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ORIGINAL ARTICLE **OPEN ACCESS**

Development and Validation of UV-Spectroscopy Method for the Determination of Dapagliflozin

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ABSTRACT
Simple and sensitive spectroscopic methods in UV region and visible region were developed for the estimation of Dapagliflozin in its pharmaceutical dosage forms. Method was based on Dapagliflozin showing its absorption maxima at 278 nm in distilled water. This method obey Beer-Lambert law at concentration ranges of 5-10 µg/ml. The percent recoveries were found out to be 98-102%.The results obtained with the proposed methods were in good agreement with the labeled amount when tablet dosage forms were analyzed.
Keywords: *Dapagliflozin, UV spectroscopy, Method Development, ICH guidelines*

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INTRODUCTION

The Dapagliflozin (DAPA) is an undoable, dynamic and particular inhibitor of sodium-glucose co-transporter 2 (SGLT2). It works by the reabsorption of glucose from the liver, resulting in more glucose excretion in the urine, thereby increasing glycemic control in individual with type 2 diabetes mellitus. It is defined in chemical terms as (1S)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl]-D-glucite. Structure of Dapagliflozin shown in Figure 1. This is an ethanol, methanol, dimethyl-sulfoxide, and dimethyl-formamide soluble white crystalline powder. Dapagliflozin is type as Category III in the Biopharmaceutical Classification System according to the European Medicines Agency being more soluble and almost impermeable [1].

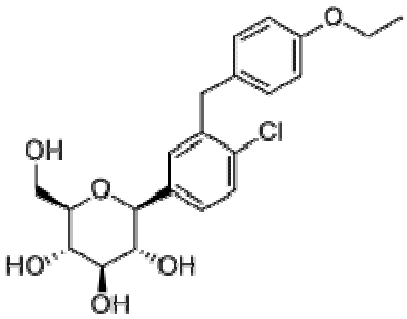

Fig.1: Structure of Dapagliflozin

These inhibitors are a new class of antidiabetic agents, called flozins. They have novel mechanism of action that is insulin-independent and depends only on plasma glucose and renal function. These inhibitors provide benefits beyond glycemic regulation, including moderate body weight and blood pressure decreases, and improved insulin sensitivity and β-cell function. Dapagliflozin is an orally available in the form of tablets.

Single agent, insulin supplement or an orally antihyperglycemic agent Dapagliflozin is effective and decreases both weight of the body and blood pressure [2]. This drug is efficient in type two diabetes

mellitus patients, both as single agent as well as in combination with other anti-diabetic agents. In addition, recent studies have shown relatively fast action of Dapagliflozin, with decreases in fasting plasma glucose levels within one week of treatment [2]. Its critical and important physic-chemical properties are showed in Table 1.

Table 1: Critical Physicochemical characterization of Dapagliflozin

Parameter	Description
CAS Number	461432-26-8
Molecular formula	$C_{21}H_{25}ClO_6$
Molecular weight	408.9
Appearance	Solid
Melting point	74-78°C
Solubility	Ethanol, Dimethyl Formamide, Distilled water
Drug type	Approved

As per the literature survey, it is revealed that the drug has been estimated by UV-Spectroscopic method and liquid chromatography analysis has been reported for the estimation in bulk and pharmaceutical dosage form [3-9]. There is new method has been developed for Dapagliflozin with using distilled water.
The aim and objective of the present work is to develop and validate spectrophotometric method for determination of Dapagliflozin in its tablet dosage form. The methods was validated in compliance with ICH Guidelines [10-11].

MATERIAL AND METHODS

Instrumentation:

An UV/Vis double beam spectrophotometer (UV-1800, Shimadzu), having 1cm matched quartz cell, loaded with UV probe software was used for recording and measuring of spectra and absorbance. All weighing was performed over 0.1mg sensitivity citizen CX 220 and a sonicater (Hicon, model 1.5L (H)) were used in the study.
The working standard of Dapagliflozin was procured from CHEMSCENE, NJ USA. The marketed formulation Forxiga (10mg) tablets were procured from local market in India. Methanol and ethanol was from merk Specialties Pvt. Ltd., Mumbai.All chemicals were at least of analytical grade and used as received.Purified water was obtained by reverse osmosis and filtration through a milli-Q® system (Millipore, Milford, MA, USA) and was used to prepare all solutions.

Materials and Reagents:

Selection of wavelength:

Dapagliflozin is freely soluble in methanol, ethanol and dimethyl sulfoxide, Distilled water so distilled water selected throughout the study. Dapagliflozin 10 µg/mL solution was scanned in between 200 nm to 400 nm and showed maximum absorption at 278nm by UV in distilled water.

Preparation of stock and working standard solution:

5mg of Dapagliflozin was accurately weighed and taken in 50 mL clean and dry volumetric flask. Drug was dissolved and diluted up to the mark using ethanol. This was considered as the standard stock solution (100 µg/mL). 5 mL of the stock solution was pipette out and made up to 10mL to get a concentration 50µg/ml and was treated as the working standard.

