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UV-Spectrophotometric Approach for Concurrent Assessment of Sitagliptin and Dapagliflozin

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Abstract

A precise, specific, and sustainable ultraviolet (UV) spectrometer technique was established and validated for measuring and determining dapagliflozin and sitagliptin in combined therapeutic dosage forms. The simultaneous equations approach to spectrophotometric assessment employed methanol as a solvent. Dapagliflozin and sitagliptin were identified to have λmax values of 224 nm and 263 nm, consequently, according to the previously mentioned approach. The concentration ranges employed to assess linearity for dapagliflozin, and sitagliptin were 1–13 µg/mL, and 5–35 µg/mL, correspondingly, The r2 values of dapagliflozin (r2 = 0.999) and sitagliptin (r2 = 0.9998) were found. ICH specifications statistically validated the developed approach's correctness, precision, and other statistical studies. Consequently, this method can be used to regularly analyze the combination dosage forms of sitagliptin and dapagliflozin.

Keywords: Polynomial approach, Synchronous, Dapagliflozin, and Sitagliptin.

1. Introduction

In today's setting, hyperglycemia remains among the most prevalent diseases affecting individuals of all ages. According to the current investigation, the risk of 'type-2' diabetes is still rising rapidly in the general population. 'Type-2' diabetes is a serious condition where the body becomes less capable of creating sufficient insulin in the pancreas and develops resistance to the hormone's ordinary effects. Type 2 diabetes is treated with many anti-diabetic drugs, including sitagliptin, glimepiride, metformin, and dapagliflozin [Chaudhury 2017; Jani 2022 and Donepudi, 2019]. Dapagliflozin, sometimes known as DG, is chemically (2S-3R-4R-5S-6R). [Phenyl-4-Chloro-3-(4-ethoxybenzyl)] Tetrahydro-2H-pyran-6-(hydroxymethyl), 2-2-6, 4,5-triol. Dapagliflozin, an inhibitor of the sodium-glucose cotransporter, stops the kidneys from reabsorbing glucose.

Dapagliflozin treatment produces extensive glycosuria which can cause tiredness and weight loss. Dapagliflozin is no longer recommended In treat diabetic ketoacidosis in patients with 'type-1' diabetes [Aswini R, 2018; Mante GV, 2018; Tripathi KD, 2001; and Ahmad S, 2021]. In a chemical way, phosphate of sitagliptin (SG) (R)3-(trifluoromethyl)-4-oxo-4-[3-(1-2-4) triazolo (4-3-a)-5,6-dihydro (pyrazin-7[8H]-yl)-1-(trifluorophenyl-2,4,5) Butan-2-amine is an anti-hyperglycemic, or anti-diabetic, oral dipeptidyl peptidase-4 "DPP-4" inhibitor. Sitagliptin has an absolute bioavailability of about 87% [Aswini R, 2018; Ahmad S, 2021; Gumieniczeka A, 2018 and Sujani PV, 2013]. By using UV spectroscopy, no simultaneous equation technique for DG and SG has been tested [Loni 2012; Ambadas 2014; Sujani 2013 and Arayne 2009]. The object of the investigation is to create and validate an analytical technique for the measurement [Altinoz 2001; Nayana 2019; Tarkase 2013 and Badyala 2015] of the pharmacological dose forms of dapagliflozin and sitagliptin using UV spectroscopy [Jani 2015; Rageeb 2020; Mante 2017; Shaikh 2021; Panwar 2008 and Joshi 2019].

2. Material and Methods

2.1 Instrumentation

All analytical work was performed with single-beam UV-visible spectroscopy (ultraviolet (UV)-117) from Systronics with a couple of 1 cm quartz cells.

2.2 Chemicals and Reagents

Exemed Pharmaceuticals PVT. Ltd., based in Vapi, Gujarat, India, provides DG and SG. Throughout the examination, water that was double-distilled and scientific-grade methanol was used.

2.3 Market Preparation

We received 'DAPANORM-TM' (DUO-10) "10 mg Dapagliflozin and 100 mg Sitagliptin" pharmaceutical tablets from an adjacent pharmacy. were obtained from the local market.

