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SPECTROPHOTOMETRIC ESTIMATION OF DAPAGLIFLOZIN AND VILDAGLIPTIN IN PHARMACEUTICAL DOSAGE FORM BY FIRST ORDER DERIVATIVE METHOD

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ABSTRACT

A Simple and precise UV- spectrophotometric first order derivative approach for estimating Dapagliflozin and Vildagliptin in tablet dose form has been developed and validated in its tablet dosage form. The standard and sample solutions of Dapagliflozin and Vildagliptin were prepared using methanol as the solvent. Dapagliflozin was estimated at 287.2 nm and Vildagliptin at 222.9 nm for the first order derivative UV- spectrophotometric method. Beer's law was obeyed in the concentration ranging from 5 to 15 µg/ml for Dapagliflozin and 50 to 150 µg/ml for Vildagliptin with coefficient of correlation value 0.998 and 0.999 respectively. The method was tested and validated for various parameters as per the ICH guidelines. The precision is expressed as relative standard deviation, which was found to be 1.58% and 0.75% respectively for the above method. The proposed method was successfully applied for the determination of Dapagliflozin and Vildagliptin in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be precise. The proposed method is simple, easy to apply, low-cost and require relatively inexpensive instruments.

Keywords: Dapagliflozin, Vildagliptin, first order derivative spectroscopy, methanol

INTRODUCTION

Dapagliflozin is a sodium-glucose co-transporter-2 (SGLT2) inhibitor. Chemically it is (2S,3R,4R,5S,6R)-2-[4-Chloro-3-(4-ethoxybenzyl) phenyl]-6-(hydroxymethyl)tetrahydro-2H- pyran-3,4,5-triol. Dapagliflozin works by

inhibiting SGLT2 selectively and potently, resulting in decreased renal reabsorption of glucose and higher glucose excretion in urine, lowering blood glucose levels. The mechanism is independent of the action of insulin **[1-2]**.

Vildagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor. Vildagliptin binds covalently to DPP-4, inhibiting the metabolism of DPP-4 and thereby preventing the breakdown of glucagon-like peptide-1 (GLP-1). There is increase in intact GLP-1 levels, and reduces glycaemia in patients with type 2 diabetes mellitus. Vildagliptin has been demonstrated to increase the secretion of insulin and decrease the production of glucagon in a glucose-dependent way **[3-4]**.

By reviewing the literature for analytical methods on dapagliflozin and vildagliptin it was concluded that no method was available for the estimation of dapagliflozin and vildagliptin. Hence the objective of this research paper is develop and validate UV spectrophotometric method for estimation of dapagliflozin and vildagliptin in in its pharmaceutical dosage form.

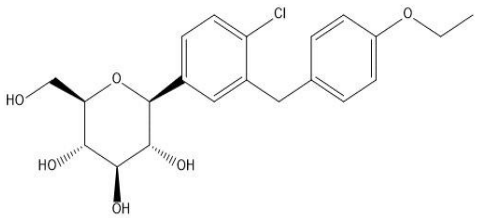

Figure 1: Chemical structure of Dapagliflozin [7]

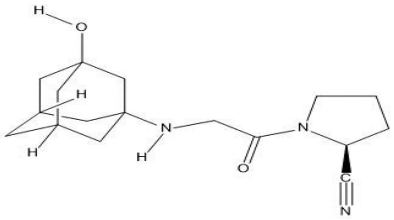

Figure 2: Chemical structure of Vildagliptin [8]

Instrument and reagents

Shimadzu UV-Spectrophotometer, UV 1800 (Japan) was used for the spectral scan. UV-probe 2.34 software was used for all spectrum measurements. Dapagliflozin and vildagliptin reference standards were obtained from a reputable firm with certificate analysis.