Preparation of calibration curve:

From this stock solution appropriate dilution were made to get final concentration of 5, 6, 7, 8, 9, 10 µg/mL and absorbance was taken at λ_{max} 278 nm (Table 2). Averages of such 10 sets of values were taken for standard calibration curve, and the calibration curve was plotted and spectra shown in figure 2.

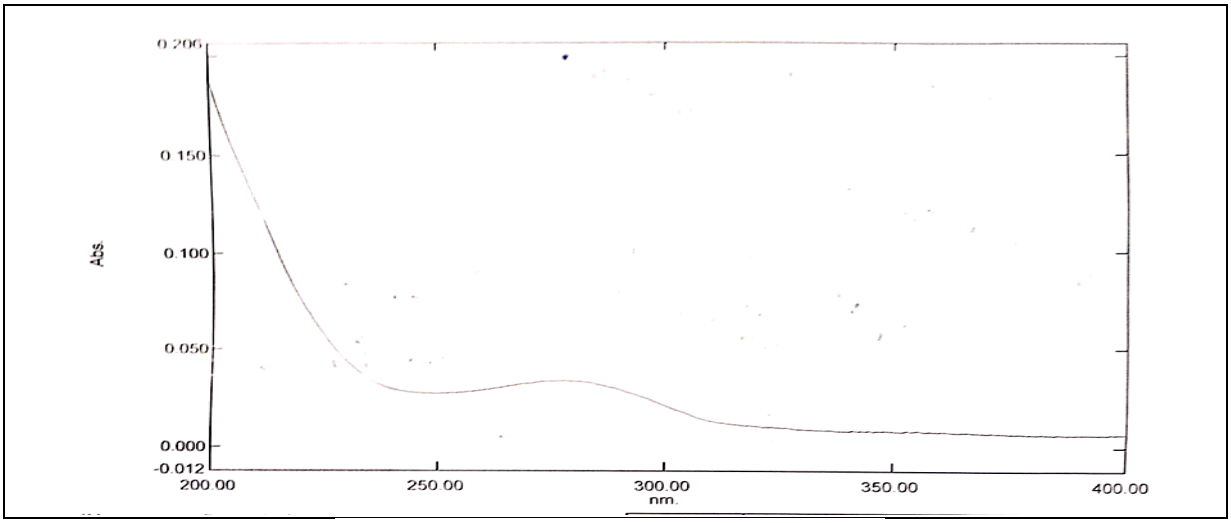

Figure 2: Absorption Spectra of Dapagliflozin

Method validation:

The development and validation of analytical procedure with respect to linearity, precision, accuracy, robustness, and ruggedness, limit of detection and limit of quantitation were developed according to ICH guidelines [10-11].

Linearity:

The linearity was established across the range and the absorbance of standard stock solution in different media in the range of 5-10 µg/mL. The calibration curves were prepared by plotting graph between average absorbance (n=3) and concentration. Linearity was determined by least square regression method.

Precision:

System precision:

Six replicates recording of absorbance at 278 nm of 10 µg/mL concentration standard solution showed %RSD (Relative Standard Deviation) less than 2 which indicates acceptable reproducibility and thereby the precision of the system.

Method precision:

It was determined by performing assay of sample under the test of (i) Intra-day precision and (ii) Inter-day precision. In the intraday study three different solution of the same concentration (10 µg/mL) were prepared and analysed thrice a day (morning, afternoon, evening). In the intraday variation study, the solution of same concentration (10 µg/mL) were prepared and analysed daily for three days, and the absorbance was recorded.

Accuracy:

Accuracy was determined by performing recovery experiments in which determination of % mean recovery of sample by percentage method at three different levels (80-120%). 80-120% of the solution were prepared as per the procedure given in the methods from the dilutions used for the linearity (10 µg/mL) in method. At each level, three different analyses were performed. Percent mean recovery was calculated. The accepted limits of recovery are 98%-102% and all observed data were within the required range which indicates good recovery values and hence the accuracy of the method developed.

Ruggedness:

Ruggedness was determined by performing the same proposed method on different instrument. Also, method was carried out by two different analysts and by performing the method on different days to check the reproducibility. In these three methods %RSD were less than 2 and that indicated the developed method is rugged.

Robustness:

Robustness is the ability of a method to remain unaffected by small deliberate variation in method parameters. It is determined by performing the analysis at slightly different wavelength from the selected wavelength. In these three methods %RSD were less than 2.

Detection limit and quantitation limit:

The detection limit and the quantitation limit were based on the slope of the calibration curve and the standard deviation of Y-intercept of regression line.

RESULTS AND DISCUSSION

The proposed method provide a simple, accurate, economical, and convenient way for the analysis of Dapagliflozin by UV- visible Spectroscopy. In the developed method, linearity was observed and calibration plot was shown in figure 3.

