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Original Article

Estimation of Dapagliflozin from its Tablet Formulation by UV-Spectrophotometry

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ABSTRACT

Aim: Simple, precise and accurate UV-spectrophotometric methods for estimation of Dapagliflozin were developed and validated as per ICH guidelines. **Experimental and Results:** These methods includes Calibration curve, Area under curve (AUC), First and Second order derivative method based on measurement of absorbance at a selected wavelengths using UV-visible spectrophotometer with 1 cm matched quartz cell and methanol with water as a solvent. All developed methods obeyed Beer's-lambert's law in the concentration range of 5-40 µg/mL, with correlation coefficient value less than 1. The percent amount of drug estimated by these methods was nearly 100%, found to be in good agreement with label claim of marketed tablet formulation. The recovery study was carried out at five different levels and results were found to be satisfactory. **Conclusion:** The results of estimation and validation parameters like accuracy, precision, ruggedness, linearity and range were studied for all the developed methods and were found to be within limits. The proposed method can be adopted for routine quality control for estimation of drug in formulation.

Key words: Dapagliflozin, UV-Visible spectroscopy, Validation, Derivative, Area under curve.

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INTRODUCTION

Dapagliflozin is a highly selective, 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 glyceamic 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. Dapagliflozin selectively block for SGLT2 over SGLT.[1]

It is chemically known as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol (Figure 1). It has a molecular formula $C_{24}H_{33}ClO_8$ with molecular weight 408.98. Dapagliflozin is a white to half white crystalline powder which is soluble in water, ethanol, methanol and dimethyl formamide.

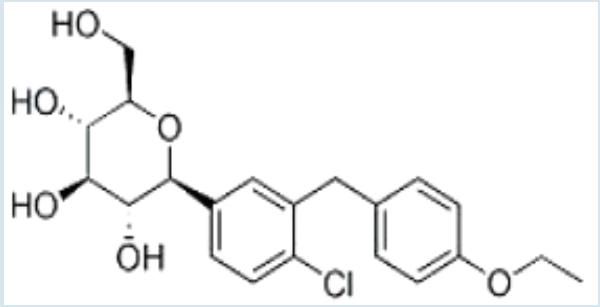

Figure 1: Chemical structure of Dapagliflozin.

OBJECTIVE

Literature survey indicated that the drug has been estimated from bulk and marketed formulation by UV-spectroscopy including zero order method.[4] The proposed work represents four new simple, economical, and rapid UV-spectrophotometric methods for the quantification of Dapagliflozin in bulk and its tablets. The developed methods were validated for accuracy, precision, ruggedness and sensitivity as per ICH guidelines.

MATERIAL AND METHODS

Chemicals and Reagents:

Pharmaceutical grade Dapagliflozin standard was obtained as generous gift from Indogo Remedies, Mumbai, Maharashtra, 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 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 appropriate wavelengths for analysis for Dapagliflozin

Method I (Zero order): The working standard solution of 10 µg/mL was prepared and scanned in the UV range 400–200 nm; Dapagliflozin shows a maximum absorbance at 224 nm.

Method II (Area under Curve): From the zero order spectrum of Dapagliflozin, the AUC between a wavelength range 218-230 nm was considered for the analysis.

Method III (First order derivative UV-spectrophotometry using amplitude), the zero order absorption spectrum of Dapagliflozin was derivatized in first order and the amplitudes was recorded at 220 nm.

Method IV (Second order derivative UV- Spectrophotometry using amplitude); the zero order absorption spectrum of Dapagliflozin was derivatized into second order and and the amplitudes were recorded at 224 nm and 235.5 nm.

The selection of wavelengths in all methods is shown in Figure 2.

Preparation of Calibration Curve

Appropriate dilutions of standard stock solution were made to get final concentration in the range of 5-40 µg/mL. Absorbances and area under curve were measured of each prepared solution at above selected wavelengths. The calibration curve was plotted between concentration *vs.* absobance/AUC, having correlation coefficient 0.998 and 0.997 respectively (Figure 3.).

Preparation of Sample Solution

To determine the content of Dapagliflozin from marketed tablets; twenty tablets were weighed, powdered and average weight was calculated. An amount of tablet powder equivalent to 10.0 mg of Dapagliflozin was weighed accurately, transferred to a 10.0 mL volumetric flask. Sufficient amount of methanol was added and sonicated for 10 min and the solution was diluted up to mark with the same solvent and filtered through whatmann filter paper (No. 41). From the filtrate, measured volume was taken and diluted with distilled water to get the final concentration of 20 µg/mL. The absorbances were measured at selected wavelengths as described above and concentrations in the sample were determined.

