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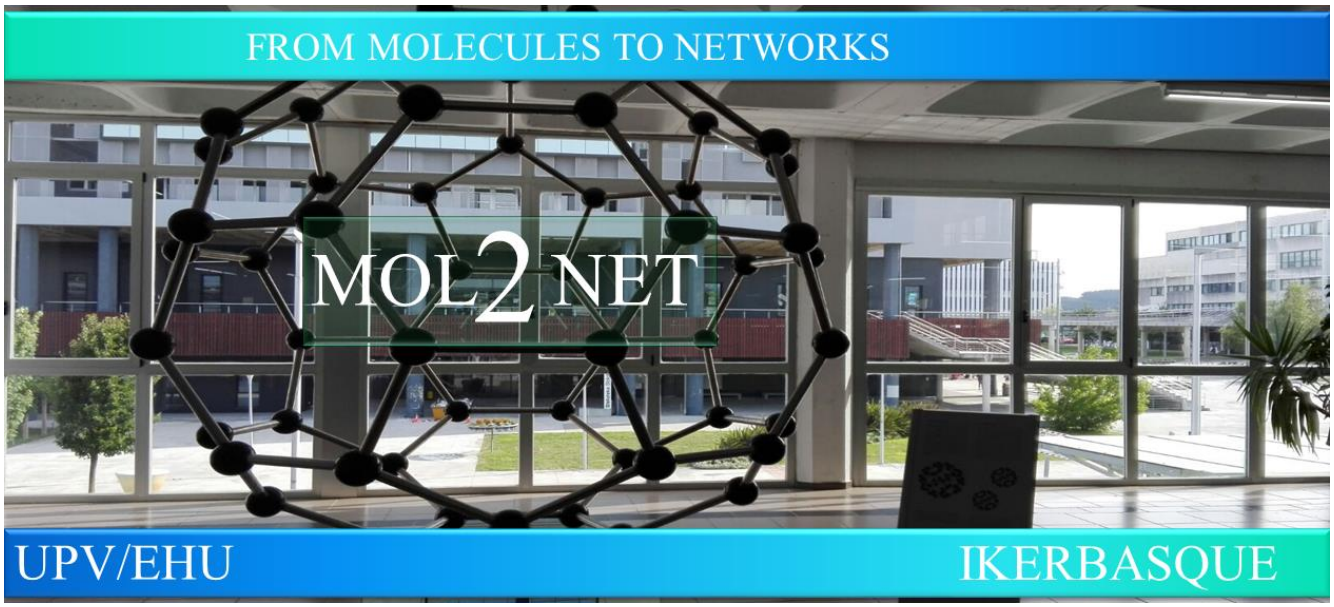

Development of UV Spectrophotometric Methods and Validation for Estimation of Dapagliflozin in Bulk and Tablet Dosage Form by Absorbance Maxima and Area Under the Curve Method

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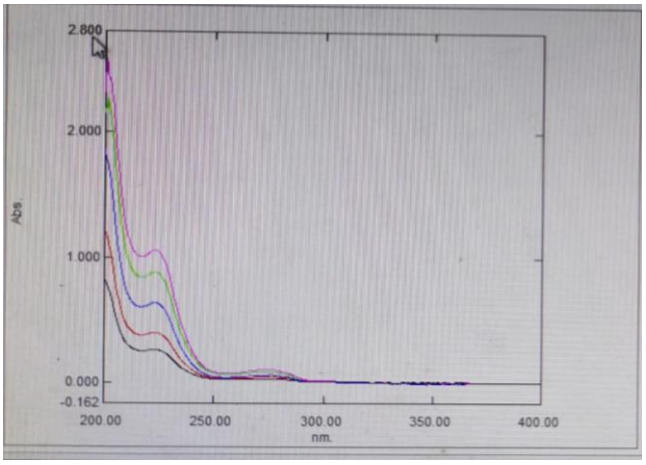
Graphical Abstract



Abstract.

UV spectrophotometric method was developed for the determination of dapagliflozin in pure form and in tablets. The method was developed using methanol and water as diluents. The results were achieved by using two methods: absorbance maxima and area under the curve method. All the parameters were chosen according to ICH [Q2(R1)] guideline and validated statistically using RSD and %RSD. The results of validation parameters were within the range. The developed method is accurate and precise.

Keywords: Dapagliflozin; antidiabetic agent; UV spectrophotometry, validation parameters

Introduction

The primary transporter responsible for renal glucose reabsorption in humans, Sodium-Glucose Co-Transporter 2 (SGLT2), is highly selectively inhibited by dapagliflozin, an oral medication that is also reversible. Patients with Type 2 Diabetes Mellitus get improved glycemic control as a result of decreased glucose reabsorption and inhibition of the Sodium-Glucose Co-Transporter 2. Since dapagliflozin's mode of action involves the kidney's direct and insulin-independent removal of glucose, it differs from and complements the mechanisms of the anti-diabetic medications now on the market. Over SGLT.1, dapagliflozin selectively blocks SGLT2[1].

