International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Development and Validation of Analytical Method for Simultaneous Estimation of Dapagliflozin Propanediol Monohydrate and Sitagliptin Phosphate Monohydrate in Tablet Dosage Form

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Abstract: ***In the present study UV spectroscopy methods were developed validated for the simultaneous analysis of dapagliflozin propanediol monohydrate and sitagliptin phosphate monohydrate in tablet dosage form. UV spectroscopy were developed using Q-Absorbance ratio method. From overlay spectra of two drugs, it was evident that DAPA and SITA have an iso-absorptive point at 222.80 nm (λ1). The second wavelength used was 266.80 nm (λ2) of λmax of SITA. DAPA and SITA showed considerable absorbance at both wavelengths. The methods were validated in terms of linearity, accuracy, precision and robustness. Both the methods were linear (R2= <1) and accurate (In UV method %recovery for DAPA was 99.66-101.45% and for SITA was 100.26-101.76%. The method was also found precise (%RSD <2%) and robust. So, the method was found to be accurate, precise, specific, robust and reproducible.***

Keywords: Dapgliflozin, Sitaglptin, UV-spectroscopy, FT-IR, Q-ABSORBANCE ratio

1. Introduction[1]

As per the World Health Organization (WHO), diabetes mellitus[5-6] is a persistent metabolic disorder marked by heightened blood glucose levels, resulting in long-term damage to the heart, blood vessels, eyes, kidneys, and nerves. T2DM, constituting over 90% of diabetes mellitus cases, is characterized by insufficient insulin secretion from pancreatic islet β-cells, tissue insulin resistance (IR), and an inadequate compensatory insulin secretory response. Hyperglycemia occurs in diabetes when the pancreas fails to produce sufficient insulin. SITA functions as an anti-diabetic agent by inhibiting Dipeptidyl peptidase-4, thereby enhancing the release of post-prandial insulin, reducing Glucagon secretion, and lower T2DM treated by two ways: 1) oral medication 2) insulin therapy. Dapagliflozin (DAPA) functions as a Sodium-Glucose cotransporter-2 (SGLT2) inhibitor. It is used in combination with dietary and exercise interventions to enhance glycaemic management in adults with type 2 diabetes mellitus[7]. Sitagliptin[8] ring both post-meal and fasting blood glucose levels in individuals with type 2 diabetes mellitus. Dapagliflozin[9] functions as a selective and reversible inhibitor of the SGLT2 transporter. In the treatment of individuals with diabetes, dapagliflozin has demonstrated additional advantages that go beyond glycaemic control. These benefits encompass blood pressure reduction, renal protection, and enhanced cardiac function. Gliflozins also contribute to a decrease in glycated hemoglobin levels and offer additional benefits, such as consistent maintenance of body weight, blood pressure, and reductions in serum uric acid. Dapagliflozin inhibits the sodium-glucose cotransporter 2 (SGLT2) which is primarily located in the proximal tubule of the nephron. SGLT2 facilitates 90% of glucose reabsorption in the kidneys and so its inhibition allows for glucose to be excreted in the urine. Gliptins represent an innovative class of medications that enhance the health of beta cells while reducing the secretion of glucagon. This leads to an improvement in both post-meal and fasting hyperglycemia.

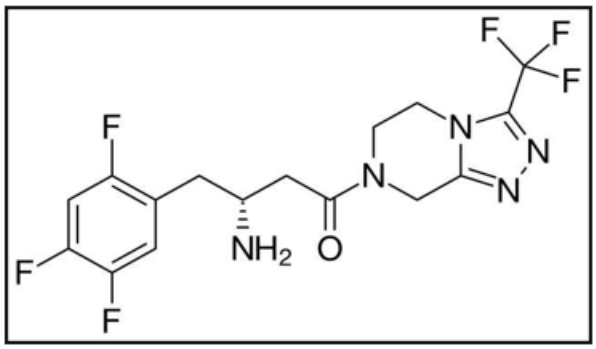

Figure 1: Sitagliptin Phosphate Monohydrate

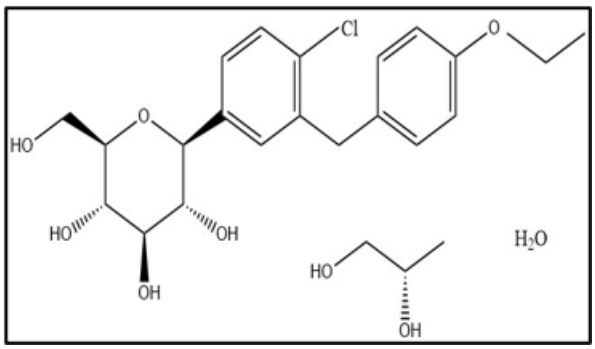

Figure 2: Dapagliflozin Propanediol monohydrate

Spectroscopic method for Dapagliflozin propanediol monohydrate and Sitagliptin phosphate monohydrate were done by using Methanol, water, methyl alcohol and phosphate buffer as common solvent. Wavelength for Dapagliflozin propanediol monohydrate and Sitagliptin phosphate monohydrate were found to be 220 and 267 nm respectively. all chromatographic method was done

Dapagliflozin propanediol monohydrate by using mobile phase as chloroform, methanol and aqueous ammonium in different proportion by using pre-coated silica gel 60 F254 as Stationary phase. For Sitagliptin phosphate monohydrate all chromatographic method was done using Methanol, toluene and ethyl acetate in different proportion by using silica gel 60 F254 as Stationary phase.