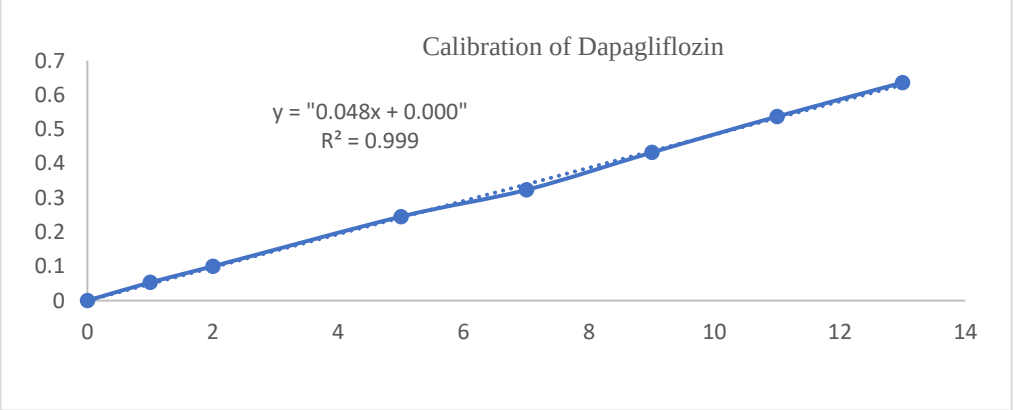
2.4 Standard Stock Solution

10 mg of each of the pharmaceutical tablets were mixed separately in 10-milliliter methanol-filled volumetric flasks to make a standard stock solution of DG and SG. A stock solution measuring 1000 µg was obtained. Standard solutions for use with a concentration of 100 micrograms per milliliter were prepared at adequate dilute solutions utilizing these stocked

solutions. To find this way, the full ultraviolet spectrum was examined using working standard solutions. DG and SG have been identified to have λmax wavelengths of 224 nm and 263 nm, correspondingly. In addition, 7 standard concentrations of each drug were prepared with an average concentration range of 1–13 µg/ml for DG, and 5–35 µg/ml for SG. Calibration graphs were generated when the absorbance of each of these reference solutions was obtained at 224, and 263 nm. Consider the absorbtivity coefficient.

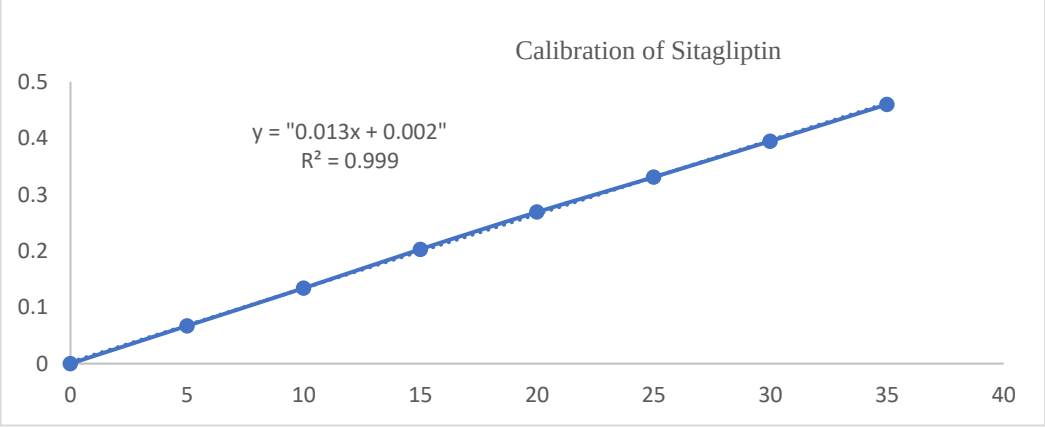
2.5 Sample Solution

In a 10-milliliter volumetric flask, 10 milligrams DG and 100 mg SG of both drugs were dissolved using methanol to produce the sample solution comprising each drug. Various concentrations have been obtained from this stock solution by dilution in appropriate ratios. Established analytical methods were applied to measure the adsorption of the given solutions at 224 and 263 nm to determine their quantities.



