlation coefficient (r^2) of 0.999. Linearity data was tabulated in Table 2.

The calibration curve was shown in Fig. 3.

Accuracy:

It is the closeness of test results obtained by the method to the true value. It was determined by percent recovery of the standard API to the blank.

The average recovery of the analyte of 50%, 100%, 150% solution was found to be 99.95%.

Precision:

It is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings.

It was determined by studying repeatability, intra-day and inter-day precision of method. The results were calculated as %RSD and were tabulated in Table 3.

Robustness:

It is the capacity of the method to remain unaffected by small but deliberate variations in method parameters.

The analysis was performed by slightly changing the temperature, mobile phase composition and flow rate. The results were calculated as %RSD and were given in Table 4.

Limit of Detection (LOD):

LOD is the lowest level of concentration of analyte in the sample that can be detected, though not necessarily quantitated. It is calculated to be 0.6µg.ml using the formula,

$LOD = 3.3\sigma/S$

Where,

σ= Standard deviation of the response,

S= Slope of calibration curve.

Limit of Quantitation (LOQ):

LOQ is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied. It was calculated to be 1.8µg/ml using the formula,

$LOQ = 10\sigma/S$

Where,

σ = Standard deviation of the response,

S = Slope of calibration curve.

Degradation studies:

The degradation studies of DAPA were carried out by following the guidelines of ICH. In order to determine the stability of DAPA, the drug was treated with acid, alkali, hydrogen peroxide, heat, water and UV light. Then the percentages of degradation were calculated and shown in Table 5.

Oxidative degradation:

To 1ml of stock solution, 1ml of 20% hydrogen peroxide (H_2O_2) was added. Then the solution was kept at 60^0c for about 30 minutes. Then the resultant solution was diluted sufficiently to obtain a concentration of 100µg/ml. From this solution, 10µl

was injected into the system and the chromatogram was recorded. Then the % of degradation was calculated.

Acid degradation:

To 1ml of stock solution, 1ml of 2N hydrochloric acid was added and refluxed at 60^{0}c for about 30 minutes. Then the resultant solution was sufficiently diluted to get 100µg/ml solution. From this, 10µl was injected into the system. From the peak area found in the chromatogram, the % of degradation was calculated.

Alkali degradation:

To 1ml of stock solution, 1ml of 2N sodium hydroxide was added and refluxed at 60^{0}c for about 30 minutes. Then the resultant solution was sufficiently diluted to get 100µg/ml solution. From this, 10µl was injected into the system. From the peak area found in the chromatogram, the % of degradation was calculated.

Dry heat degradation:

In this dry heat degradation study, 1ml of standard stock solution was kept in an oven which is maintained at 105^{0}c for about 6hrs. Then the solution was diluted sufficiently to obtain a concentration of 100µg/ml. Then the chromatogram was recorded and from this, the % of degradation was calculated.

Photo stability study:

In order to determine the photo stability of the drug, 1ml of stock solution was exposed to UV light in UV chamber for about 7 days. Then the same procedure was followed as mentioned above.

Neutral degradation study:

To determine the neutral stability of the drug, the drug was treated with water and then the solution was refluxed at a temperature of 60^{0}c. The remaining procedure was followed as mentioned above.

Results and Discussion:

Table 1: System suitability data [a]

Parameter	Dapagliflozin
Tailing factor	1.25
Theoretical plates	4866
%RSD of peak area	0.2

a: System suitability was estimated by determining the parameters such as tailing factor and theoretical plates.

Table 2: Linearity data [b]

Parameter	Result
Range	25-150µg/ml
Regression equation	17296x+3129
Slope	17296
Intercept	3129
Correlation co-efficient	0.999

b: The linearity plot showed to be linear with a correlation co-efficient (r^2), 0.999.

Table 3: Precision study [c]

Concentration (100µg/ml)	Repeatability	Intra-day	Inter-day	
			Day 1	Day 2
SD	1429	4787	4787	1429
%RSD	0.7	0.2	0.2	0.7

c: Precision was estimated by studying repeatability, intra-day and inter-day tests. The results were calculated as Standard deviation and Relative standard deviation.

Table 4: Robustness data [d]

Concentration (100µg/ml)	Flow rate (ml/min)		Temperature(^{0}C)		Mobile phase	
	0.8	1.2	25	35	+2ml	-2ml
SD	2632	6484	9277	16801	1343	2320
%RSD	0.1	0.3	0.5	0.8	0.07	0.1

d : Robustness was studied by performing the analysis at flow rate (0.8ml/min and 1.2ml/min), temperature (25^{0}C and 35^{0}C) and mobile phase (+2ml and -2ml). All the results were calculated as Standard deviation and %RSD.

Table 5: Degradation study data [e]

Degradation Condition	% of degradation
Hydrogen peroxide	5.1
Acid	7.4
Alkali	6.4
Thermal (Dry heat)	4.4
UV	1.4
Neutral (Water)	0.6

e: Degradation percentages of degradable conditions such as treatment with hydrogen peroxide, acid, alkali, dry heat, UV light, were calculated.

Fig. 2: Chromatogram of Dapagliflozin standard solution

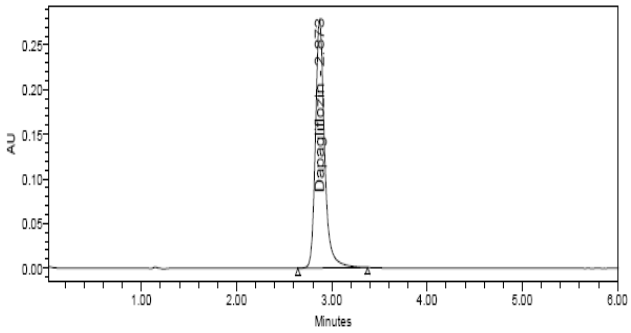
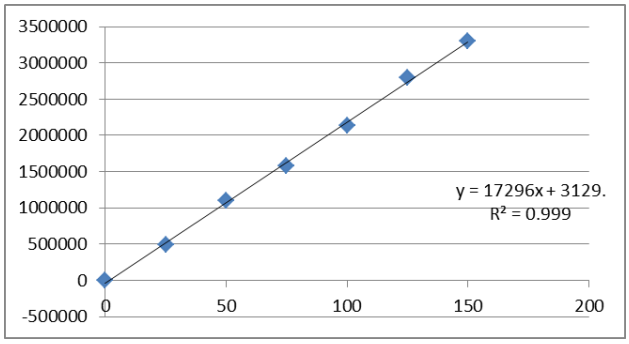


Fig. 3: Calibration plot for Dapagliflozin



CONCLUSION

In conclusion, a simple, selective, sensitive and accurate stability indicating RP-HPLC method was developed and validated for the analysis of Dapagliflozin API. Further the method was found to be linear, precise, accurate and robust. The degradation studies reveal the stability of the drug. Hence the proposed method can be safely and successfully used for the estimation of Dapagliflozin API in routine analysis.

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