2. Materials and Methods

Melting Point Determination:
Melting point of Dapagliflozin propanediol monohydrate and Sitagliptin Phosphate monohydrate was carried out by melting point apparatus.10mg of powdered drug was filled in capillary that was attached with the tip of thermometer in melting point apparatus. Temperature at which the drug powder melted was noted down. It was performed in triplicate.

Solubility Study:
Solubility of Dapagliflozin Propanediol Monohydrate (DAPA) and Sitagliptin Phosphate Monohydrate (SITA) was performed using various solvents like water, methanol, acetonitrile etc.

IR Spectra:
Drug was placed in sample compartment of FT-IR instrument, where it was scanned in the range of 4000 - 650 cm-1. Principle IR peaks were observed for drug are shown in table and from this data it was concluded that drugs were found to be authentic.

UV Absorption Study: [2-4]
Accurately weighed 10 mg of Dapagliflozin propanediol monohydrate (DAPA) and Sitagliptin Phosphate (SITA) were transferred separately in 10 ml volumetric flasks, dissolved in small volume of methanol and thenvolume was adjusted to the mark with methanol to obtain concentration of 1000µg/ml. These solutions were further diluted to obtain concentration of 10µg/ml. These standard solutions of Dapagliflozin propanediol monohydrate (DAPA) and Sitagliptin Phosphate monohydrate (SITA) in methanol were scanned in UV range, 200-400 nm in 1 cm cell using methanol as blank and maximum absorbance was measured for selection of λmax of Dapagliflozin propanediol monohydrate (DAPA) and Sitagliptin Phosphate monohydrate (SITA).

UV spectrum of Drug
Both drug Dapagliflozin propanediol monohydrate (DAPA) and Sitagliptin Phosphate monohydrate (SITA) scan in the range of 200-400nm and wavelength maxima found were similar to reference.

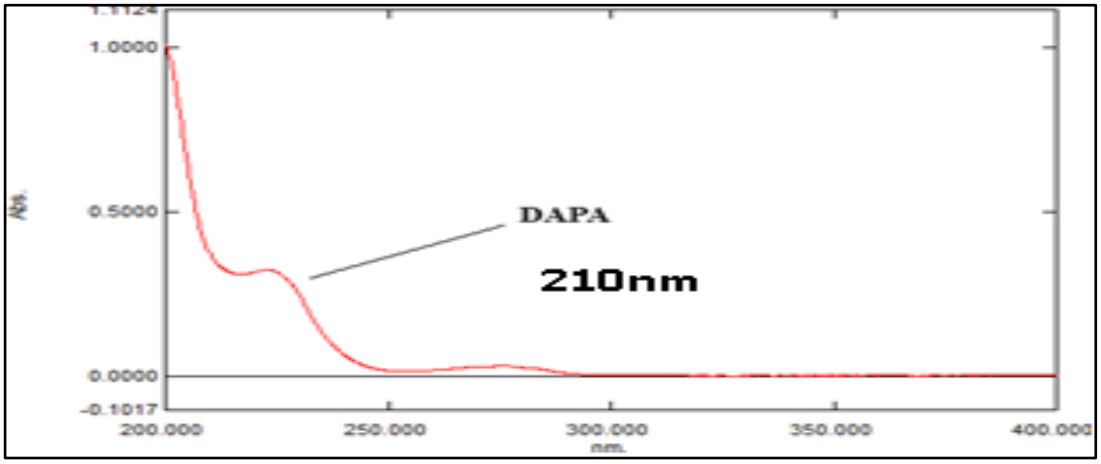

Figure 3: UV Spectrum of 10 µg/ml of Dapagliflozin propanediol monohydrate (DAPA)

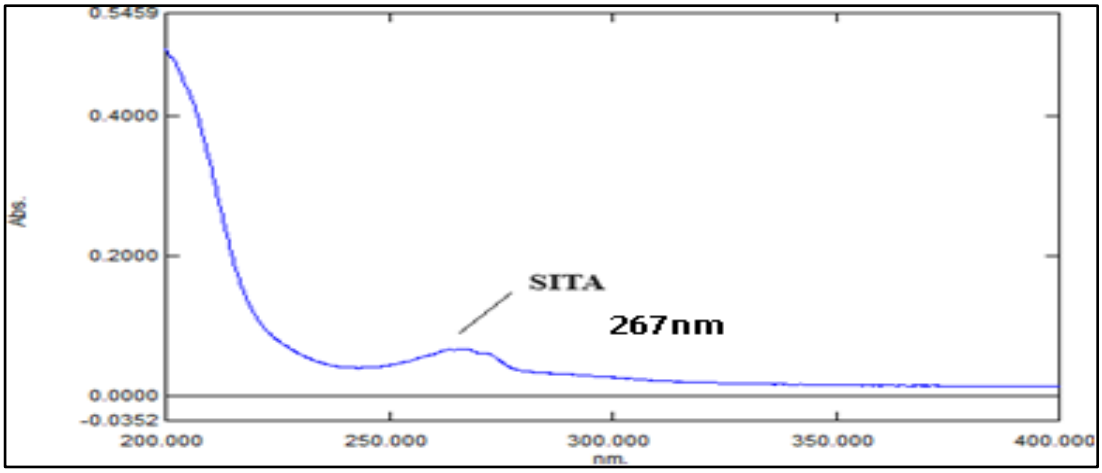

Figure 4: UV Spectrum of 10 µg/ml of Sitagliptin phosphate monohydrate (SITA)

Standard solution of Dapagliflozin propanediol monohydrate (DAPA)

Stock-A: DAPA (1000 µg/ml): Accurately weighed quantity of Dapagliflozin propanediol monohydrate (DAPA) 10mg was transferred to 10 ml volumetric flask, add some methanol and sonicate for 10min and diluted up to the mark with methanol to give a stock solution having strength of 1000µg/ml.

