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Analytical method Development and Validation of Dapagliflozin by UV-spectroscopy

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ABSTRACT

A simple, specific, accurate, and precise spectroscopy method was developed and validated for the estimation of dapagliflozin in pure form. The Standard solution was prepared by weighing 100 mg of dapagliflozin in 100 ml volumetric flask with 0.1N Nitric Acid. The final Standard solution was made to produce 1000 µg / ml with 0.1N Nitric Acid. Further dilutions were prepared as per procedure and were scanned at 232 nm. The linearity was found in the concentration range of 10-60 µg / ml. The Correlation coefficient was 0.996. The regression equation was found to be Y = 0.043 X = 0.336. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation, ruggedness and robustness. The limit of detection and limit of quantitation for estimation of dapaliflozin was found to be 5.36 (µg / ml) and 17.08 (µg / ml), respectively. The percentage recovery of dapaliflozin was found to be in the range of 98.49 ± 0.0001 to 101.3 ± 0.003. Proposed method can be successfully applied for the quantitative determination of dapagliflozin in pharmaceutical pure form.

KEYWORDS: Dapagliflozin, 0.1N Nitric Acid, UV/VISIBLE spectroscopy.

INTRODUCTION

The Dapagliflozin (DAPA) is an undoable, dynamic and particular inhibitor of sodium-glucose cotransporter 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.

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

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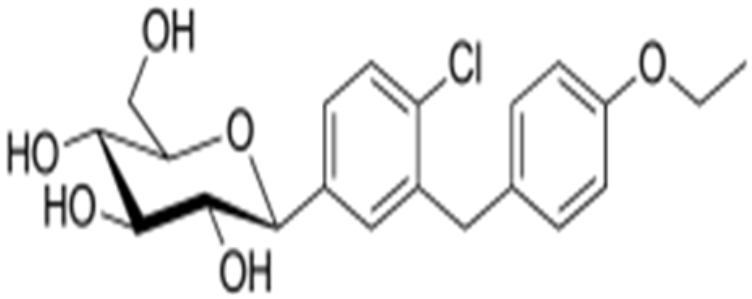
function. These inhibitors provide benefits beyond glycemic regulation, including moderate body weight and blood pressure decreases, and improved insulin sensitivity and β-cell function. Dapagliflozin is an orally available in the form of tablets. Single agent, insulin supplement or an orally antihyperglycemic agent Dapagliflozin is effective and decreases both weight of the body and blood pressure. 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.

Drug Profile

Molecular formula: $C_{21}H_{25}CLO_6$



Structure:

Chemical name: BMS -512148;[1S]-1,5-anhydro-1-C-{4-chloro -3-[4-ethoxy Phenyl] methyl]phenyl}-D-glucitol

Category: Antidiabetic **Molecular weight**: 408.873mg/ml **Uses**: Used as antidiabetic drug.

MATERIALS AND INSTRUMENTS

The following materials used were either analytical reagent (AR) or laboratory reagent (LR) grade or the Possible Pharma grade available as supplied by the manufacturer or supplier without further purification or investigation.

Table no. 1: Instruments.

S. No.	Equipments	Source
1	UV Spectrophotometer	Systronics Limited
2	Sonicator	Wensar

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METHODOLOGY

Selection of solvents: In order to select suitable solvent for determination of Dapagliflozin various solvent methanol, glacial acetic acid, sodium hydroxide, sulphuric acid, Nitric acid, ethanol tried for the solubility studies and it was found that Dapagliflozin was freely soluble in 0.1N HNO_3 the present investigation distilled water was selected as a solvent.

Selection of wavelength: 10mg/ml of Dapagliflozin was scanned in the range of 200-400nm.