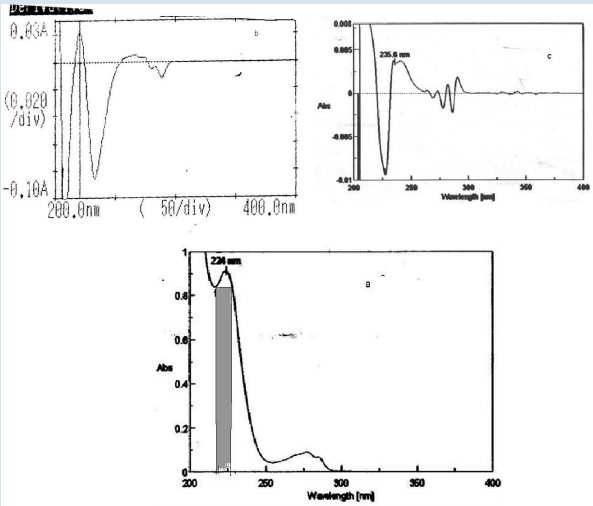

Figure 2: (a) Zero order spectrum of Dapagliflozin showing AUC between selected wavelengths, (b) First order derivative spectrum and (c) second order derivative spectrum.

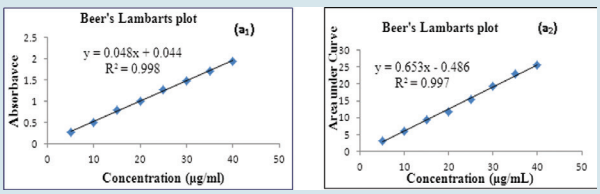

Figure 3: Beer's Lambart's plots at 224nm (a_1) and Area under curve at a selected wavelength region (a_2).

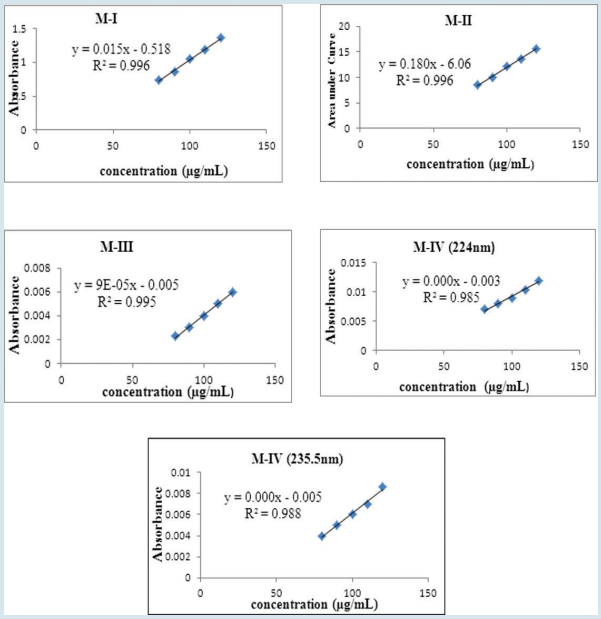

Figure 4: Plot of linearity and range for Method I-IV.

Validation of Methods

The proposed method was validated as per ICH guidelines.

Accuracy: The accuracy of proposed method was ascertained on the basis of recovery studies. Weighed the pre-analyzed tablet powder equivalent to 2.5 mg; a known amounts of standard drug was added at different levels 50-150 %. The resultant solutions were then re-analyzed by the developed methods. At each concentration, each sample was analyzed thrice at each level to check repeatability and from the data it was analyzed that the methods were found to accurate.

Precision: The precision of the analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the methods can be studied as; intra-day variation, inter-day variation studies. Intra-day study was carried out by analyzing the 20 µg/mL of sample for three times in the same day while in inter-day study same solution analyzed for five different days.

Ruggedness: Ruggedness of proposed methods was performed to examine effect of non-procedure related factors such as instruments and analysts. For this study Dapagliflozin (20 µg/mL) was analyzed by proposed methods using two different analyst and two different UV-spectrophotometers (Jasco V-630 and Shimadzu-1700) restraining similar operational and environmental conditions.

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.

RESULTS AND DISCUSSION

Dapagliflozin was found to be highly soluble in methanol and stable in methanol-water, using these solvents working standard solutions were prepared of desired concentration throughout experimentation. All developed methods obeyed Beer's-lambert's law in the concentration range of 5-40 µg/mL with correlation coefficient value less than 1. In order to test the appropriateness of the developed methods to the pharmaceutical formulation, an assay of dapagliflozin tablets was performed at working concentration. The recovery study was carried out at five different levels 50-150 %. All developed methods were validated as per ICH guidelines.