Stock 1: DAPA (100 µg/ml): Aliquot of 1 ml from above standard stock solution was pipette out into 10 ml of volumetric flask and diluted up to the mark with methanol to give a stock solution having strength of 100µg/ml.

Standard solution of Sitagliptin Phosphate Monohydrate (SITA)

Stock-B: SITA (1000 µg/ml): Accurately weighed quantity of Sitagliptin Phosphate Monohydrate (SITA) 10mg was transferred to 10 ml volumetric flask, dissolved and diluted upto mark with methanol to give a stock solution having strength of 1000µg/ml.

Preparation of Working Standard Solution of Dapagliflozin propanediol monohydrate (DAPA): From the above stock solution of 100 µg/ml of DAPA pipette out 0.5ml, 1 ml, 1.5ml, 2ml and 2.5ml of solution and transferred to 10 ml volumetric flask and make up the volume up to 10 ml with methanol to produce concentration 5,10, 15, 20 and 25 µg/ml respectively.

Preparation of Working Standard Solution of Sitagliptin Phosphate Monohydrate (SITA): From the above stock solution of 1000 µg/ml of SITA pipette out 0.5ml, 1 ml, 1.5ml, 2ml and 2.5ml of solution and transferred to 10 ml volumetric flask and make up the volume up to 10 ml with methanol to produce concentration 50, 100, 150, 200 and 250 µg/ml respectively.

Determination of Iso-absorptive Point and Wavelength of Maximum Absorbance: Concentration of 10 µg/ml of DAPA and 100 µg/ml of SITA each solutionwas scanned between 200-400 nm against methanol as a blank reagent. The spectrum of each solution was obtained. The wavelength maximums were found to be 223.20 nm and 266.80 nm for DAPA and SITA respectively. The overlaying spectrum was also obtained to determine iso-absorptive point. Iso-absorptive point at 222.80 nm.

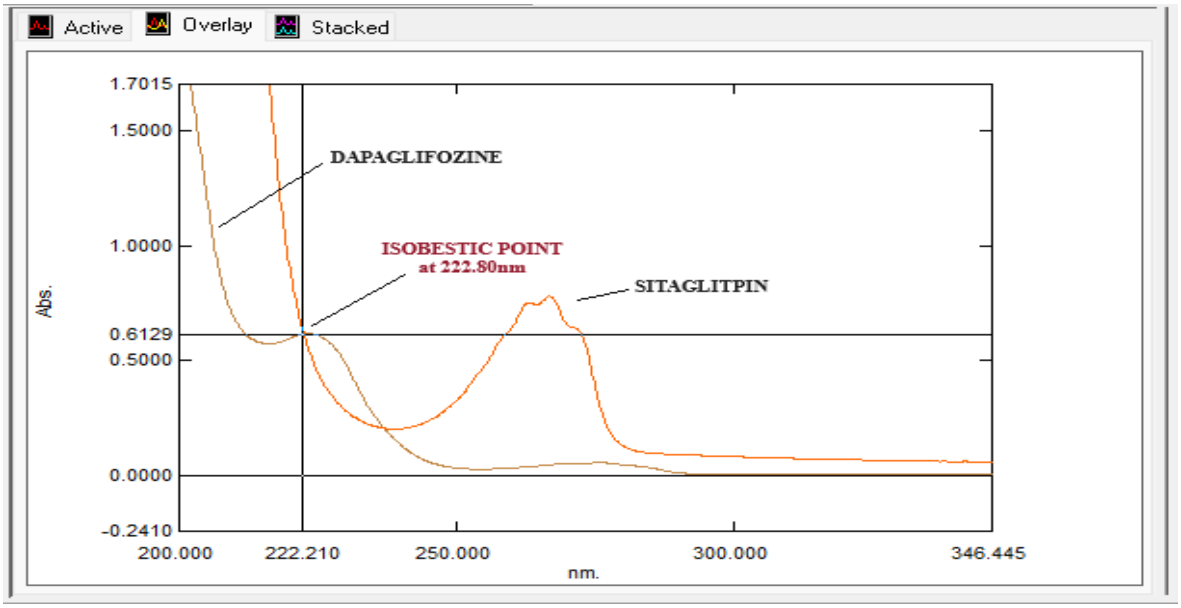

Figure 5: Overlay spectra of Dapagliflozin propanediol monohydrate (DAPA)and Sitagliptin Phosphate Monohydrate (SITA) in methanol showing their iso- absorptive point at 222.80 nm.

Assay of Marketed Dosage form (Tablet): The quantity of the tablet powder equivalent to 10 mg of Dapagliflozin (DAPA) and 100 mg of Sitagliptin Phosphate Monohydrate (SITA) was transferred to a 100 ml volumetric flask.The content was mixed with Methanol (70 ml) and sonicated for 20 min to dissolve the drug as completely as possible.The solution was then filtered through a Whatman filter paper no. 41. The volume was adjusted up to mark with methanol to get 100 µg/ml of DAPA and 1000 µg/ml of SITA.An aliquot of this solution (1 ml) was transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol to make finalconcentration of 10 µg/ml of DAPA and 100 µg/ml of SITA.The absorptivity coefficient of both drugs was determined and the individual concentration of DAPA and SITA was determined using the following equations:

