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Method Development and Validation of Dapagliflozin in API by RP-HPLC and UV-Spectroscopy

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ABSTRACT
In the present study, two analytical methods were developed for the estimation of Dapagliflozin in API. Method A: RP-HPLC method, Method B: UV spectroscopic method. In method A, the drug showed linearity in the range of 25-150µg/ml with a correlation coefficient (r^2) of 0.999, where as in method B, the linearity range was found to be 1-5µg/ml with a correlation coefficient of (r^2) 0.999. Both the methods were validated for different validation parameters such as linearity, accuracy, precision, detection limit, quantitation limit, robustness and ruggedness and the results were found to be within the acceptance limits as per the guidelines of International Conference on Harmonization (ICH).

Keywords: Dapagliflozin, UV-spectroscopy, RP-HPLC, International Conference on Harmonization.

INTRODUCTION

Dapagliflozin belongs to a new class of oral ant diabetic drugs, called Sodium Glucose Co-Transporter 2 (SLGT2) inhibitors. These Sodium Glucose Co-Transporters are responsible for glucose reabsorption in the kidney. Hence inhibiting the SLGT2 have been proposed as a new strategy in the treatment of diabetes. [1] By suppressing the SLGT2, dapagliflozin reduces plasma glucose concentration intern by elevating the renal glucose excretion. It is a C-aryl glucoside derivative which has a molecular structure as shown in Figure 1. It is chemically known as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol.

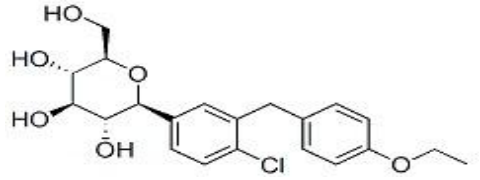

Fig. 1: Structure of Dapagliflozin

The literature survey revealed that dapagliflozin was estimated in biological fluids like human plasma and rat plasma by LC-MS/MS. [2] Moreover the pharmacologic action of the drug was estimated by several methods. [3-11] But the drug was not estimated by any of the techniques, UV spectroscopy and RP-HPLC. Hence the present work was aimed to develop and validate an RP-HPLC method and a UV spectroscopic method.

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MATERIALS AND METHODS

Method A (RP-HPLC Method)

Drugs, solvents and chemicals

Dapagliflozin pure API was gifted by Manus Aktteva Biopharma, Gujarat. Methanol and distilled water which were of HPLC grade were used for the analysis. All other chemicals used which were of analytical grade and were procured from local market.

Instrument

Waters HPLC system controlled with software Empower 2, fitted with a Photo Diode Array detector and a gradient run was used for resolving the drug. The column used for the separation of the drug was BDS column and the column temperature was maintained at the ambient temperature.

Preparation of mobile phase

A mixture of Acetonitrile and Ortho phosphoric acid was used as the mobile phase. The optimized method consists of the mobile phase in the ratio of 55:45v/v. Methanol (HPLC grade) was used for dissolving the drug and further dilutions were made using water (HPLC grade).

Preparation of Standard stock solution

Dapagliflozin API was transferred to a dry, clean 10 ml volumetric flask, dissolved in 5 ml of methanol and then the volume was made up to the mark with methanol to get a concentration of 1000µg/ml.

Preparation of working standard solutions of Dapagliflozin for linearity

Appropriate dilutions were made with water to produce 25-150µg/ml solutions. The calibration curve was plotted and was shown in Figure 2.

The chromatogram of Dapagliflozin which showed the retention time 2.87minutes was shown in Figure 3.