In terms of chemistry, it is referred to as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] penyl]-D-glucitol. Its molecular weight is 408.98 and its formula is $C_{24}H_{33}ClO_8$. White to half-white crystalline powder, dapagliflozin dissolves in methanol, water, ethanol, and dimethyl formamid[2].

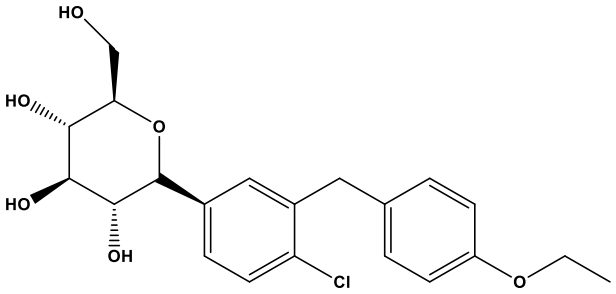


Figure 1. Structure of Dapagliflozin

Objective

Literature survey reveals that UV spectrophotometric methods for Dapagliflozin[3] are available but solvent used was only methanol which is an organic solvent and harmful for human beings. In this method, solvent used was water: methanol to decrease the concentration of methanol and also two methods for calculation were used to develop more accurate method: absorbance maxima[4] and area under the curve method[5].

Materials and Methods

Chemicals and Reagents

Pharmaceutical grade Dapagliflozin standard was obtained as generous gift from Alembic Pharma, Baroda, Gujarat, India.

Instruments

UV-Spectrophotometer: Jasco V-630 and Shimadzu-1700 double beam
Sonicator: PCi Mumbai, Model No.3.5L 100H
Weighing balance: Shimadzu AUX220 and Analytical Balance

Preparation of Standard Stock Solution

The standard stock solution was prepared by dissolving 10.0 mg of Dapagliflozin in 10.0 ml of methanol: water in 60:40 ratio to acquire a concentration of 1000 µg/ml. The working standard solution of 10 µg/ml was prepared by appropriate dilution of the stock solution with distilled water.

Selection of wavelength

Method 1: For, area under curve method, the range of the wavelength considered was 216.40 and 228.60 nm

Method 2: For first order derivative method,

Preparation of Sample Solution

Twenty tablets were weighed, powdered, and the average weight was computed to ascertain the amount of dapagliflozin contained in the commercially available tablets. An amount of tablet powder equivalent to 10.0 mg of Dapagliflozin was weighed accurately, transferred to a 10.0 mL volumetric flask. The solution was diluted up to the mark with the same solvent (methanol: water in ratio of 60:40) and filtered through what Mann filter paper after being added to and sonicated for 10 minutes. A determined volume of the filtrate was taken, and the final concentration of 20µg/mL was obtained by diluting it with distilled water. As previously mentioned, the absorbance was measured at specific wavelengths, and the quantities in the sample were calculated.

Preparation of calibration curve

Appropriate dilutions of standard stock solution were made to get final concentration in the range of 0.5-2.5 µg/mL. Absorbance and area under curve were measured of each prepared solution at above selected wavelengths. The calibration curve was plotted between concentration vs. absorbance/AUC, having correlation coefficient 0.996 and 0.993 respectively (Figure 3.)

