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Development and Validation of UV-Spectrophotometric Techniques Using Zero-order, Derivative, and AUC Approaches to Determine Dapagliflozin in Plasma

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ABSTRACT

To develop and validate a novel, sensitive, and precise UV spectrophotometric method for quantifying dapagliflozin in plasma. Four UV-spectroscopic approaches zero-order (Method-A), first-order derivative (Method-B), second-order derivative (Method-C) and AUC were employed by utilizing a protein precipitation technique, with distilled water selected as the optimal solvent. The linearity range to all four methods is 5-25 μg/mL. The λ_{max} and r^2 for all methods were, Method A (zero-order, λ_{max} = 278nm, $r^2 = 0.996$), Method B (first-order derivative, λ_{max} = 278 nm, $r^2 = 0.9963$), Method C (second-order derivative, λ_{max} = 239nm, $r^2 = 0.9951$), and Method D (area under the curve (AUC), λ_{max} = 268–288nm, $r^2 = 0.9945$). The method demonstrated excellent precision, robustness (%RSD<2), and recovery rates ranging from 98% to 102%, including quantification limits (LOQ) and detection limits (LOD) were established. All four validated methods met ICH criteria, confirming the method's suitability for routine quality control and bioanalytical analysis of dapagliflozin in both pure and pharmaceutical dosage forms.

Keywords: Dapagliflozin, Daparyl 10, Spectrophotometric Analysis, Validation, Plasma.

INTRODUCTION

Dapagliflozin is a white to off-white crystalline powder, the medication dissolves in solvents such methanol, ethanol, dimethyl sulfoxide (DMSO), and dimethylformamide (DMF). A reversible, highly specific oral inhibitor of the human sodium-glucose co-transporter 2 (SGLT2), a crucial protein that the kidneys use to reabsorb glucose. Dapagliflozin efficiently reduces blood sugar levels in individuals with type 2 diabetes mellitus by blocking SGLT2, which increases glucose excretion and inhibits glucose reabsorption.

Despite the fact that its pharmacological characteristics have been thoroughly investigated, especially by LC-MS analysis of biological fluids such as human plasma, no UV-spectrophotometric technique for bulk quantification has been published.

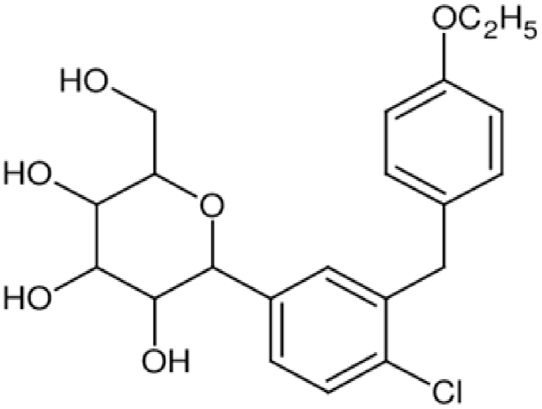

Fig. 1. Structure of Dapagliflozin

MATERIALS AND METHODS

Materials

A gift sample of dapagliflozin API (99.9% purity) was acquired from JLS Laboratories Pvt. Ltd and all the solvents purchased were analytical grade and used shimadzu double beam UV-spectrophotometer-UV-1800 model (Serial number-A11635581266).

Method Validation

Standard Stock Solution (1000 *µ*g/ml) preparation

Weighed a 10 mg sample of pure dapagliflozin transferred into a dry, clean 10 mL volumetric flask, dissolved in a small volume of distilled water, and the final volume was adjusted to 10 mL, resulting in a concentration of 1000 µg/mL.

General Procedure for Preparation of Plasma Samples

A solution of dapagliflozin was added to a 1.0 mL aliquot of the plasma that had been placed into a vortex tube for one minute, the solution was vortexed for another 30 seconds until a precipitate developed after adding 1.0 mL of methanol to precipitate the proteins. The mixture was centrifuged for 10 min at 4°C at 5000rpm. Following centrifugation, the precipitate was kept undisturbed by carefully collecting the supernatant, then the absorbance of the solution was analysed by UV Spectroscopy.

Linearity

(i) **Zero-Order Spectroscopy:** Measured volumes of the plasma sample solution were moved among several 10 mL volumetric flasks. After that, distilled water was added to each flask until it reached the 10 mL threshold, resulting in dapagliflozin concentrations ranging from 5 to 25 µg/mL. The generated solutions absorbance values were analysed using a UV-Vis spectrophotometer at wavelength 278nm with distilled water serving as the blank. Calibration curves could be created by plotting concentration on the X-axis and corresponding absorbance on the Y-axis.