Preparation of Standard solution

Dapagliflozin [9-12]: 1 mg of Dapagliflozin was taken in 10 ml volumetric flask. This volumetric flask was sonicated for 2-3 minutes after a small amount of methanol was added. The flask was shaken, and made upto mark with methanol to make a solution containing 100 µg/ml of Dapagliflozin.

Vildagliptin [10-17]: 10 mg of Vildagliptin was taken in 10 ml volumetric flask. This volumetric flask was sonicated for 2-3 minutes after a small amount of methanol was added. The flask was shaken, and made upto mark with methanol to make a solution containing 1000 µg/ml of Vildagliptin.

First order derivative method

All zero-order spectrum's (D^0) were transformed to first order derivative spectrum's (D^1) using delta lambda 2.0 and a scaling factor of one. At various doses, the overlay of first order derivative spectrum's of Dapagliflozin and Vildagliptin was recorded. Dapagliflozin and Vildagliptin have zero-crossing points (ZCPs) of 222.9 nm and 287.2 nm, respectively. The overlay UV spectra of Dapagliflozin (10 µg/ml) and Vildagliptin (100 µg/ml) in methanol (First order D1) represented in **Figure 3**.

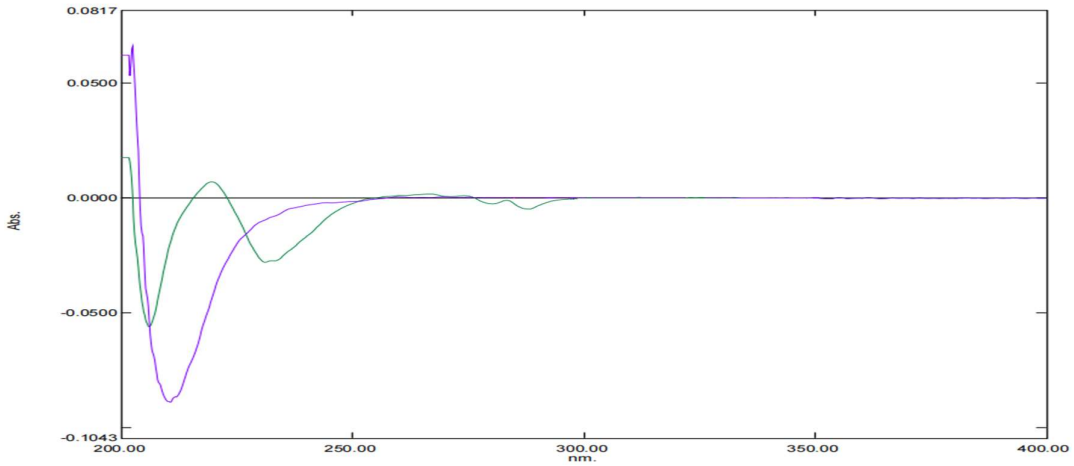

Figure 3: Overlain UV Spectrum of Dapagliflozin (10 µg/ml) and Vildagliptin (100 µg/ml) (First order D1) showing zero crossing point of Dapagliflozin (222.9 nm) and zero crossing point of Vildagliptin (287.2 nm)

Method Validation [6]: The method was validated in accordance with the International Conference on Harmonization (ICH) guideline Q2(R1). Specificity, Linearity and range, Accuracy, Precision, Detection limit, Quantification limit, Robustness, and System suitability tests have all been thoroughly validated.

Linearity and Range

The linearity was determined by analyzing independent levels of concentrations ranging from 5-15 µg/ml for DAPA and 50-150 µg/ml for VILDA. Using the specified method, the absorbance of DAPA solution was measured at 287.2 nm (ZCP of Vildagliptin) and 222.9 nm for VILDA (ZCP of Dapagliflozin). The absorbance vs. concentration calibration curve was plotted. DAPA and VILDA correlation coefficients and regression line equations were obtained.

Accuracy

The method's accuracy was proven in triplicate utilizing a recovery study of formulation at three distinct degrees of standard addition (80%, 100%, and 120%) of label claim. % The method's accuracy was justified by a recovery rate of 98-102% with a low SD.