(Concentration µg/mL)

Fig 1: The calibration curve of DG



(Concentration µg/mL)

Fig 2: The calibration curve of SG

3. Simultaneous equations approach

The technique used relied on Vierodt's simultaneous equations method. This technique can be applied in cases where a sample consists of multiple medications both absorbed at different

λmaxes relative to one another [Kalyan S *et al.*, 2019]. Based on the concept that the absorption of the DG, and SG combination at (224 nm and 263 nm) correlates to the total of the absorbances at cooresponding wavelengths, two equations were created. Absorbativity coefficient statistics were employed to develop two equations [Ambadas RR *et al*., 2014].

"$C_D = (A_2ay_1\text{-}A_1ay_2/ax_2ay_1\text{-}ax_1ay_2)$"

"$C_S = (A_1ax_2\text{-}A_2ax_1/ax_2ay_1\text{-}ax_1ay_2)$"

The concentrations of DG and SG are denoted by CD and CS, accordingly. The absorption measurements for DG are 224 and 263 nm, while the absorption values for SG are 263 and 224 nm, separately. The absorbances of the diluted samples at (224 & 263 nm), accordingly, are A1 and A2, respectively.

"$C_D = (A_20.1051–A_10.0133/0.051\times0.1051 –0.3403\times0.0133)$"---1

"$C_S = (A_10.0510\text{-}A_20.3403/0.0510\times0.1051–0.3403\times0.0133)$"---2

Absorptivity values

S. N0.	Concentration		abs at 224		absorptivity at224		abs at 263		absorptivity at 263	
	C_D	C_S	DG	SG	AX_1	AX_2	DG	SG	AY_1	AY_2
0	0	0	0	0	0	0	0	0	0	0
1	1	5	0.053	0.075	0.053	0.015	0.053	0.067	0.053	0.0134
2	2	10	0.105	0.15	0.0525	0.015	0.1	0.134	0.05	0.0134
3	5	15	0.215	0.227	0.043	0.015133333	0.251	0.203	0.0502	0.013533333
4	7	20	0.323	0.301	0.046142857	0.01505	0.35	0.269	0.05	0.01345
5	9	25	0.432	0.37	0.048	0.0148	0.47	0.331	0.052222222	0.01324
6	11	30	0.537	0.445	0.048818182	0.014833333	0.56	0.395	0.050909091	0.013166667
7	13	35	0.636	0.535	0.048923077	0.015285714	0.665	0.46	0.051153846	0.013142857
average					0.340384116	0.105102381			0.051069308	0.013333265
SD					0.003475502	0.000167427			0.001164197	0.000139012
%RSD					1.021052972	0.159298959			2.279642144	1.042594096

Table1: Linear regression analysis with their absorptivity.

In the given solution, CD and CS indicate the concentrations of Dapagliflozin and Sitagliptin, accordingly, determined at µg/mL. At 224 and 263 nm, respectively, are the mixture's absorbances, A 1 and A 2. The concentrations of CD and CS can be readily determined by applying these two equations. Table 1 illustrates the absorptivity measurements.

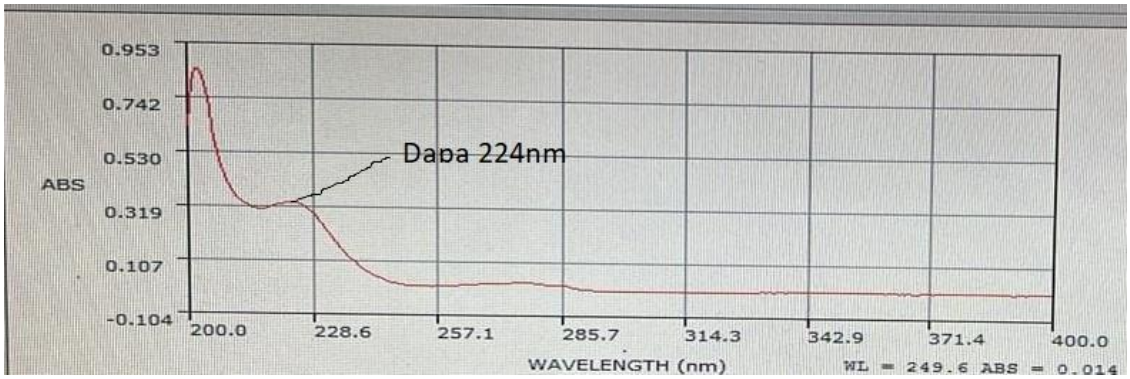


Fig 3: UV Spectra of "Dapagliflozin"