Analysis of marketed Tablets

The percentage amounts of Dapagliflozin estimated from tablet formulation using Method I-IV was found to be 99.89, 99.51, 100.01, and 100.01 respectively. The % amount estimated from tablet formulation indicates that there was no interference from excipients present in it. (Table 1)

METHOD VALIDATION

Developed methods were validated for linearity, accuracy, precision, ruggedness and sensitivity as per the ICH guidelines.

Accuracy

The solutions were re-analyzed by proposed methods; results of recovery studies were reported in Table 2. The % RSD value was found to be less than 2 indicate that the methods were accurate (Table 2.)

Precision

The precision of the method was expressed in terms of % RSD. The obtained results showed reproducibility of the assay. The % RSD values were found within limit, so this indicates that the methods were precise. (Table 3a and 3b)

Linearity

From the linear regression data, it is clear that the calibration curves showed good linear relationship over the concentration range of 80-120% of label claim. The calibration curves were shown in Figure 4.

Ruggedness

The results of ruggedness study were found in the acceptable range with % RSD values less than 2 by all the methods as shown in Table 4a and 4b. The results showed no statistical differences between different operators and instruments suggesting that the developed methods were rugged.

STATISTICAL ANALYSIS OF RESULTS

When new analytical method is developed, it is usual practice to compare the values obtained from sets of results with either a true value or mean or other sets of data to determine whether the analytical procedure has been accurate and/or precise, or if it is superior to another method. This can be achieved by using a) Student's t test and b) variance ratio test (F-test). In present study, the F value and t-value is determined by comparing two standard deviation and sample means x_1 and x_2 respectively as shown in Table 5.

Table 1: Results of % estimation from tablets

Sr. No	Wt. of tablet powder (mg)	% Label claim				
		M-I	M-II	M-III	M-IV 224nm	M-IV 235.5nm
1.	244.8	100.6	100.04	99.91	99.91	99.91
2.	243.90	99.43	99.71	100.28	100.28	100.28
3.	244.61	98.73	99.96	99.99	99.99	99.99
4.	244.60	100.8	99.21	99.99	99.99	99.99
5.	244.70	100.25	99.61	99.95	99.95	99.95
6.	244.68	99.53	98.53	99.99	99.99	99.99
	Mean	**99.89**	**99.51**	**100.01**	**100.01**	**100.01**
	±SD	**0.7935**	**0.5628**	**0.1321**	**0.1321**	**0.1321**
	%RSD	**0.79**	**0.57**	**0.13**	**0.13**	**0.13**

Table 2: Results of recovery study

Sr. No	Total Amt. of drug estimated (mg)				Amt. of pure drug recovered (mg)				% Recovery			
	M-I	M-II	M-III	M-IV 224nm, 235.5nm	M-I	M-II	M-III	M-IV 224nm, 235.5nm	M-I	M-II	M-III	M-IV 224nm, 235.5nm
1.	4.95	4.97	5.00	5.00	2.51	2.53	2.56	2.56	100.5	100.2	102.4	102.4
2.	7.45	7.48	7.50	7.50	5.01	5.04	5.06	5.06	100.2	100.8	101.2	101.2
3.	10.2	10.0	10.0	10.0	7.84	7.59	7.56	7.56	104.6	101.2	100.8	100.8
4.	12.5	12.5	12.5	12.5	10.1	10.0	10.0	10.0	101.1	100.6	100.6	100.6
5.	15.0	15.0	15.0	15.0	12.5	12.5	12.5	12.5	100.7	101.5	100.4	100.4
				Mean					**101.4**	**100.8**	**101.0**	**101.09**
				±SD					**1.805**	**0.545**	**0.778**	**0.778**
				%RSD					**1.77**	**0.54**	**0.77**	**0.77**

Table 3a: Results of Instrument to instrument variations

Sr. No	Instruments	Wt. of tablet powder taken (mg)	% Label claim				
			M-I	M-II	M-III	M-IV	
						224nm	235.5nm
1.	Shimadzu 1600	244.5	101.24	99.84	100.03	100.03	100.03
2.	Jasco V-630		99.86	99.91	99.94.	99.45	101.28
	Mean		**100.55**	**99.87**	**99.98**	**99.74**	**100.65**
	±SD		**0.9758**	**0.0494**	**0.0636**	**0.4101**	**0.8838**
	%RSD		**0.97**	**0.04**	**0.06**	**0.41**	**0.87**