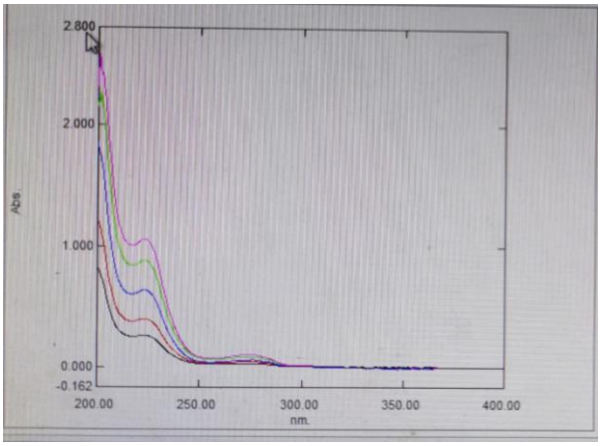


Figure 2. First order derivative spectrum for dapagliflozin

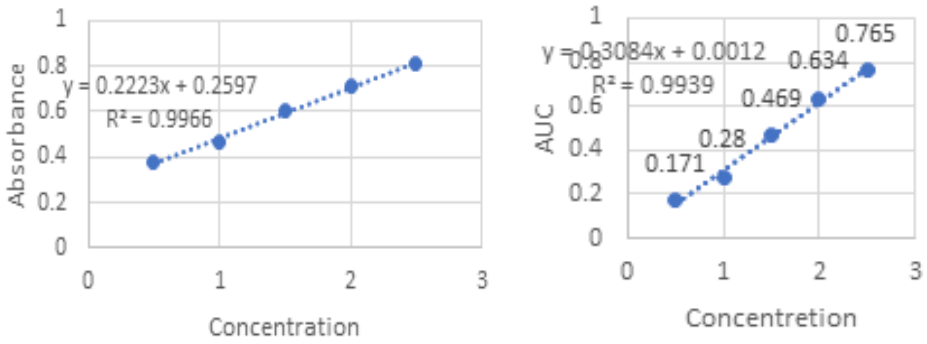


Figure 3. Calibration Curve

Method Validation

The method was validated as per ICH guidelines.

Linearity: The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration (amount) of an analyte in the sample within a given range. For linearity study, five solutions of Dapagliflozin of different percent of label claim (80-120 %) were

prepared, analyzed by proposed methods and the obtained data were utilized to plot calibration curves.

Precision: The degree to which a set of measurements taken from multiple samplings of the same homogenous sample under the specified conditions agree with one another is expressed as the precision of the analytical procedure. Studies on intra-day variation and inter-day variation can be used to examine the precision of the approaches. The 20 µg/mL sample was examined three times in the same day for the intra-day study, and five times for the inter-day study using the same solution.

LOD & LOQ: limit of detection can be defined as the minimum concentration at which peak appears and limit of quantification can be defined as the minimum concentration at which the peak area can be calculated or quantified.

Accuracy: Recovery studies were used to determine the suggested method's accuracy. 1 mg of the pre-analyzed tablet powder were weighed, and between 80 and 120 percent of the known amount of the standard medication was added. The proposed approaches were then used to re-analyze the resulting solutions. To ensure repeatability, each sample was examined three times at each concentration level. Based on the data analysis, it was determined that the procedures were correct.

Results and Discussion

Dapagliflozin was discovered to be quite soluble in methanol and water; during the course of the experiment, working standard solutions at the appropriate concentration were created utilizing these solvents. In the concentration range of 0.5–2.5 µg/mL, the devised approach complied with Beer-Lambert's law, with a correlation coefficient value of less than 1. A working concentration assay of dapagliflozin tablets was conducted to evaluate the suitability of the developed methodologies for the pharmaceutical formulation. There were five distinct levels at which the recovery study was conducted: 80–120%. The created procedure was verified in accordance with ICH criteria.

Results of validation parameters

Linearity: Table 1 shows the results of linearity of the absorbance maxima method and table 2 shows the results of area under the peak method. In both the table as the concentration of the solution increases, the absorbance and area under the curve increases that shows the linearity of the developed method.

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Concentration	Absorbance
0.5	0.376
1.0	0.466
1.5	0.603
2.0	0.710
2.5	0.810

Table 1. Linearity study for absorbance maxima method

Concentration	Starting wavelength	Ending wavelength	Area
0.5	216.40	228.60	0.171
1.0	216.40	228.60	0.280
1.5	216.40	228.60	0.469
2.0	216.40	228.60	0.634
2.5	216.40	228.60	0.765

Table 2. Linearity study for area under the curve method

Precision: Intraday and interday precision results are shown in table 3 and 4. Percentage RSD of repeatability were <2% for drugs, indicates that the method is precise.

Concentration	Absorbance mean*±S.D	%RSD
1.0	0.467±0.0049	0.85
1.5	0.605±0.0077	1.15
2.0	0.711±0.0093	1.26

Table 3. Intraday Precision

Concentration	Absorbance mean*±S.D	%RSD
1.0	0.455±0.0051	1.12
1.5	0.590±0.0081	1.37
2.0	0.715±0.0090	1.25

Table 4. Interday Precision

LOD and LOQ: LOD for dapaglipflozin was found to be 0.1 ppm and LOQ for dapagliflozin was 0.5 ppm.

Accuracy: The solutions were reanalyzed using the suggested technique, and Table 5 presents the findings of the recovery investigations. The accuracy of the procedures was indicated by the % RSD number being less than 2. (Table 5.)

Accuracy level	Amount of DAPA sample	Amount of DAPA sample added	Total amount of DAPA	Total amount of DAPA found mean (n=3)	%Recovery (n=3)
Pre Analyzed	1	0.0	1.0	0.9615	-
80%	1	0.8	1.8	1.804	98.8%
100%	1	1.0	2.0	2.0245	101.1%
120%	1	1.2	2.2	2.2185	100.4%

Table 5. Accuracy Study/%recovery study`

Conclusions

The UV spectrophotomettric method developed for the determination of Dapagliflozin is based on calibration curve, results of absorbance maxima and area under the curve method. The method was validated as per ICH guidelines and all the validation parametaers were within the range.

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