Cx = (Qm-Qy)*A1 / (Qx-Qy)*ax1Cy = (Qm-Qx)*A2 / (Qy-Qx)*ay1

Where, **CX and CY** = concentrations of Dapagliflozin (DAPA) and Sitagliptin Phosphate Monohydrate (SITA) respectively.
A1 = Absorbance of the mixture at Iso absorptive Point.
A2 = Absorbance of the mixture at other drug maximum wavelength.
Qx = ax2 (Absorptivity of DAPA at 266.80nm) / ax1 (Absorptivity of DAPA at222.80nm)
Qy = ay2 (Absorptivity of SITA at 266.80nm) / ay1(Absorptivity of SITA at222.80nm)

Qm = A2 (Absorbance of sample at 266.80nm) / A1 (Absorbance of sample at222.80nm)
ax1 = Absorptivity of DAPA at isoabsorptive point.
ax2 = Absorptivity of DAPA at λmax of DAPA.
ay1 = Absorptivity of SITA at isoabsorptive point.
ay2 = Absorptivity of SITA at λmax of SITA.

Validation of Proposed Method:
Parameters to be considered for the validation of methods are:

1) **Linearity and Range:** The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 5-25 µg/ml and 50-250 µg/ml for DAPA and SITA respectively (n=6). Iso absorptive at 222.80nm.

2) **Precision:**
   a) **Repeatability:** Aliquots of 1.5 ml of working standard solution of DAPA (100 µg/ml) were transferred to a 10 ml volumetric flask. Aliquots of 1.5 ml of working standard solution of SITA (1000 µg/ml) were respectively transferred to a 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 15 µg/ml solution of DAPA and 150µg/ml solution of SITA. The absorbance of solution was measured spectrophotometry six times and % RSD was calculated.
   b) **Intraday precision:** The intraday precision of the developed method was assessed by analyzing solutions containing concentrations 5, 15 and 25µg/ml for DAPA (100 µg/ml) and 50, 150 and 250µg/ml for SITA (1000 µg/ml) and three replicate (n=3) each on same day.
   c) **Interday precision:** The interday precision of the developed method was assessed by analyzing solutions containing concentrations 5, 15 and 25µg/ml for DAPA (100 µg/ml) and 50, 150 and 250µg/ml for SITA (1000 µg/ml) and three replicate (n=3) each on different day.

3) **Accuracy**
The accuracy of the method was determined by calculating recovery of DAPA and SITA by the standard addition method. Aliquots of 1 ml of working stand solution of DAPA (10 µg/ml) and SITA (100 µg/ml) were added at 50, 100 and 150 % level to pre-analyzed 1 ml sample solutions of DAPA (10 µg/ml) and SITA (100 µg/ml) transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 5, 10, 15 µg/ml solution of DAPA and 50, 100, 150 µg/ml solution of SITA. Absorbance of solution was measured at selected wavelengths for DAPA and SITA. The amount of DAPA and SITA was calculated at each level and % recoveries were calculated by measuring the absorbance and fitting the values in equation. Accuracy was assessed using three concentrations and three replicates of each.

4) **LOD (Limit of Detection) and LOQ (Limit of Quantification)**
The method's sensitivity was assessed based on the limit of detection (LOD) and limitof quantitation (LOQ). As per ICH guidelines, the LOD represents the minimum analyte concentration detectable in a sample, while the limit of quantitation signifies the lowest analyte concentration in a sample that can be accurately and precisely quantified.The LOD and LOQ of the developed method was calculated from the six- calibration curve. The LOD and LOQ were calculated by using this formula.
LOD = 3.3 × σ/slopeLOQ = 10 × σ/slope
Where, σ = standard deviation of intercept of 6 calibration curves
Slope = the mean slope of the 6 calibration curves

3. Result and Discussion

Determination of Iso-absorptive Point and Wavelength of Maximum Absorbance
The overlain derivative spectra of Dapagliflozin propanediol monohydrate (DAPA) and Sitagliptin Phosphate Monohydrate (SITA) [**(Figure 03 & 04)]** at different concentrations revealed that 10 µg/ml of Dapagliflozin propanediol monohydrate (DAPA) and 100 µg/ml Sitagliptin Phosphate Monohydrate (SITA) possess iso- absorptive point at 222.80 nm and 266.80 nm λmax of SITA.Considering above facts, wavelength 222.80 nm (λ1) and 266.80 nm (λ2) were selected for the estimation of both the drugs by absorbance ratio method.

Calibration data at 222.80 nm and 266.80 nm for DAPA and SITA are shown in Table 1 and 2. The following equations for straight line were obtained for DAPAand SITA.

Linear equation for DAPA at 222.80nm,
$$y = 0.0305x + 0.0339 \quad (1)$$

Linear equation for DAPA at 266.80nm,
$$y = 0.0025x + 0.0071 \quad (2)$$

Linear equation for DAPA at 222.80nm,
$$y = 0.0031x + 0.0231 \quad (3)$$

Linear equation for DAPA at 266.80nm,
$$y = 0.0037x + 0.0113 \quad (4)$$

Method Validation:
1) Linearity and Range
Different concentrations of Dapagliflozin propanediol monohydrate (DAPA) (5- 25µg/ml) and Sitagliptin Phosphate Monohydrate (SITA) (50-250µg/ml) were prepared from respective stock solutions.

