



Method Development and Validation of Dapagliflozin API by UV Spectroscopy

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ABSTRACT

In the present study, a simple, novel, safe, sensitive and economic UV- Spectrophotometric method for the estimation of a Type II anti diabetic drug, Dapagliflozin was developed and assessed. The developed method was validated as per ICH guidelines. The drug showed two different wavelengths of maximum absorption, at 203nm and 237nm. This method can be successfully applied for the estimation of Dapagliflozin in bulk for routine analysis with UV detection at 237nm. A Labindia UV-Visible spectrophotometer with 1cm matched quartz cells and ethanol solvent were employed in this method. The Developed method obeyed Beer's-Lambert's law in the concentration range of 0.5-0.9µg/ml, having correlation coefficient of 0.994. Different validation parameters like, precision (intra-day and inter-day studies), limit of detection, limit of quantitation, ruggedness and robustness were studied and were found to be within the limits.

Keywords: Correlation coefficient, Dapagliflozin, Ethanol, UV-Visible spectrophotometer.

INTRODUCTION

Dapagliflozin is a highly selective, potent, orally active and reversible inhibitor of the human Sodium-Glucose Co-Transporter 2 (SGLT2) the major transporter responsible for the renal glucose reabsorption. It improves glycaemic control in patients with Type 2 Diabetes Mellitus by inhibiting the Sodium-Glucose Co-Transporter 2, intern by reducing glucose reabsorption.

Dapagliflozin's mechanism of action is complementary to and different from the mechanisms of currently available antidiabetic drugs as it involves the direct and insulin-independent elimination of glucose by the kidney.

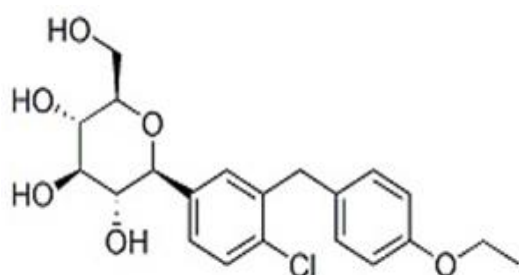


Figure 1: Chemical structure of Dapagliflozin

Dapagliflozin has the structural formula 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. It has a molecular formula of $C_{27}H_{25}ClO_6$ and a molecular weight of 408.87. Dapagliflozin is a white to half white crystalline powder which is soluble in water, ethanol, methanol, DMSO, dimethyl formamide.

Literature survey revealed that the drug has been estimated by LC-MS method in biological fluids like human plasma and rat plasma.¹ Moreover the

pharmacologic action related aspects of Dapagliflozin were determined,²⁻⁸ but no UV-Spectrophotometric method was reported for the estimation of Dapagliflozin in bulk till now.

Hence the present work was aimed to develop and validate a simple, sensitive, precise, and specific UV-Spectrophotometric method for estimation of Dapagliflozin in its API.

MATERIALS AND METHODS

Instrumentation

The absorbance and spectral measurements were done on a double-beam Labindia UV-Visible spectrophotometer with software UV Win. 1cm quartz cells were used for sample handling. A digital analytical balance was used for weighing.

Materials and Reagents

Dapagliflozin API was obtained from Manus Aktteva Biopharma, Gujarat as a gift sample. Ethanol and Distilled water were procured from local market. All the chemicals and solvents used were of analytical grade.

Preparation of standard stock solution (100µg/ml)

Accurately weighed 5mg of pure Dapagliflozin was taken in clean, dry 50ml volumetric flask and dissolved in few ml of ethanol, and the volume was made up to 50ml to obtain a concentration of 100µg/ml.

Preparation of working standard solution (10µg/ml)

From standard stock solution (100µg/ml), 5ml was pipette out and diluted to 50ml with distilled water to produce working standard solution (10µg/ml).

Selection of Analytical wavelength

Different aliquots (0.5, 0.6, 0.7, 0.8 and 0.9 ml) of working standard solution were transferred to a series of 10 ml volumetric flasks and then made up to 10ml with distilled water to obtain a concentration range of 0.5-0.9 µg/ml. One of the solutions was scanned in UV range of 190-400 nm using water as a blank and the wavelength of maximum absorption was found to be 237nm and 203nm. 237nm is selected for further analysis. The UV spectrum of 0.7µg/ml solution was shown in Figure 2.

Method validation

Linearity

Different aliquots 0.5, 0.6, 0.7, 0.8, 0.9 ml of working standard were transferred to a series of 10 ml volumetric flasks and then made up to 10ml with distilled water to obtain 0.5, 0.6, 0.7, 0.8, 0.9 µg/ml respectively. Then their absorbance was measured at 237nm against blank. The calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis. The calibration plot was shown in Figure 3. And the optical characteristics and other parameters were shown in table 1.

Precision

The precision of the method was demonstrated by intraday and inter-day studies. In the intra-day study, three different solutions of the same concentrations (0.7µg/ml) were prepared and analyzed thrice a day (morning, afternoon, and evening).

In the inter-day variation study, the solutions of same concentration (0.7µg/ml) were prepared and analyzed daily for three days, and the absorbance was recorded. The results of precision, intra-day and inter-day study were shown table 2 and 3.

Ruggedness

The ruggedness of the method was determined by performing the same method by using different analysts at similar operational and environmental conditions. The results were reported in the table 4.

Robustness

Robustness is the ability of a method to remain unaffected by small deliberate variations in method parameters.

