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Various innovative UV spectroscopic methodologies for concurrent estimation of dapagliflozin and vildagliptin in combined tablet

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ABSTRACT

Improved glycemic regulation in people with diabetes mellitus can be achieved with a fixed-dose combination (tablets) comprising dapagliflozin 10 mg and vildagliptin 100 mg. The proposed research suggests five spectrophotometric methodologies namely simultaneous equation, absorbance ratio, second derivative zero crossing, ratio difference, and first derivative of ratio spectra methods for the simultaneous assessment of the combined tablet that are straightforward, fast, easy, accurate, and reproducible. The concentration series for DPZ showed a strong linear correlation between 0.5 and 10 µg/ml for the first, second, third, and fourth methods and between 1 and 15 µg/ml for the fifth method. However, VGT displayed exceptional linear association in the sequence of 5–100 µg/ml for the first, second, third, and fourth methods; 10–150 µg/ml for the fifth method. The outcome of precision studies was evaluated in terms of % RSD, following International Conference on Harmonization guideline acceptable limits (<2), which shows good repeatability, low intra, and interday variability, indicating an excellent precision of the developed methods. The outcome of recovery studies ranged from 96% to 103% for both the drug suggests the suitability of the proposed methods. Percentage recovery indicates that there was no interference from tablet excipients. Moreover, the low limit of detection and the limit of quantification values prove the sensitivity of the proposed methods. The projected methods were successfully applied for the quantitative determination of both drugs. Sample solutions were analyzed six times and experimental values were found to be within 98% and 101% for both the drugs. Proposed methods were compared with reported methods in terms of their name of methods, range, sensitivity, specificity, solvents used, and application. The proposed methods are found to be comparable with the reported methods and can cover up shortcomings and thus can be utilized as alternative methods for the simultaneous assessment of dapagliflozin and vildagliptin in the combined formulation.

INTRODUCTION

It was predicted that by the end of 2019, 463 million people would have diabetes worldwide. By 2030, this number is assumed to raise to 578 million globally. By 2045, this number is projected to increase once more, this time to 700 million. Those in urban regions and citizens of high-income countries are at greater risk. Approximately half of those who have diabetes are unaware of their disorder. The number of persons with diminished glucose tolerance is expected to increase from its current 374 million in 2019 to 454 million in 2030 and 548 million in 2045, as per current projections (Saeedi *et al*., 2019). Several studies have been conducted to find anti-diabetic drugs that are more successful at controlling glucose levels while also having less negative side effects. Inhibitors of sodium-glucose cotransporter-2 (SGLT-2) have recently been sanctioned for usage in the management of type 2 diabetes either on its own or in addition to other diabetic treatments (Kalra, 2014). Dapagliflozin (DPZ) bearing the chemical name (2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyphenyl) methyl]phenyl]-6-(hydroxymethyl)-oxane-3,4,5-triol has been

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categorised as SGLT2 inhibitor. The ability of kidney to reabsorb glucose is mostly dependent on a transporter called SGLT2. Often used in conjunction with other drugs, it can be helpful in controlling type 2 diabetes mellitus if combined with a healthy food intake and regular workout (Donepudi and Achanta, 2019; Gundala *et al*., 2019). Vildagliptin (VGT) chemically identified as (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile which is an antihyperglycemic agent acts by inhibition of the dipeptidyl peptidase-4 enzyme selectively. Incretin hormones like glucagon-like peptide-1 and glucose-reliant insulinotropic polypeptide are protected from breakdown by this mechanism. Glycated hemoglobin and fasting plasma glucose concentrations go down and alpha and beta-cell activity in the pancreas improves as a result of this. In patients with poor glycemic control following monotherapy, VGT is authorized in the European Union and internationally for the management of type 2 diabetes mellitus in conjunction with metformin, a sulfonylurea, a thiazolidinedione, or an SGLT2 inhibitor. Figure 1 provides the chemical structures of both the analytes (Boovizhikannan and Palanirajan, 2013; Donepudi and Achanta, 2019; Gundala *et al*., 2019).