Table 1: Linearity data of DAPA and SITA at 222.80 nm

DAPA			SITA		
Conc. µg/ml	Mean Abs. ± SD	% RSD	Conc. µg/ml	Mean Abs. ± SD	% RSD
5	0.1914 ± 0.0034	1.78	50	0.1926 ± 0.0032	1.66
10	0.3220 ± 0.0052	1.61	100	0.3234 ± 0.0051	1.58
15	0.5205 ± 0.0051	0.98	150	0.4784 ± 0.0042	0.88
20	0.6193 ± 0.0069	1.11	200	0.6363 ± 0.0067	1.05
25	0.8059 ± 0.0045	0.56	250	0.8118 ± 0.0049	0.60

The Linearity curves and calibration curves of these two drugs at 222.80nm and 266.80nm are shown in **Figure 3 and 4.**

Table 2: Linearity data of DAPA and SITA at 222.80 nm

DAPA			SITA		
Conc. µg/ml	Mean Abs. ± SD	% RSD	Conc. µg/ml	Mean Abs. ± SD	% RSD
5	0.1914 ± 0.0034	1.78	50	0.1926 ± 0.0032	1.66
10	0.3220 ± 0.0052	1.61	100	0.3234 ± 0.0051	1.58
15	0.5205 ± 0.0051	0.98	150	0.4784 ± 0.0042	0.88
20	0.6193 ± 0.0069	1.11	200	0.6363 ± 0.0067	1.05
25	0.8059 ± 0.0045	0.56	250	0.8118 ± 0.0049	0.60

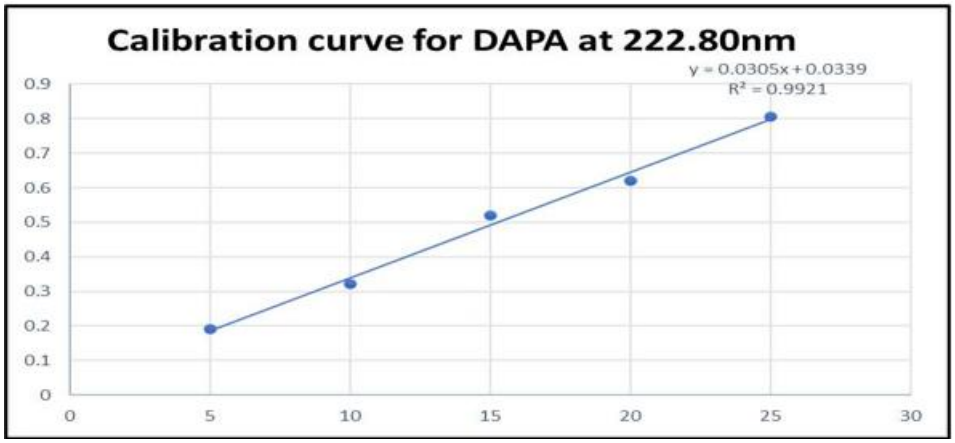

Figure 6: Calibration graph of Dapagliflozin propanediol monohydrate(DAPA) at 222.80nm

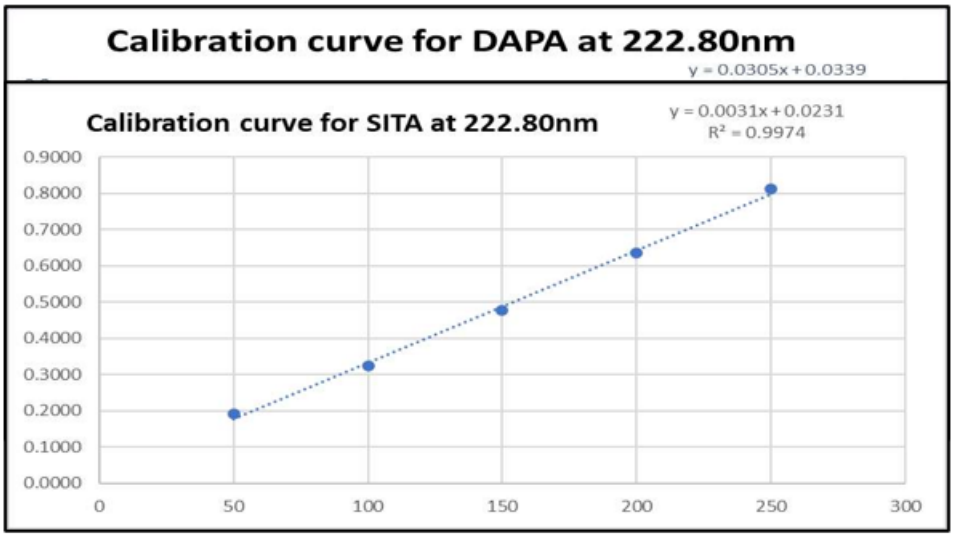

Figure 7: Calibration graph of Sitagliptin Phosphate Monohydrate (SITA) at 222.80 nm

Table 3: Linearity data of DAPA and SITA at 266.80 nm

DAPA			SITA		
Conc. µg/ml	Mean Abs. ± SD	% RSD	Conc. µg/ml	Mean Abs. ± SD	% RSD
5	0.0204 ± 0.0004	1.96	50	0.2095 ± 0.0027	1.29
10	0.0298 ± 0.0004	1.34	100	0.3682 ± 0.0039	1.06
15	0.0477 ± 0.0006	1.26	150	0.5701 ± 0.0041	0.72
20	0.0588 ± 0.0011	1.87	200	0.7798 ± 0.0080	1.03
25	0.0695 ± 0.0006	0.86	250	0.9410 ± 0.0084	0.89