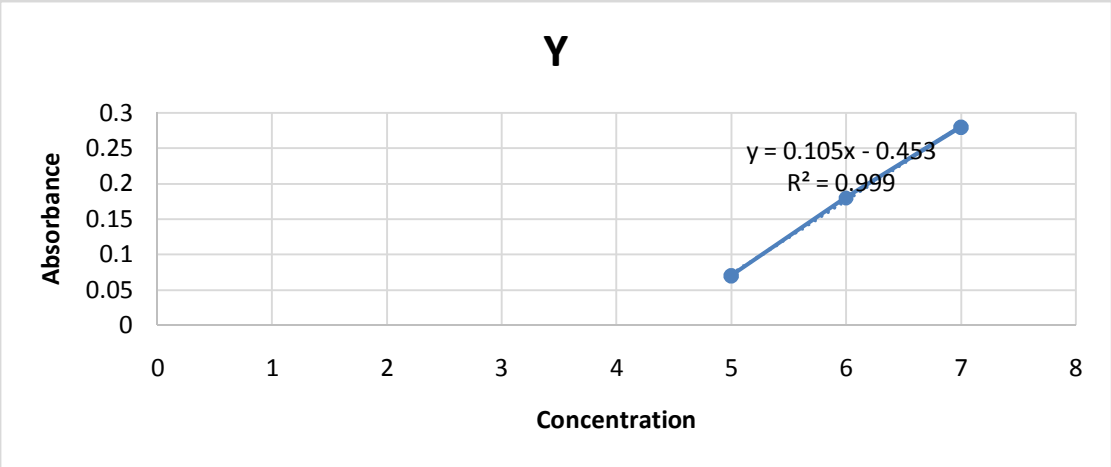

Figure 3 Linearity graph of Dapagliflozin

Concentration (µg/mL)	Absorbance
5	0.07
6	0.18
7	0.28
8	0.39
9	0.47
10	0.58

Table 2 Calibration plot of Dapagliflozin

The accuracy of the proposed methods checked by standard addition method and was found within the specified range thus indicated the accuracy of the methods (Table 3).

Level	Abs	%Recovery	Mean % recovery
80	0.55	98.21	99.37
80	0.54	98.88	
80	0.54	100.04	
100	0.61	100.4	100.2
100	0.60	100.2	
100	0.59	100.1	
120	0.62	101.2	101.7
120	0.63	102.0	
120	0.61	102.1	

Table 3: Accuracy of Dapagliflozin

The precision of the methods were found for 10 µg/mL samples within the limit (<2% RSD) proves the precision of methods (Table 4).

Method A	
Concentration µg/mL	Absorbance
10	0.61
10	0.60
10	0.61
10	0.59
10	0.60
10	0.59
Mean	0.60
SD	0.00198
%RSD	0.330

Table 4: A. Precision (system)

Concentration 10 µg/ml	Intra-day study			Inter-day study		
	Morning	Afternoon	Evening	Day 1	Day 2	Day 3
Avg. Abs	0.58	0.61	0.60	0.61	0.60	0.61
SD	0.001817	0.001810	0.001531	0.001	0.00210	0.00201
%RSD	0.313	0.303	0.255	0.163	0.350	0.329

Table 4: B. Precision (method)

The ruggedness of the methods were determined by performing the same method by using different analysts at similar operational and environmental condition. The result was reported in Table 5.

Method			
Analyst	**Abs**	**SD**	**%RSD**
Analyst 1	0.61	0.0010	0.163
	0.60		0.166
	0.59		0.169
Analyst 2	0.62	0.0020	0.322
	0.58		0.344
	0.62		0.322

Table 5: Ruggedness of Dapagliflozin

The robustness of the proposed method was established by percent recovery and percent RSD of the sample on the same day. The result proves the robustness of the methods (Table 6).

Wavelength (in nm)	Sample Abs	Standard Abs	SD	%RSD
277	0.60	0.60	0.0051	0.836
	0.61			
	0.60			
279	0.59	0.61	0.0032	0.524
	0.61			
	0.61			

Table 6: Robustness of Dapagliflozin

The limit of detection of the method was found to be0.018µg/mL.The limit of quantitation of the method was found to be 0.052 µg/mL. The summary of optical characteristics and validation parameters of method was shown in Table 7.

Table 7: summary of optical characteristics and validation parameters

Parameters	Result
Detection wavelength (nm)	278nm
Beer's Law limits(µg/ml)	5-10
Regression equation	Y=0.105x-0.4533
Correlation coefficient	0.9992
Slope (m)	0.105
Intercept (c)	0.4533
Precision (%RSD)	
Intra-day	0.10-0.32
Inter-day	0.10-0.41
Accuracy (%mean recovery)	
80% level	99.37
100% level	100.2
120% level	101.7
Ruggedness	
2 Analyst (% RSD)	<2
Robustness	
Wavelength (+2nm,-2nm) %RSD	<2

CONCLUSION

The reproducibility, repeatability, and accuracy of these methods were found to be good, which is evident by low standard deviation values. The percent recovery experiment values obtained indicates non-interference from the excipients used in the formulations. The percentage recovery was close to 100% for these methods. Thus, it can be concluded that the method developed was simple, accurate, sensitive and precise. Hence, these can be successfully applied in the estimation of Dapagliflozin. The proposed method can be used for routine quality control analysis of Dapagliflozin in its pharmaceutical formulation. The most striking feature of these methods is its simplicity and low cost.

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