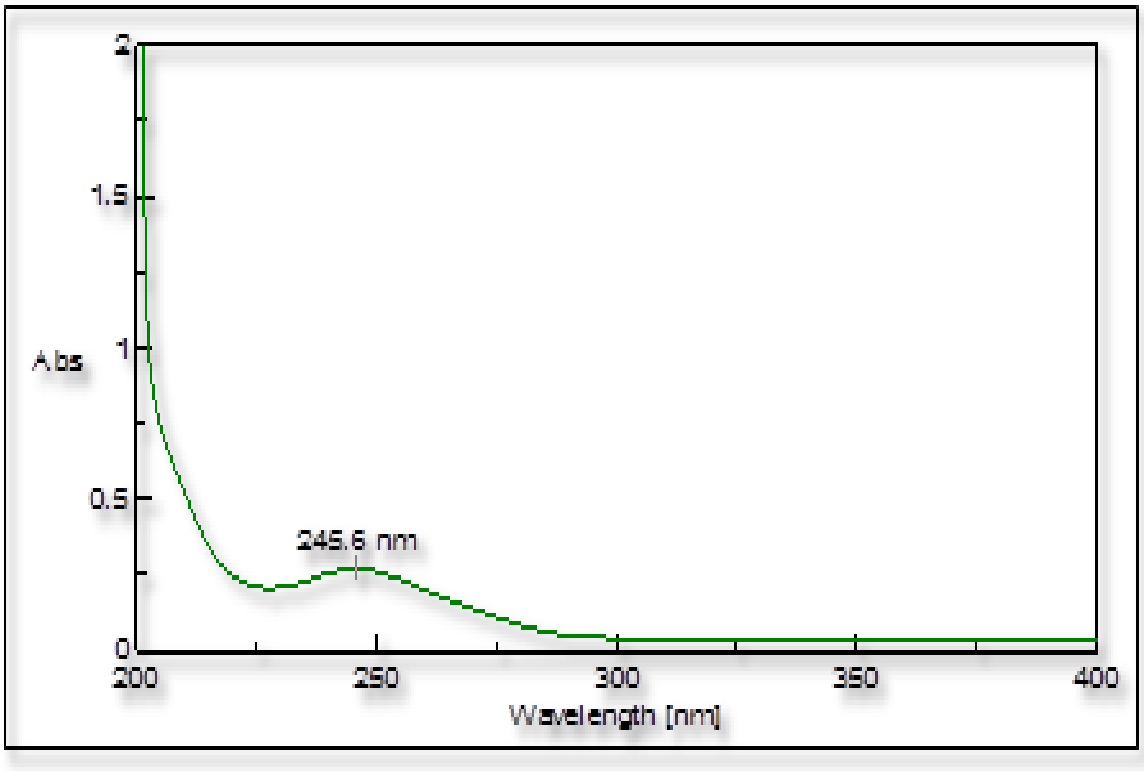

Fig 2: UV spectrum of Dapagliflozin

Validation of the method: The method was validated in terms of parameters like linearity, accuracy, precision, Limit Of Detection (LOD), Limit of Quantitation (LOQ), ruggedness, and robustness.

Preparation of 0.1n nitric acid: Take 63ml of Concentrated Nitric Acid and make upto to 1000 ml with water.

Preparation of stock solution: 100mg of Dapagliflozin was dissolved in 0.1n nitric acid in a 100 ml volumetric flask and solution was made upto volume with nitric acid.

Preparation of standard working solution: 10ml of standard solution was dissolved in 100ml of volumetric flask and the solution was made up to volume with 0.1N HNO_3.

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Linearity: To evaluvate the linearity, serial dilution of analyte were prepared from the standard working solution was diluted with solvent to get a series of concentration ranging from 10,20,30,40,50 and 60 micro gram\ml. the prepared solution were filtered through whatman filter paper. Calibration curve was constructed by plotting the absorbance y-axis against the concentration on x-axis.

Precision:- The precision of analysed method was studied by analysis of multiple sampling of homogeneous sample. The precision is expressed as standard devition [or] relative standard deviation. The presicion of the method was demonstrated by intra-day and inter- day variation studies.

Intraday-Precision: In The Intraday Studies, the Standard Solutions (40mg/ml) Was Analysed for 6 Times in different time Interval with in day. %RSD was Calculated presented in table no:2 2.2 Inter day Precision : In the Inter-day variation studies, the standard solution (40mg/ml) was Analysed for 6 times n different days. %RSD was Calculated Presented In table no:3

Accuracy:- Recovery Studies By The Standard Addition Method Performed with a View to Justify the accuracy of proposed method . previously analysed sample of Dapagliflozin (45,55 and 65microg/ml) were spiked with 80,100,120% extra Dapagliflozin standard and the mixture were analysed by the proposed method. The experiment was performed in triplicate and recovery of the pure. %RSD was calculated and reported in table no:4.

Ruggedness:-Ruggedness is the measure of the reproducibility of a test result under normal expected operating condition from instrument to instrument and analyst to analyst. The ruggedness of the method was determined by carrying out the experiment by different operations. The result of ruggedness testing is reported in the table no: 5

Robustness:-Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variation in the method condition, and is indication of the reliability of the method. A method is robustness, if it is unexpected by small changes in operating condition. To determine the robustness of this method, the experimental condition where deliberately altered at 3 different levels and responses were evaluated. Variation of wave length[230nm and 234nm] had no significant effect and the absorbance of 40 µg/ml Solution, indicating that the method was robustness. The result are shown in table no: 6.