(ii) **First order and second order derivative spectroscopy:** The instrument's built-in software was used to convert the dapagliflozin zero-order spectrum into its first-order and second-order derivative spectra. The linearity absorbance values of method-A was measured at 239nm and the linearity absorbance values of Method-B was measured at 278nm.

(iii) **Area under curve:** The dapagliflozin zero-order spectrum, which was acquired using the previously mentioned approach, was converted into its area under curve using the instrument's built-in software. The wavelength range was discovered between 268-288nm. Plotting absorbance vs concentration for each approach yielded a calibration curve.

Precision

At a concentration of 15 µg/mL, precision was evaluated at 278nm for Method-A, 278nm for Method-B, 239nm for Method-C and 268-288nm wavelength range for Method-D and percentage relative standard deviation was computed.

Accuracy

Accuracy was carried out by adding sample solution at 50%, 100% and 150% level to the 15 µg/ml standard solution, and the percentage relative standard deviation (RSD) was calculated.

Assay of Dapagliflozin tablets

Ten tablets were weighed, determined the average weight of the tablets and weighed the powdered tablet equalent to 10 mg of dapagliflozin, it was put into a 10 mL volumetric flask, added few ml of water, sonicatedand bring the final volume down to 10 mL by adding water.

RESULTS AND DISCUSSION

For the analysis of dapagliflozin, four novel techniques have been created. The absorption maxima for methods A, B, C and D are 278nm, 278nm, 239nm, and 268-288nm respectively.

Zero order spectroscopy

Dapagliflozin's absorption spectrum has a λ_{max} at 278nm. Dapagliflozin conforms to Beer-Lambert's law for all methods within the 5–25 µg/mL concentration range (Table 1). The equations for linear regression at zero order were determined to be y = 0.044x-0.148 (R^2=0.996). All four methods' precision and accuracy studies' percentage RSD was tallied and determined to be less than 2, demonstrating the methods' were accurate and precise.

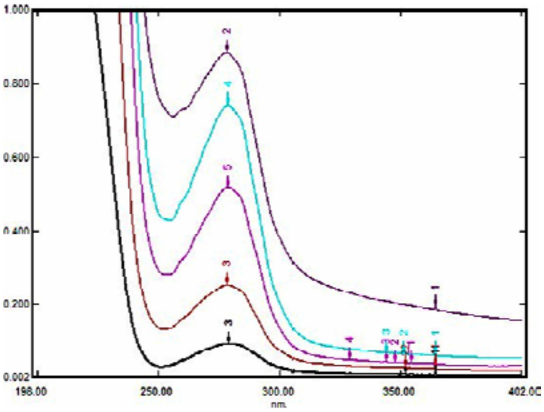

Fig. 2. Overlay spectrum of Zero orderspectroscopy

First order derivative spectroscopy

Dapagliflozin's absorption spectrum has a λ_{max} at 278nm. The equations for linear regression at first order were determined to be y = -0.221x+0.0769 (R^2 = 0.9963). The precision percentage RSD was calculated and found to be less than 2, demonstrating that the method was accurate and precise.

Second order derivative spectroscopy

Dapagliflozin's absorption spectrum reveals a λ_{max} at 239nm. Regression value was determined as 0.9973, linear regression equations for second order, which are y = -0.001x-0.001. The precision percentage RSD was calculated and found to be less than 2, demonstrating the method was accurate and precise.

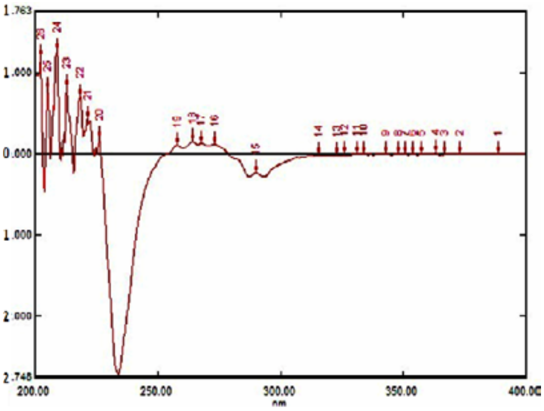

Fig. 3. Spectrum of first orderderivative spectroscopy

Area Under Curve

Dapagliflozin's absorption spectra was found to be in the 268–288nm range. The linear regression equations were determined to be y =.0.1251x-0.3744 (R^2 = 0.9947). The precision and accuracy of the procedures are demonstrated by the %RSD values, which was less than 2.