Diabetic individuals typically require a combination of drugs to control their blood sugar levels (Sen *et al*., 2022). In late 2022, the newer combination comprising of DPZ and VGT was made available in the Indian market. Fixed dose combination consisting of DPZ (10 mg) and VGT (100 mg) formulated as tablets (sustained release that aids people with keeping blood sugar levels in check when suffering from type 2 diabetes) (Joshi *et al*., 2022; Orme *et al*., 2014; Sen *et al*., 2022; 1mg.com, 2023). Ultra violet (UV) spectrophotometry (Attimarad *et al*., 2023; Banik *et al*., 2015; Manasa *et al*., 2014; Mante *et al*., 2017; Sen *et al*., 2023), high-performance liquid chromatography (Deepan and Dhanaraju, 2018; Gundala *et al*., 2019; Mante *et al*., 2018; Usman *et al*., 2020; Vankalapati *et al*., 2022), and high-performance thin layer chromatography (Abdelrahman *et al*., 2020; Ahmed *et al*., 2020; Bendale *et al*., 2018; Patil *et al*., 2020; Suma *et al*., 2019) were only some of the analytical methods described in the literature for determining DPZ and VGT concentrations in single and mixed dosage forms.

Out of all the reported methods, only Attimarad *et al*. (2023) detailed the determination of DPZ and VGT in mixed formulation simultaneously by three different UV spectroscopic methods, namely ratio difference spectroscopic method (RSM), derivative ratio spectroscopic technique (DRS) and constant ratio substation coupled with multiplication with divisor spectrum method (CSM). Whereas, the proposed study describes five different alternative UV spectroscopic methods namely simultaneous equation method (SEM), absorbance ratio method (ARM), second derivative zero crossing method (^{2}DR), ratio difference method (RDM), and the first derivative of ratio spectra method (FDR). Proposed methods were compared with reported methods in terms of their name of methods, range, the limit of detection (LOD), limit of quantification (LOQ), specificity, solvents used, and application. Results of the comparison suggests that the proposed methods are quite similar in terms of various outcomes. However, the proposed methods stand out against the reported methods in terms of range, sensitivity, and solvent used. Furthermore, the reported approaches (Attimarad *et al*., 2023) have certain shortcomings, such as the absence of validation results for DPZ in the CSM method (Table 4). Additionally, there are instances where the method name does not correspond accurately in certain places. All of the presented methods have been validated in line with International Conference on Harmonization (ICH) standards and they each have their own set of advantages, including a broad concentration range, great sensitivity, and a low barrier in terms of reference and sample preparation. For routine drug quality assessments, UV-spectroscopic methods are regarded as simple, fast, and cost-effective analytical processes. The main disadvantage of direct UV spectroscopic techniques is the influence of multicomponent formulations and formulation additives. As a result, several methods were developed to counteract these effects, including SEM, ARM, ^{2}DR, RDM, and FDR, all of which were validated as suitable for the simultaneous assessment of DPZ and VGT with no interference (Attimarad *et al*., 2012). However, the main limitation of UV-visible spectroscopy is that it can only be used to measure solutions and it cannot be used to measure solid or gaseous samples.

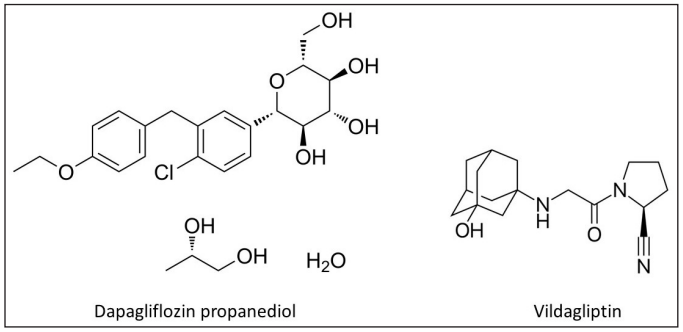

Figure 1. Chemical constructions of DPZ propanediol and VGT.

MATERIALS AND METHODOLOGIES

Chemicals and reagents

Dalton PharmaChem, located in Vadodara, Gujarat, India, generously supplied us with a gift sample of DPZ (98.8% *w*/*w*) and VGT (99.25% *w*/*w*) as our reference standard throughout the course of our study. Loba Chemie Pvt. Ltd., Mumbai, India supplied all other solvents, chemicals, and excipients (specificity) made used in this research.