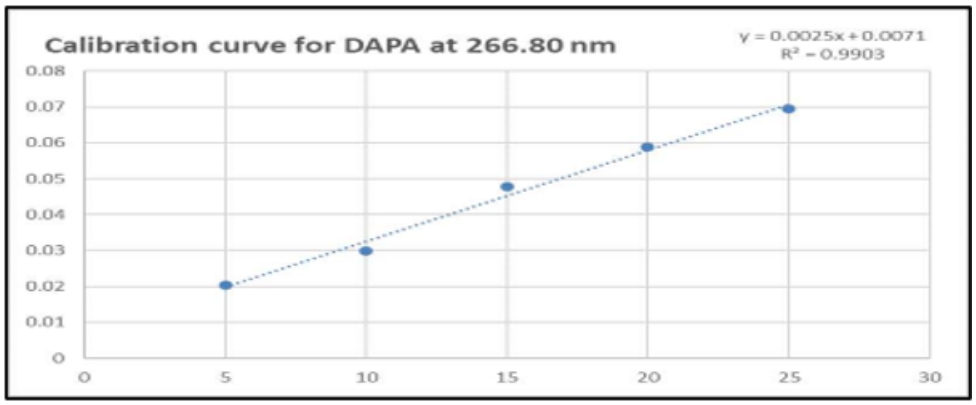

Figure 8: Calibration graph of Dapagliflozin propanediol monohydrate (DAPA)at 266.80 nm

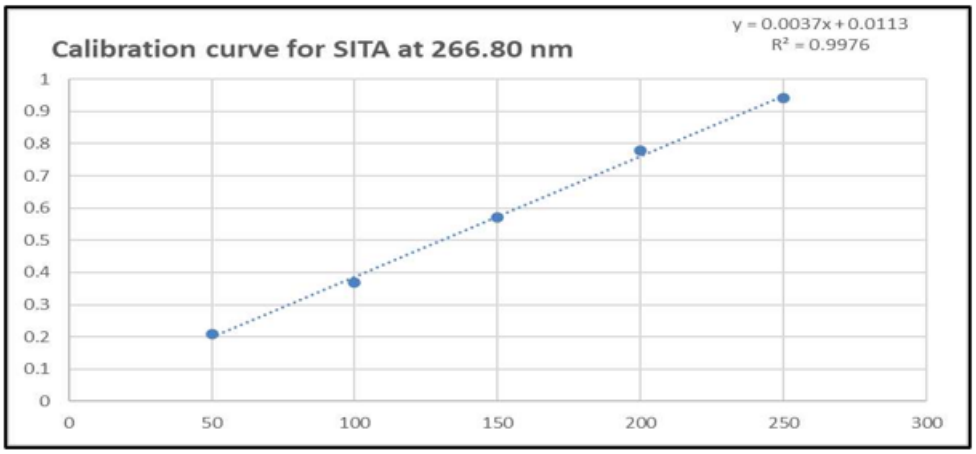

Figure 9: Calibration graph of Sitagliptin Phosphate Monohydrate (SITA) at266.80nm

Table 4: Average of absorptivity's at 222.80 and 266.80 nm

at 222.80 nm		at 266.80 nm	
ax1	0.0305	ax2	0.0025
ay1	0.0031	ay2	0.0037

Table 5: Absorbance of Mixture at 222.80 nm and 266.80 nm

Conc. of DAPA and SITA (µg/ml)	At 222.80 nm	At 266.80 nm
5+ 50	0.2295 ± 0.0026	0.3855 ± 0.0043
10 +100	0.3990 ± 0.0039	0.6516 ± 0.0091
15+150	0.6118 ± 0.0042	0.9991 ± 0.0081
20 +200	0.8364 ± 0.0078	1.2510 ± 0.0057
25 + 250	1.0116 ± 0.0089	1.6127 ± 0.0043