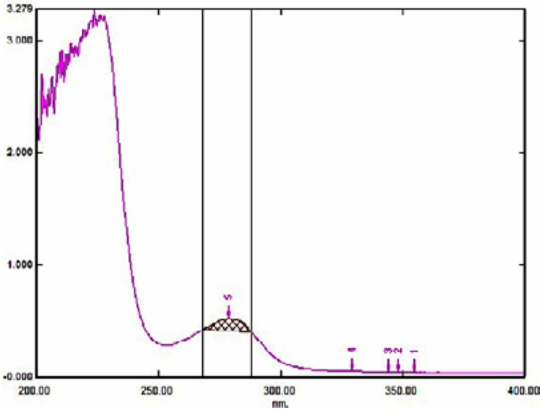

Fig. 4. Spectrum of Area under Curve

Table 1: Results of Linearity

Conc. (µg/mL)	Absorbance Values of Method-A	Absorbance Values of Method-B	Absorbance Values of Method-C	Absorbance Values of Method-D
5	0.094	-0.042	0.058	0.299
10	0.261	-0.129	0.157	0.762
15	0.517	-0.254	0.314	1.555
20	0.742	-0.376	0.462	2.176
25	0.958	-0.470	0.577	2.720

Table 2: Results of Intraday precision

Conc. (µg/mL)	Absorbance Values of Method-A	Absorbance Values of Method-B	Absorbance Values of Method-C	Absorbance Values of Method-D
	0.515	-0.255	0.316	1.505
	0.517	-0.257	0.3181	1.499
15 µg/mL	0.521	-0.256	0.3174	1.511
	0.524	-0.260	0.3222	1.544
	0.515	-0.262	0.3246	1.546
	0.517	-0.262	0.3240	1.548
Average	0.518166	-0.25866	0.3203	1.5255
SD	0.003600	0.003076	0.0036	0.02281
%Rsd	0.6949	1.1894	1.1467	1.4952
Limits	%RSD: <2.0	%RSD: <2.0	%RSD: <2.0	%RSD: <2.0

Note: Intraday Precision % RSD to all four methods were less than 2

Table 3: Results of Interday precision

Conc.(µg/mL)	Absorbance Values of Method-A	Absorbance Values of Method-B	Absorbance Values of Method-C	Absorbance Values of Method-D
	0.521	-0.253	0.3127	1.46
	0.524	-0.254	0.3140	1.492
15	0.523	-0.255	0.3160	1.505
	0.531	-0.254	0.3140	1.492
	0.535	-0.257	0.3181	1.499
	0.536	-0.253	0.3120	1.46
Average	0.52833	-0.25433	0.314466	1.48466
SD	0.0065	0.00150	0.002244	0.019714
%Rsd	1.2305	-0.5919	0.7138	1.32788
Limits	%RSD: <2.0	%RSD: <2.0	%RSD: <2.0	%RSD: <2.0

Note: Interday Precision %RSD to all four methods were less than 2

Table 4: Results of LOD & LOQ

Sr. No	Method-A		Method-B		Method-C		Method-D	
	LOD µg/mL	LOQ µg/mL	LOD µg/mL	LOQ µg/mL	LOD µg/mL	LOQ µg/mL	LOD µg/mL	LOQ µg/mL
1	0.48759	1.4774	0.51996	1.5747	0.2247	0.6847	0.2747	0.8327

Note: LOD & LOQ valueswere 1:3 range

Table 5: Assay results

Label claim(mg)	Weight found				%Assay			
	Method A	Method B	Method C	Method D	Method A	Method B	Method C	Method D
10 mg	10.077 mg	10.1574 mg	10.1910 mg	10.1256 mg	100.77%	101.54%	101.9%	101.25%

DISCUSSION

This study presents the development and validation of a new analytical method for the quantification of dapagliflozin in bulk and pharmaceutical formulations using multi-technique UV-spectrophotometry, including zero-order, first-order, second-order derivative, and area under the curve (AUC) methods. The methods demonstrated precision, and robustness meeting the validation criteria outlined in the ICH Q2 (R1) guidelines. Recovery studies confirmed that the method is free from interference caused by excipients present in the formulation. Therefore, the methods have potential, to be utilized in future quality control analyses for dapagliflozin formulations.

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Conflict of Interest

No conflict of interest.

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