Repeatability (n=6)

1 ml of working standard solution of DAPA was transferred into separate 10 ml volumetric flasks and diluted up to mark with methanol to get 10 µg/ml of DAPA and 1 ml of working standard solution of VILDA was transferred into 10 ml volumetric flask to get 100 µg/ml of VILDA. Each concentration was prepared 6 times. The absorbance of each solution was measured at selected wavelengths and % RSD was calculated.

Intraday precision (n=3)

As part of the procedure, solutions containing 7.5, 10, and 12.5 µg/ml DAPA and 75, 100, and 125 µg/ml VILDA were tested three times on the same day. The absorbance of DAPA and VILDA solutions was measured at 287.2 nm and 222.9 nm, respectively. SD and %RSD were determined.

Interday precision (n=3)

According to the method, solutions containing 7.5, 10, and 12.5 µg/ml DAPA and 75, 100, and 125 µg/ml VILDA were evaluated on three different days. The absorbance of DAPA and VILDA solutions was measured at 287.2 nm and 222.9 nm, respectively. SD and %RSD were determined.

Limit of Detection

A calibration curve was repeated six times, and the standard deviation (SD) of the intercepts was measured, after which the LOD was determined using the formula.

LOD = 3.3 σ/S, Where, σ = the standard deviation of Y-intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves**.**

Limit of Quantitation

A calibration curve was repeated six times, and the standard deviation (SD) of the intercepts was measured, after which the LOQ was determined using the formula.

LOQ = 10 σ/S, Where, σ = the standard deviation of Y-intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Estimation from tablets

Twenty tablets of Dapagliflozin 10 mg and Vildagliptin 100 mg were weighed, and the average weight of each tablet was determined. The powdered equivalents of DAPA and VILDA were weighed and placed into a volumetric flask of 100 ml. Methanol was poured to the flask and sonicated for 15 minutes before being filtered. Methanol (100 µg/ml, 1000 µg/ml) was used to make up the volume to the mark. 1ml of the previously mentioned solution was added to 10 ml of volumetric flask and made up to the mark with methanol (10 µg/ml, 100 µg/ml). Such solution was used for analysis.

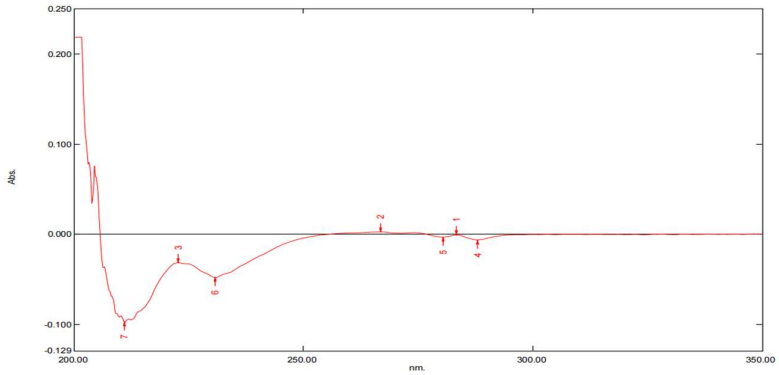

Figure 4: First order derivative spectrum of tablet mixture (10 µg/ml, 100 µg/ml) showing absorbance at 287.2nm and 222.9nm

VALIDATION

Linearity: Linearity was evaluated using ranged concentrations of solution. DAPA was prepared at a concentration of 5-15 µg/ml and VILDA at a concentration of 50-150 µg/ml from a master stock solution in a 10 ml volumetric flask.

The calibration curve for dapagliflozin and vildagliptin was plotted in the concentration range of 5 to 15 µg/ml and 50 to 100 µg/ml respectively was given in **Figure 6**.