It is determined by performing the analysis at slightly different wavelengths from the selected wavelength of maximum absorption. The results were recorded in table no. 5.

Limit of Detection (LOD)

Limit of Detection (LOD) of the method was found to be 0.037µg/ml which is calculated from the following formula,

$$LOD=3.3 \sigma/S$$

Where,

σ =Standard deviation of the response of the analyte,

S=Slope of the linearity plot of the analyte.

$$LOD=0.0925\mu\text{g/ml}$$

Limit of Quantitation (LOQ)

Quantitation limit is the concentration that can be quantitated reliably with a specified level of accuracy and precision. Limit of Quantitation can be calculated from the following formula,

$$LOQ=10\sigma/S$$

Where,

σ =Standard deviation of the response,

S=Slope of the linearity plot of the analyte.

$$LOQ=0.28\mu\text{g/ml}$$

RESULTS AND DISCUSSION

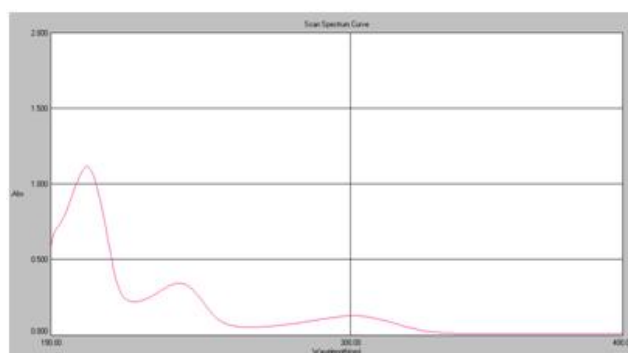


Figure 2: UV spectrum of 0.7µg/ml Dapagliflozin solution

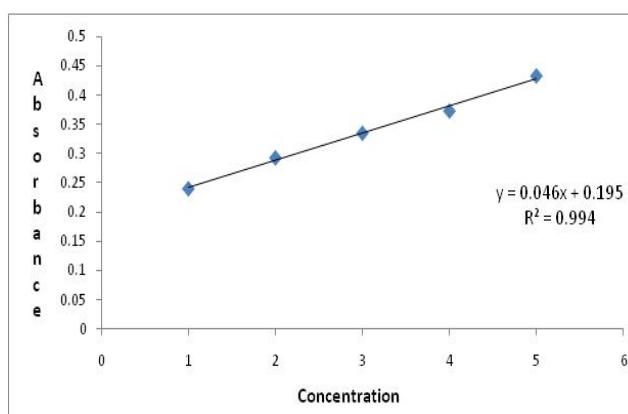


Figure 3: Calibration curve of Dapagliflozin

Table 1: Optical characteristics and other parameters

Parameters	Results
Absorption maxima	237nm
Beer's-Lambert's range (µg/ml)	0.5-0.9µg/ml
Regression equation (y)	0.046x+0.195
Slope(m)	0.046
Intercept(c)	0.195
Correlation Co-efficient	0.994
Limit of detection(µg/ml)	0.0925µg/ml
Limit of quantitation(µg/ml)	0.00129µg/ml



Table 2: Precision result

Concentration ($\mu\text{g/ml}$)	Absorbance
0.7	0.326
0.7	0.325
0.7	0.326
0.7	0.325
0.7	0.326
0.7	0.326
Mean	0.325
SD	0.00051
% RSD	0.51

Table 3: Intra-day and Inter-day precision study results

Concentration ($0.7\mu\text{g/ml}$)	Intra-day study			Inter-day study		
	Morning	After noon	Evening	Day 1	Day 2	Day 3
Avg. Abs	0.33*	0.326*	0.324*	0.331*	0.26*	0.189*
SD	0.0011	0.0005	0.0005	0.001	0.001	0.0005
%RSD	0.34	0.17	0.17	0.3	0.38	0.3

*Average of three determinations

Table 4: Ruggedness study

Concentration ($0.7\mu\text{g/ml}$)	Analyst 1	Analyst 2
Mean Abs	0.326	0.329
SD	0.0015	0.0005
% RSD	0.46	0.17

Table 5: Robustness study

Concentration ($0.7\mu\text{g/ml}$)	At 235nm	At 239nm
Mean Abs	0.320*	0.322*
SD	0.002	0.002
% RSD	0.9	0.89

The UV spectrum of $0.7\mu\text{g/ml}$ Dapagliflozin solution was shown in Figure 2. From this spectrum, 237nm was selected as the wavelength of maximum absorption (λ_{max}). The linearity of the method was found to be within the range of $0.5\text{-}0.9\mu\text{g/ml}$ with a correlation coefficient of 0.994. The linearity plot was shown in Figure 3. Precision of the method was determined by repeatability, intra-day and inter-day studies and the results were shown in Table 2 and Table 3. The LOD and LOQ of the method were calculated as 0.0925 and $0.00129\mu\text{g/ml}$ respectively. Ruggedness of the method was estimated by performing the same analysis by two different analysts and the results were found to be within the limits and were shown in Table 4. Robustness was estimated by performing analysis at slightly different wavelengths from

the actual wavelength of maximum absorption and the results were reported in Table 5.

CONCLUSION

The proposed UV- Spectrophotometric method for estimation of Dapagliflozin in API and its validation was carried out as per ICH guidelines. By studying various parameters finally we conclude that the method is simple, precise, sensitive, economic, and specific and can be applied for the determination of Dapagliflozin in API. The method was found to be linear in the specified range. All the required validation parameters were estimated and were found to be within the limits.

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