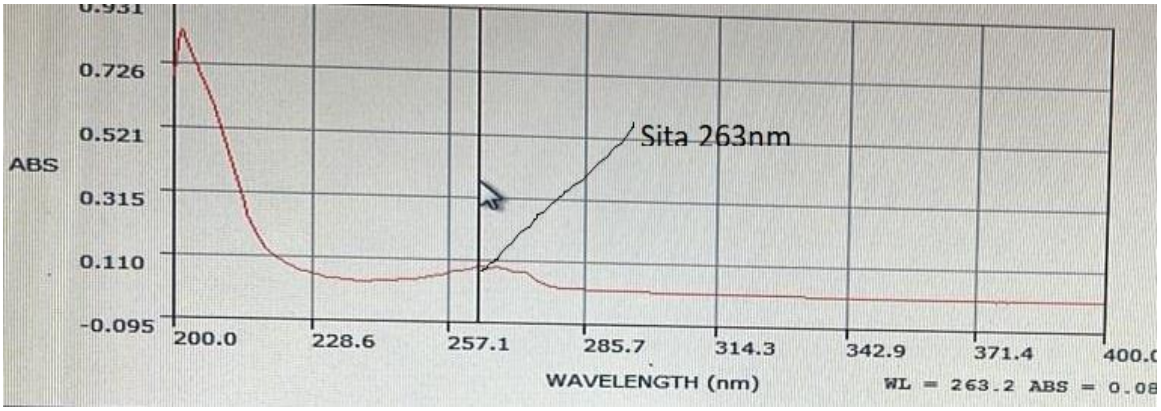


Fig 4: UV Spectra of "Sitagliptin"

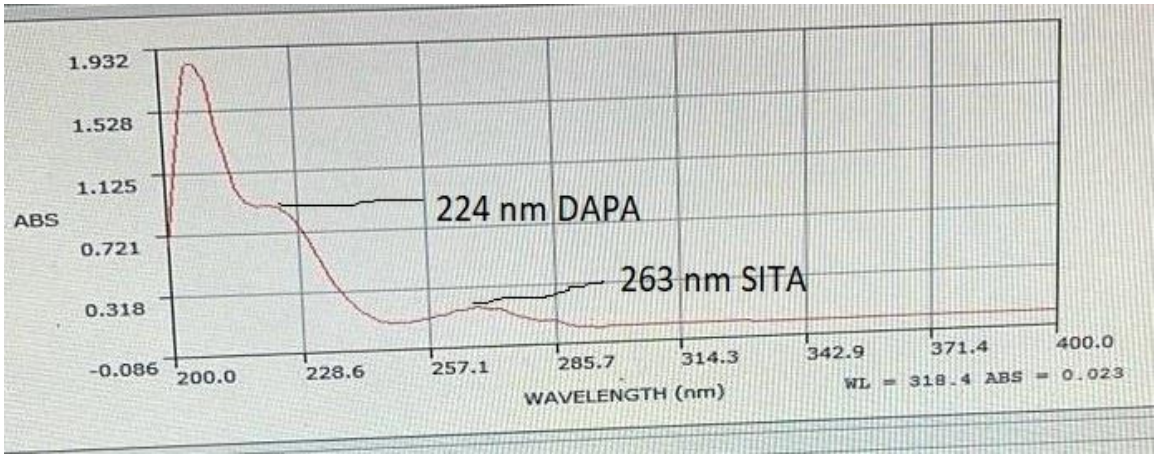


Fig 5: UV Spectra of both "Dapagliflozin and sitagliptin"

4.1 Market formulation analysis

A finely ground powder was obtained by crushing (20) tablets after weighing each. After accurately measuring and transferring a powder sample equal to (10) miligram of dapagliflozin into a 10 ml volumetric flask, the sample was dissolved in 5 ml of methanol, and shaken for ten minutes. The volume of the sample was subsequently adjusted with methanol. Next, Whatman filter paper no 1 was applied to filter the solution. Methanol was added to the solution to dilute it more. Dapagliflozin (5µg/mL) and sitagliptin (50µg/mL) were the final concentrations in the mixture. Simply applying solutions (1) and (2), the amount of the two drugs was determined by applying this method by measuring the absorbance of the sample solution, or [A_1] along with [A_2], at (224 & 263 nm), separately.