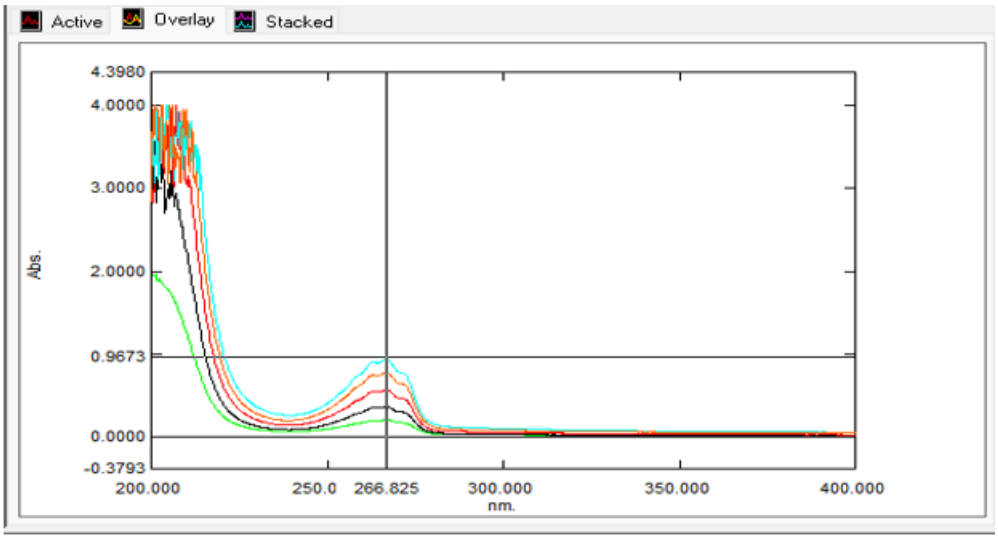

Figure 10: Overlain spectra of SITA at 266.80 nm

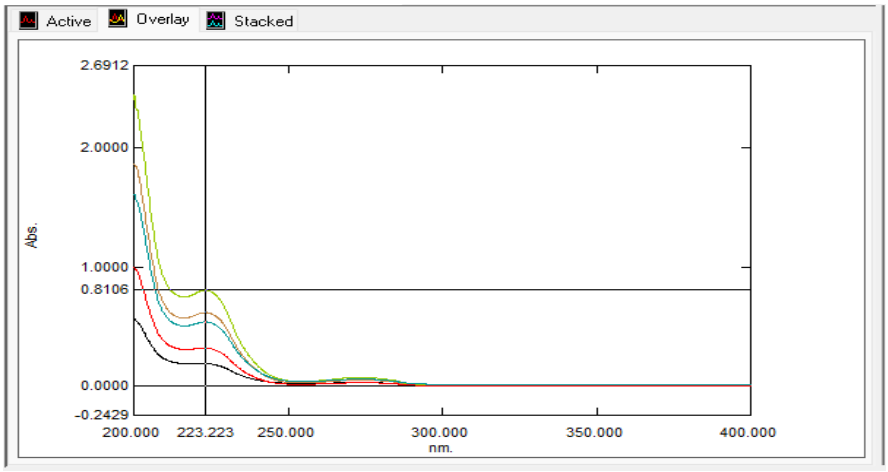

Figure 11: Overlain spectra of DAPA at 222.80 nm

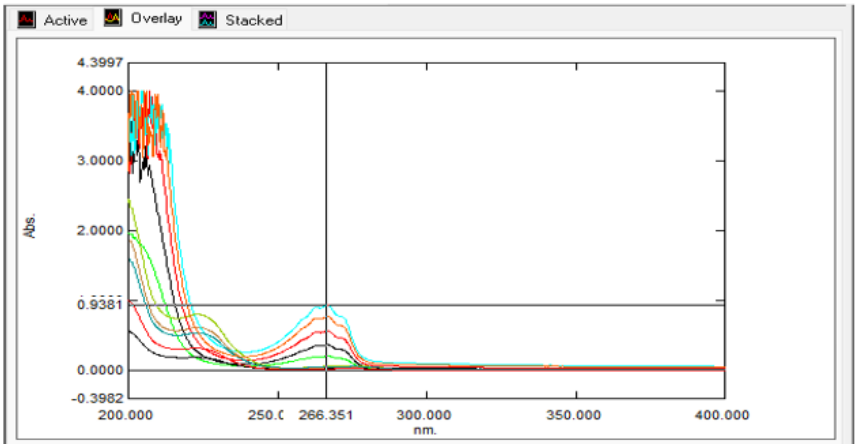

Figure 12: Overlain spectra of DAPA and SITA

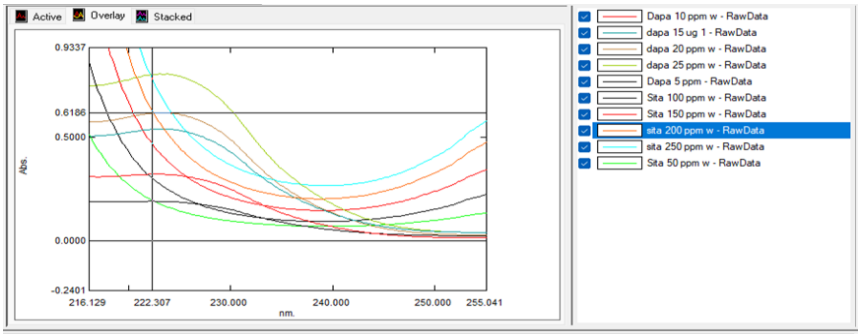

Figure 13: Isosbestic point of DAPA and SITA

2) Precision

(A) Repeatability

The result of repeatability of DAPA (15 µg/mL) and SITA (150 µg/mL) are presented in table 6. The %RSD for repeatability was found to be less than 2 for DAPA and SITA.

Table 6: Data of repeatability of DAPA and SITA

	Absorbance at 222.80 nm		Absorbance at 266.80 nm	
	DAPA 15 µg/mL	SITA 150 µg/mL	DAPA 15 µg/mL	SITA 150 µg/mL
1	0.5196	0.4719	0.0458	0.5657
2	0.5205	0.4747	0.0457	0.5644
3	0.5199	0.4803	0.0452	0.5629
4	0.5177	0.4783	0.0452	0.5645
5	0.5147	0.4747	0.0456	0.5605
6	0.5177	0.4763	0.0459	0.5658
Average	0.5184	0.4760	0.0456	0.5640
Std. Dev	0.0022	0.0029	0.0003	0.0020
% RSD	0.41	0.62	0.66	0.35

(B) Intraday precision

Mixed solutions of DAPA and SITA containing 5, 15 and 25 µg/ml and 50, 150 and 250 µg/ml respectively series were analyzed three times on the same day using developed spectroscopic method and %RSD was calculated. The %RSD value was found to be less than ±2.0 indicated that the method is precise.

Table 7: Intraday precision data for estimation of DAPA and SITA (n=3)

Drug	Conc. (µg/ml)	Absorbance at 222.80nm	% RSD	Absorbance at 266.80nm	% RSD
DAPA	5	0.1899 ± 0.0013	0.68	0.0205 ± 0.0003	1.46
	15	0.5282 ± 0.0024	0.45	0.0449 ± 0.0007	1.56
	25	0.8002 ± 0.0070	0.87	0.0697 ± 0.0002	0.29
SITA	50	0.1905 ± 0.0012	0.63	0.2084 ± 0.0025	1.20
	150	0.4810 ± 0.0080	1.66	0.5677 ± 0.0029	0.51
	250	0.8101 ± 0.0069	0.85	0.9435 ± 0.0031	0.33

Table 8: Interday precision data for estimation of DAPA and SITA (n=3)