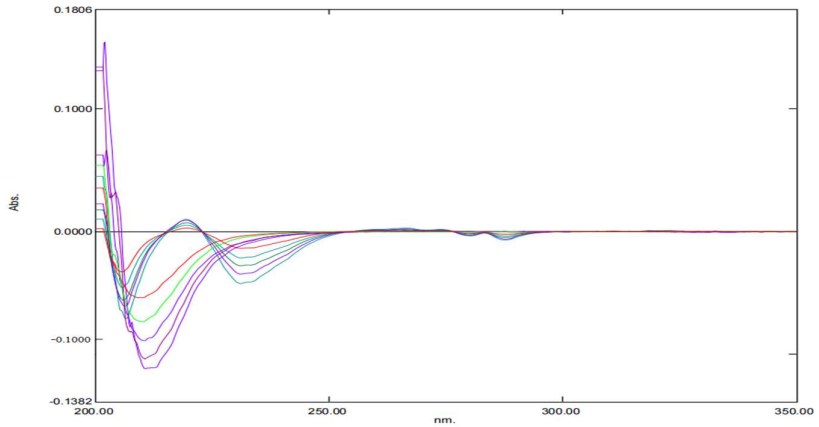

Figure 5: The overlay spectra of dapagliflozin and vildagliptin standard solution (DAPA:5-15 µg/ml, VILDA :50-150 µg/ml)

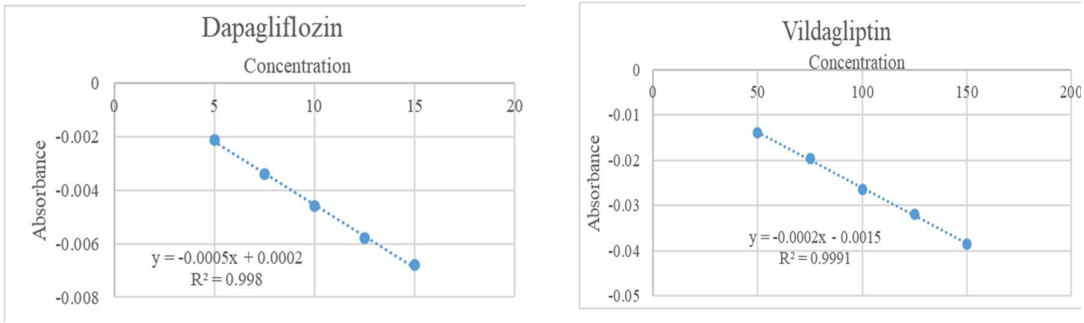

Figure 6: Calibration curve for dapagliflozin and vildagliptin by first order derivative spectroscopy (Abs vs Conc.)

Accuracy: Accuracy of the proposed methods was performed as per recovery studies. The proposed approach was used to perform recovery studies by adding standard drugs at different amounts to the pre-analysed tablets powder solution. From the amount of the drug estimated, the percentage recovery was calculated.

Table 1: Results of recovery studies of Dapagliflozin and Vildagliptin for first order derivative method

Drug	% Spiking	Amount of sample taken (practical) (µg/ml)	Amount of Standard spiked (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	%Mean Recovery ±SD	% RSD
DAPA	80%	10	8	-0.0085	17.8775	99.31± 1.18	1.1976
		10	8	-0.0084	17.6634		
		10	8	-0.0086	18.0916		
	100%	10	10	-0.0094	19.80	100.09± 1.07	1.07
		10	10	-0.0095	20.02		
		10	10	-0.0096	20.23		
	120%	10	12	-0.0104	21.95	99.75± 0.97	0.98
		10	12	-0.0103	21.73		
		10	12	-0.0105	22.16		
VILDA	80%	100	80	-0.0425	178.87	99.90 ±0.57	0.57
		100	80	-0.0430	180.90		
		100	80	-0.0427	179.68		
	100%	100	100	-0.0475	199.20	100.07 ±0.51	0.51
		100	100	-0.0480	201.23		
		100	100	-0.0477	200.01		
	120%	100	120	-0.0526	219.94	100.34± 0.37	0.37
		100	120	-0.0530	221.56		
		100	120	-0.0528	220.75		

Method precision: To determine repeatability, interday and intraday precision, % RSD was monitored at a specified concentration level and found to be less than 2, indicating that the method was precise for estimating DAPA and VILDA.