Table 3b: Results of Analyst to analyst variations

Sr. No	Different Analyst	Wt. of tablet powder taken (mg)	% Label claim				
			M-I	M-II	M-III	M-IV	
						224nm	235.5nm
1.	Analyst-I	244.5	101.24	99.84	100.03	100.03	100.03
2.	Analyst-II	244.4	99.24	99.99	100.03	100.03	100.03
	Mean		**100.24**	**99.91**	**100.03**	**100.03**	**100.03**
	±SD		**1.4142**	**0.1060**	**0.01**	**0.01**	**0.01**
	%RSD		**1.14**	**0.10**	**0.01**	**0.01**	**0.01**

Table 4a: Results of Intraday study

Sr. No	Time Interval (Hrs)	Wt. of tablet powder (mg)	% Label claim				
			M-I	M-II	M-III	M-IV	
						224nm	235.5nm
1.	0	244.5	101.24	99.84	100.03	100.03	100.03
2.	1		100.63	99.79	100.03	100.03	100.03
3.	2		101.23	99.82	100.03	100.03	100.03
4.	3		102.14	99.93	100.03	100.03	100.03
	Mean		**101.31**	**99.84**	**100.03**	**100.03**	**100.03**
	±SD		**0.6225**	**0.0602**	**0.01**	**0.01**	**0.01**
	%RSD		**0.61**	**0.06**	**0.01**	**0.01**	**0.01**

Table 4b: Results of Interday study

Sr. No	Day Interval	Wt. of tablet powder (mg)	% Label claim				
			M-I	M-II	M-III	M-IV	
						224nm	235nm
1.	1	244.5	100.13	99.86	100.03	100.03	100.03
2.	2		102.82	100.06	107.34	97.81	101.70
3.	3		107.50	105.70	116.32	108.92	113.37
4.	4		106.23	108.33	111.66	97.81	108.37
5.	5		106.34	108.41	118.64	95.58	110.03
	Mean		**104.58**	**104.47**	**110.79**	**100.03**	**106.70**
	±SD		**3.03**	**4.2613**	**7.4269**	**5.2116**	**5.6536**
	%RSD		**1.90**	**4.07**	**6.70**	**5.21**	**5.29**

Table 5: Statistical Analysis of Results

Sr. No	Comparison of Methods	Statistical results			
		Assay		Accuracy	
		t-value	F-value	t-value	F-value
1.	M-I Vs. M-II	0.95	1.98	0.73	11.11
2.	M-I Vs. M-II, III, IVa and IVb	0.36	36.08	0.45	5.46
3.	M-II Vs. III, IVa and IVb	1.43	3.62	0.52	2.03
4.	M -III Vs. IVa and IVb	Not much difference	1.00	Not much difference	1.00

Note: Tabulated t-value corresponds to 5% probability for 10 degrees of freedom is 2.22 and tabulated F-value corresponds to 5% probability for 5 degrees of freedom are 5.05

CONCLUSION

The UV-Spectrophotometric methods that were developed for the determination of Dapagliflozin are based on Calibration curve, Derivative and Area under curve techniques. The methods were validated and found to be simple, sensitive, accurate, and precise. Hence, it can be used successfully for routine analysis of Dapagliflozin from its tablets.

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CONFLICT OF INTEREST

No conflict of interest are declared.

ABBREVIATION USED

AUC: Area under curve, **API**: Active pharmaceutical ingredient, **RSD**: Relative standard deviation, **SD**: Standard deviation.

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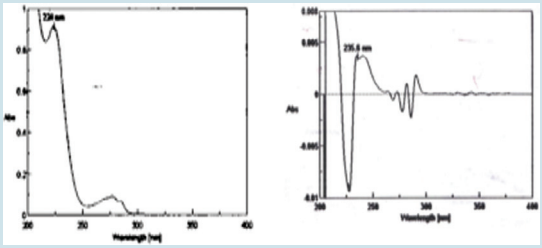
PICTORIAL ABSTRACT



SUMMARY

- UV-Spectrophotometric methods developed for the determination of Dapagliflozin are based on Calibration curve, Derivative and Area under curve technique.
- Percentage amounts of Dapagliflozin estimated from tablet formulation using Method I-IV was found to be nearly 100.00%
- Methods were validated as per guidelines
- Statistical analysis of results were done using student t test and variance ratio test (F-test)

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