Drug	Conc. (µg/ml)	Absorbance at 222.80nm	% RSD	Absorbance at 266.80nm	% RSD
DAPA	5	0.1960 ± 0.0037	1.89	0.0210 ± 0.0004	1.90
	15	0.5220 ± 0.0042	0.80	0.0453 ± 0.0006	1.32
	25	0.8055 ± 0.0074	0.92	0.0694 ± 0.0008	1.15
SITA	50	0.1949 ± 0.0033	1.69	0.2101 ± 0.0013	0.62
	150	0.4805 ± 0.0058	1.21	0.5676 ± 0.0086	1.51
	250	0.8155 ± 0.0074	0.91	0.9510 ± 0.010	1.05

(C) Interday precision

Mixed solutions of DAPA and SITA containing 5, 15 and 25 µg/ml and 50, 150 and 250 µg/ml respectively series were analyzed three times on the three different days using developed spectroscopic method and %RSD was calculated.

The %RSD value was found to be less than ±2.0 indicated that the method is precise.

3) Accuracy

The developed UV spectroscopic method was checked for the accuracy. It was determined by calculating the recovery of DAPA and SITA from tablet dosage form by standard addition method in the combined mixture solution. The spiking was done at three levels 50 %, 100 % and 150 %.

Table 9: Recovery data of DAPA (n=3)

Conc. of DAPA from formulation(µg/ml)	Amount ofStd. DAPA added (µg/ml)	Total amount of DAPA (µg/ml)	Total amount ofDAPA found (µg/ml) Mean ± SD	% Recovery
10	5	15	15.22 ± 0.05	101.47 ±0.33
10	10	20	19.93 ± 0.34	99.66 ± 1.70
10	15	25	25.12 ± 0.23	100.48 ± 0.91

Table 10: Recovery data of SITA (n=3)

Conc. of SITA from formulation (µg/ml)	Amount ofStd. SITA added (µg/ml)	Total amount ofSITA (µg/ml)	Total amount ofSITA found (µg/ml) Mean ± SD	% Recovery
100	50	150	150.39 ± 0.78	100.26 ± 0.52
100	100	200	203.51 ± 0.49	101.76 ± 0.24
100	150	250	251.09 ± 0.69	100.44 ± 0.27

4) Limit of detection and quantitation

The obtained LOD and LOQ results are presented in Table 11.

Table 11: LOD and LOQ data of DAPA and SITA

	DAPA (µg/ml)	SITA (µg/ml)
Std. dev. of Intercept	0.0050	0.0046
Average of Slope	0.0315	0.0326
LOD at 222.80nm	0.52	0.46
LOQ at 222.80nm	1.59	1.41
Std. dev. of Intercept	0.0059	0.0054
Average of Slope	0.00252	0.0375
LOD at 266.80nm	7.72	0.48
LOQ at 266.80nm	23.41	1.44

5) Assay

A zero-order spectrum of the test solution was recorded and measure the absorbance at 222.80nm (λ1) and 266.80nm (λ2) for estimation of DAPA and SITA. The concentrations of DAPA and SITA in formulation were determined using the absorption ratio equation.

Table 12: Analysis data of formulation *(n=3)

Sr. No.	Drug	Formulation (µg/ml)	% Assay* ± SD
1	DAPA	10	100.5 ± 0.87
2	SITA	100	96.20 ± 1.20

Table 13: Summary of validation parameters

Parameters	Q-Absorption Ratio Method			
	DAPA at 222.80nm	DAPA at 266.80nm	SITA at 222.80nm	SITA at 266.80nm
Concentration range (µg/ml)	5-25	5-25	50-250	50-250
Regression equation	y = 0.0305x + 0.0339	y = 0.0025x + 0.0071	y = 0.0031x + 0.0231	y = 0.0037x + 0.0113
Correlation Coefficient(r^2)	0.9921	0.9903	0.9974	0.9976
Accuracy (%Recovery) (n=3)	99.66- 101.45 %		100.26-101.76 %	
Intra-day Precision (%RSD) (n=3)	0.45-0.87%	0.29-1.56 %	0.63-1.66	0.33-1.20
Inter-day precision (%RSD) (n=3)	0.80-1.90%	1.15- 1.90 %	0.91- 1.69	0.62- 1.51
LOD(µg/ml)	0.520	7.72	0.46	0.48
LOQ(µg/ml)	1.57	23.41	1.40	1.43
%Assay(n=3)	95.92 ± 1.43		96.20 ± 1.20	

4. Conclusion

The simple, precise, accurate, and sensitive UV- visible spectrophotometric method for simultaneous estimation of Dapagliflozin propanediol monohydrate (DAPA) and Sitagliptin phosphate monohydrate (SITA) in a bulk drug and tablet powder mixture was developed and validated. The % recovery Dapagliflozin propanediol monohydrate (DAPA) and Sitagliptin phosphate monohydrate (SITA) were found to be in range of 99.66- 101.45 % and 100.26-101.76 %, respectively. The recovery result confirms the high accuracy of proposed method. From overlain spectra 222.80 nm (isobestic point) and 266.80 nm (λmax of Sitagliptin phosphate monohydrate) are selected for the formation of Q absorbance equation. Further, it was found that proposed method could be used for routine analysis of pharmaceutical formulation comprising Dapagliflozin propanediol monohydrate(DAPA) and Sitagliptin phosphate monohydrate (SITA).

The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification. Assay results obtained by proposed method are in fair agreement. All the parameters for two substances met the criteria of the ICH guidelines for the method validation and found to be suitable for routine quantitative analysis in pharmaceutical dosage forms.

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