Repeatability: Concentration of 10 µg/ml of DAPA and 100 µg/ml of VILDA was prepared 6 times. The absorbance of each solution was measured at selected wavelengths and % RSD was calculated. The results obtained are calculated in **Table 2**.

Table 2: Results of repeatability of Dapagliflozin and Vildagliptin for first order derivative method

Drug	Target conc. (µg/ml)	Absorbance	Mean±SD	%RSD
DAPA	10	-0.0046	-0.00457± 0.00005	1.13080
	10	-0.0045		
	10	-0.0046		
	10	-0.0046		
	10	-0.0046		
	10	-0.0045		
VILDA	100	-0.0266	-0.02652± 0.00019	0.73191
	100	-0.0264		
	100	-0.0265		
	100	-0.0262		
	100	-0.0267		
	100	-0.0267		

Intraday precision (n=3): Solutions containing 7.5,10 and 12 µg/ml DAPA and 75,100 and 125 µg/ml VILDA were analyzed 3 times on the same day as per the procedure. The absorbance of solutions was measured at 287.2 nm and 222.9 nm of DAPA and VILDA respectively. SD and %RSD were calculated. The results obtained are calculated in **Table 3**.

Table 3: Results of intraday precision of Dapagliflozin and Vildagliptin for first order derivative method

Drug	Target conc. (µg/ml)	Mean Abs. ± SD	Mean % RSD
DAPA	7.5	-0.0034± 0.000058	1.58
	10	-0.0045± 0.000058	
	12.5	-0.0057± 0.000058	
VILDA	75	-0.01973±0.00015	0.55
	100	-0.02657±0.00015	
	125	-0.03220± 0.00010	

Interday precision (n=3): Solutions containing 7.5,10 and 12.5 µg/ml DAPA and 75,100 and 125 µg/ml VILDA were analyse on three different days as per the procedure. The absorbance of solutions was measured at 287.2 nm and 222.9 nm of DAPA and VILDA respectively. SD and %RSD were calculated. The results obtained are calculated in **Table 4**.

Table 4: Results of interday precision of Dapagliflozin and Vildagliptin for first order derivative method

Drug	Target conc. (µg/ml)	Mean Abs. ± SD	Mean % RSD
DAPA	7.5	-0.0033± 0.000058	1.34
	10	-0.0046± 0.000058	
	12.5	-0.0056± 0.000058	
VILDA	75	-0.0196±0.00020	0.75
	100	-0.0266±0.00020	
	125	-0.0321± 0.00015	

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$LOD = 3.3\ \sigma/S$

$LOQ = 10\ \sigma/S$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibration graphs. The values of LOD and LOQ are given in **Table 5**.

Table 5: Results of LOD and LOQ of Dapagliflozin and Vildagliptin for first order derivative method

Parameter	DAPA (at 287.2 nm)	VILDA (at 222.9 nm)
Linear Range (µg/ml)	5-15	50-150
Mean of Slope	-0.00047	-0.00025
Standard deviation of intercept	0.000037	0.000149
Limit of Detection (µg/ml)	0.2635	2.0003
Limit of Quantitation (µg/ml)	0.7984	6.0614

4. SUMMARY AND CONCLUSION

The first order derivative spectroscopic method was developed and validated as per ICH Q2 R1 guidelines and was successfully applied for determination of DAPA and VILDA from its pharmaceutical dosage form. The present approach has been found to be cost and time effective. The method was also proven to be precise and repeatable.

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