RESULTS

Validation of analytical method:-Validation of an analytical method is the process of method meets the requirements for the intended .Performance characteristic were expressed in terms of analytical parameters.

Linearity: Calibration graph were plotted using absorbance of standard drug versus concentration of standard drug solution. Linear regression data showed a good linear relationship over a Concentration range 10-60µg/ml.

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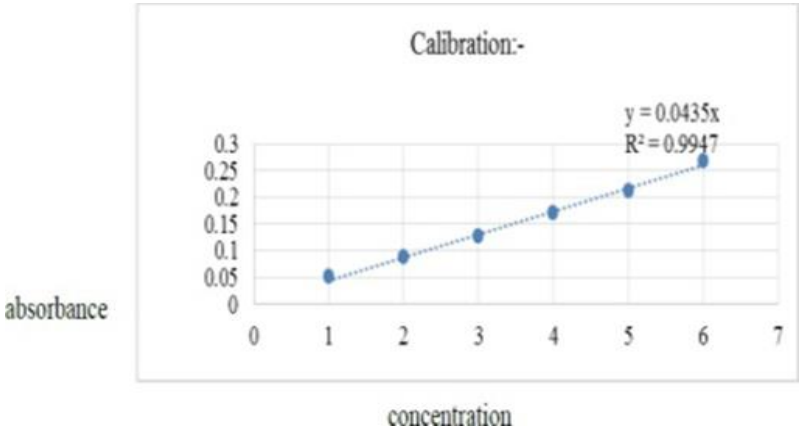

Fig-3: Calibration Curve of Dapagliflozin

1. The linearity range for Dapagliflozin was found to be 10-60µg/ml.

2. Precision

Table 2: Intraday and Interday Precicion study

Intraday precision					Inter day precision		
S. No	**Conc µg/ml**	**AVG**	**SD**	**%RSD**	**AVG**	**SD**	**%RSD**
1	40	0.1660	0.00352	1.121	0.17641	0.0016	0.9369
2	40	0.1763	0.00235	1.334	0.17513	0.002	1.2573
3	40	0.1746	0.00222	1.518	0.17508	0.0032	1.8345
4	40	0.1753	0.00279	0.594	0.17416	0.0015	0.9008
5	40	0.1730	0.00191	1.104	0.17706	0.0018	1.0250
6.	40	0.175633	0.002573	1.4664	0.174433	0.00274	1.575

Acceptance criteria: %RSD of the six replicate injections should not more than 2.0%

3. Accuracy

Table no. 3: Accuracy studies

	Initial Amount (µg/ml)	**Amount added(µg/ml)**	**Amount recovered (µg/ml)**	**%Recovery ±SD***	**%RSD**
80	40	5	44.90	99.77±0.000	0.453
80	40	5	44.84		
80	40	5	44.96		
100	50	5	55.71	101.38±0.003	0.011
100	50	5	55.74		
100	50	5	55.84		
120	60	5	64.57	99.29 ±0.016	0.405
120	60	5	64.64		

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120	60	5	64.42		

4. Ruggedness

Table no. 4: For Ruggedness (Analyst to Analyst)

S. No	Analyst-1		Analyst-2	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	40	0.1743	40	0.1711
2	40	0.1708	40	0.1738
3	40	0.1722	40	0.1705
	AVG	0.1724	AVG	0.1718
	SD	0.001762	SD	0.001758
	%RSD	1.022	%RSD	1.023

Acceptance criteria: %RSD of the six replicate injections should not be more than 2.0%**6.**

5. Robustness

Table no. 5: Robustness summary.

S. No	Condition	Modification	Mean absorbance±SD*	%RSD for absorbance
1	Wavelength (nm)	230	0.144±0.0022	1.56
		234	0.159±0.0010	0.62

*Average of the three determinations.

Acceptance criteria: %RSD should not be more than 2.0%

DISCUSSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost UV-Visible Spectroscopic method. It is successfully applied for the determination of Dapagliflozin in pharmaceutical preparations without the interferences of other constituent in the formulations. The optimum wavelength for detection was 245.6 nm at which better detector response for the drug were obtained. The calibration was linear in concentration range of 10-60µg/ml**.** The mean recoveries were found in the range of 98-102% for Dapagliflozin. Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % R.S.D. reported was found to be less than 2 %. Therefore, there is no significant difference in the results achieved by the proposed method. Hence it is

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suggested that the proposed is UV/VIS Spectrophotometric method can be effectively applied for the routine analysis of Dapagliflozin in bulk.

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 noninterference 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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