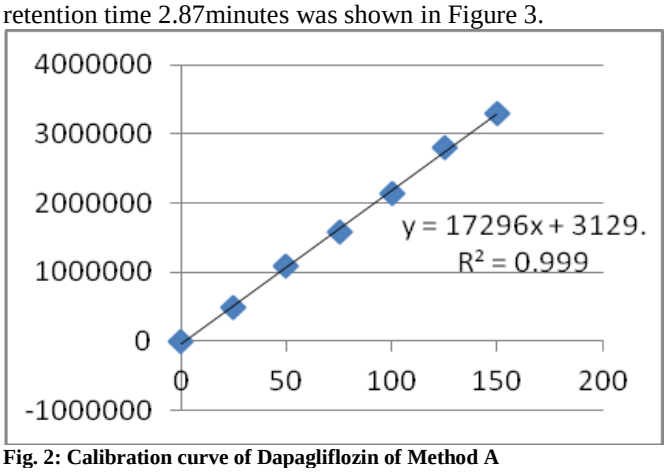

Fig. 2: Calibration curve of Dapagliflozin of Method A

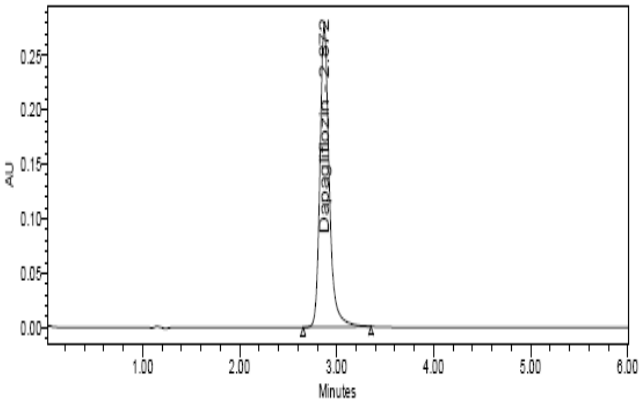

Fig. 3: Chromatogram of Dapagliflozin standard solution

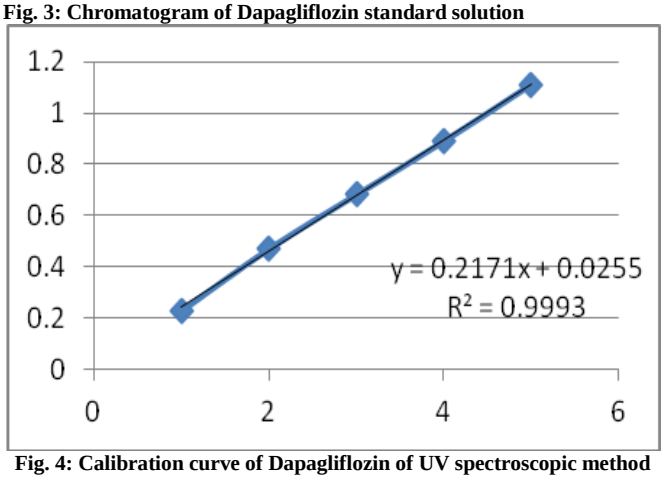

Fig. 4: Calibration curve of Dapagliflozin of UV spectroscopic method

Method Validation

Linearity

In this method, Dapagliflozin showed to be linear in the range of 25-150µg/ml with a correlation of coefficient (r^2) 0.999.

Accuracy

It was determined by standard addition method and the results were calculated as %recovery. The average % recovery of the drug was found to be 99.8% and was found to be satisfactory.

Precision

To determine the precision, intra-day and inter-day analysis was performed. The results were calculated as %RSD values and were found to be satisfactory and were shown in Table 1.

Robustness

Robustness of the method was determined by performing the same analysis at slightly different parameters from the optimized conditions of the method. The results were calculated as %RSD values as shown in Table 2.

Detection limit and Quantitation limit

The detection limit and quantitation limit of the method were calculated as the 0.60µg/ml and 1.81µg/ml respectively.

Method B (UV Spectroscopic Method)

Instrumentation

A double beam UV-Visible spectrophotometer (Make: Labindia, Model: UV-VIS 3082, Software: UV Win) was used for measuring the spectral absorbance.

Selection of analytical wavelength

Appropriate dilutions were made from the standard stock solution (1000µg/ml) to obtain different solutions of 1-5µg/ml. One of the above solutions was scanned in the UV range of 190-400nm. From the spectrum, the wavelength of maximum absorption (λ max) was selected as 203nm. The optical characteristics of the method were given in Table 3.

Method Validation

Linearity

The method showed to be linear in the range of 1-5µg/ml with a correlation coefficient of 0.999. The calibration curve was shown in Figure 4.

Precision

The precision of the method was studied by considering the results of intra-day and inter-day studies.

Detection limit and Quantitation limit

The detection limit and quantitation limit of the method were calculated as 0.016µg/ml and 0.05µg/ml respectively.

Ruggedness

In order to determine this parameter the analysis was performed at same operational conditions and same environmental conditions but using different analysts.

Robustness

To determine robustness the analysis was performed by slightly modifying the wavelength from the actual λ max.

All the validation parameters of the UV spectroscopic method were given in Table 5.

Table 1: Precision result

Precision	%RSD
Repeatability	0.78
Intra-day precision	0.26
Inter day precision	
Day 1	0.26
Day 2	0.78

Table 2: Robustness study

Parameter	Flow rate	Mobile phase	Temperature
	1.2ml/min	+2ml	350°C
	0.8ml/min	-2ml	250°C
%RSD	0.35	0.07	0.83
	0.14	0.12	0.48

Table 3: Optical characteristics of the UV spectroscopic method

S. No	Parameter	Result
1.	Absorption maxima	203nm
2.	Linearity range	1-5µg/ml
3.	Correlation coefficient	0.999
4.	Regression equation	0.217x+0.02
5.	LOD	0.01µg/ml
6.	LOQ	0.05µg/ml

Table 4: Validation parameters of UV spectroscopic method

Parameter	Precision		Robustness		Ruggedness	
	Intra-day	Inter day	201nm	205nm	Analyst 1	Analyst 2
Result	0.678± 0.22	0.673± 0.36	0.682± 0.08	0.66±0 .71	0.68±0. 22	0.683± 0.47

RESULTS AND DISCUSSION

In the present work we have developed and validated an RP-HPLC method and a UV spectroscopic method. Both the methods were validated as per ICH guidelines. The linearity was found to be 25-150µg/ml and 1-5µg/ml for RP-HPLC and UV spectroscopic method respectively, showing the correlation coefficient of 0.999. The RP-HPLC method was validated for accuracy, linearity, precision, LOD, LOQ, system suitability and robustness. The results were given in Table 2. The UV spectroscopic method was validated for precision, linearity, LOD, LOQ, robustness and ruggedness and the results were tabulated in Table 5. All the results were found to be within the limits as per ICH guidelines and hence the proposed two methods can be successfully employed for the determination of Dapagliflozin in its API for regular and routine analysis.

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