5. Method validation

Following the recommendations provided by ICH [Beckett A *et al.*, 2001], the newly developed procedure was verified for accuracy, linearity, precision, repeatability, limit of quantitation, and limit of detection.

5.1 Range and linearity

To carry out the linearity investigation, a standard solution was developed at 7 distinct concentrations (that is, 1-13µ/mL for dapagliflozin and 5-35µ/mL for sitagliptin). The experiment was performed in triplicate. The absorbance measurement was employed to evaluate the response, and by graphs of absorbance vs concentration, calibration curves were produced.

5.2 Precision and exactitude

During investigations of intra- and inter-day fluctuations, the procedure's precision was assessed. In intra-day examinations, the percentage comparative standard deviation (% RSD) was measured after thrice daily assessments of the standard and sample solution. The operational solution of the standard and sample was investigated for three days in a row as part of the inter-day variation examinations, and the (percentage, %) relative standard deviation (%-RSD) was measured. The precision of the recommended recuperation research. The standard additional method was applied to recovery experiments at 80% and 100%, and a pre-analyzed sample of a table dosage form was individually added to 120% of the label claim. At each step of recovery, recovery investigations were put to the test at least three times.

5.3 Limitations on quantitation and detection

The standard deviation of the y-intercept of regression lines or the residual deviation of the regression line were employed separately to measure the LOD, and LOQ. The D represents the standard deviation of the regression line's y-intercept and the S represents the calibrated curve's slope. The following two methods were used to calculate LOD =3.3XD/S & LOQ =10XD/S.

Drug Name	LOD	LOQ
Dapagliflogin	0.6626 µ/ml	1.008 µ/ml
Sitagliptin	0.34 µ/ml	5.3 µ/ml

Table 2: Result of LOD, and LOQ

6. Results and Discussion

The current research performed the concurrent equations technique, referred to as Vierordt's technique, for estimating dapagliflozin, and sitagliptin separately in available table dosage forms. Acceptable standards for dapagliflozin, and sitagliptin were concentrations in the ranges of 1–13 µg/mL, and 5–35 µg/mL, accordingly. The optimum precision, and accuracy were obtained by utilizing two sampling wavelengths: 263 nm (λmax of SG) and 224 nm (λmax of DG). Table 4 summarizes the findings. By applying a known concentration of a standard drug to the pre-analyzed samples, recovery studies were carried out at various concentrations of substances, and the contents were analyzed using recommended techniques. The findings of the marketed formulation analyses are shown in Table 3. The range precision, linearity, accuracy, repeatability, LOD and LOQ Table were all scientifically validated for this technique. Based on recovery examinations, accuracy was calculated. The inter, and intraday variance for each drugs was employed to calculate precision. The percentage recoveries for this technique were found to be 98.19-99.44 for dapagliflozin and 98.93-99.93 for sitagliptin.

Results

Drugs	Label claim	[Vierordt's method]	
	(Mg/tab)	(% found ± RSD*)	(% recovery ± RSD)
'DAPA' (D)	10 mg	98-98.96	98.19-99.44
'SITA' (D)	100 mg	98.99-101.01	98.93-99.93

Table 3: Validation parameters of the Vierordt's method.

Parameters	**Dapagliflozin**	**Sitagliptin**
Range of Linearity; (µg/mL)	1-13 µg/mL	5-35 µg/mL
'Correlation coefficient' (r^2)	0.999	0.9998
'Inter day' (n=3)	0.83-1.26 %	0.73-1.42 %
'Intra day' (n=3)	1.00-1.45 %	1.01-1.50 %
'Accuracy' (%)	98.91-101.4	98.93-101.50
'LOD'	0.6626 µ/ml	3.4 µ/ml
'LOQ'	1.008 µ/ml	5.3 µ/ml

Table 4:Validation parameters for the Vierordt's method.

Conclusion

The investigation described above leads to the conclusion that Vierordt's process is straightforward, quick & repeatable for the simultaneous estimation of sitagliptin, and dapagliflozin without prior separation. The invention received approval from ICH guidelines [*ICH et al*., 1996].

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