Instruments

For the experiment, a Shimadzu UV visible spectrophotometer (double beam) with a paired quartz cell having a 1 cm path length (UV-1800, UV Probe, Shimadzu Corporation, Kyoto Japan) was utilized. Weighing was carried out using the Ohaus Corporation's Adventurer Pro AVG264C electronic balance.

Preparation of standard solution

Both DPZ (10 mg) and VGT (10 mg) were weighed carefully and shifted to separate 10 ml volumetric flasks for stock solution preparation. Water was used to dilute standard medicines to 10 ml to attain a solution strength of 1,000 µg/ml. To achieve the desired concentration, further dilutions in water was performed.

Reference stock solutions of DPZ and VGT mixture

In a sequence of 10 volumetric flasks, DPZ and VGT standard solutions were blended to produce mixture of desired concentrations, and the capacity was brought up to the level with water. With water, additional diution was carried out in order to acquire the necessary concentration.

Preparation of sample solution

Marketed formulation (ENCELIN D 10, Torrent Pharmaceuticals Ltd., India) were broken into powder and weighed out to be equivalent amounts (DPZ: 5 mg; VGT: 50 mg) before being added to a 100 ml standard bottle. Further, 50 ml of water was poured into a 100 ml standard flask, stirring it for 10 minutes, and then adding water to bring the total volume to 100 ml before passing across Whatman filter paper no. 41. After that, 1 ml of the resulting liquid was transferred to a 10 ml reference bottle, and the remaining space was loaded with the same solvent to the appropriate strength (DPZ: 5 µg/ml; VGT: 50 µg/ml).

Methodology

Simultaneous equation method

The simultaneous equation method was applied to evaluate DPZ and VGT in the tablet formulation. The UV spectra of the reference analytes were recorded between 200 and 400 nm. In order to determine the proposed analytes in the tablet formulation, the overlapping UV spectra were used to determine the optimal wavelength for analysis. Substantial absorbance was seen at 223 and 210 nm in the overlaid zero-order spectra of DPZ and VGT, correspondingly (Fig. 2). The analyte absorptivity was then determined and tabulated (Table 1). The below-mentioned formula [(1) and (2)] was used to determine the exact amount of medication in each tablet.

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Cx and *Cy* represent the amounts of DPZ and VGT found in the sample solutions, respectively, in the aforementioned equation.

The absorbances of the test solution at 223 nm is A_1 and at 210 nm is A_2. Absorptivity of DPZ at 223 and 210 nm are denoted by *ax*1 and *ax*2, correspondingly. The absorptivity of VGT is denoted by *ay*1, and that at 210 nm by *ay*2 (Beckett and Stenlake, 2005; Puranik *et al*., 2006; Sen *et al*., 2016a, 2016b).

Absorbance ratio method

Excellent linearity was found between the wavelengths of 219.2 nm (the isosbestic point) and 223 nm (the λ_{max} of DPZ), hence these two wavelengths were chosen for simultaneous determination using the AR method. The formulas [(3) and (4)] below were used to determine the concentrations of DPZ and VGT in the test solution using ARM.

$$C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{a_{x1}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

$$C_y = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{a_{y1}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

*ax*1 and *ax*2 stand for the DPZ absorptivities at 223 and 219.2 nm, respectively. As opposed to this, VGT has absorptivities of *ay*1 and *ay*2 at 223 and 219.2 nm, respectively (Table 1). Absorbances A1 and A2 were measured at 223 and 219.2 nm, respectively, for the sample solution. Both DPZ and VGT were found in the sample solution at detectable quantities represented by *Cx* and *Cy*, respectively (Beckett and Stenlake, 2005; Puranik *et al*., 2006; Sen *et al*., 2016a, 2016b).

$$Q_m = \frac{A_2}{A_1} \qquad Q_x = \frac{a_{x2}}{a_{x1}} \qquad Q_y = \frac{a_{y2}}{a_{y1}}$$

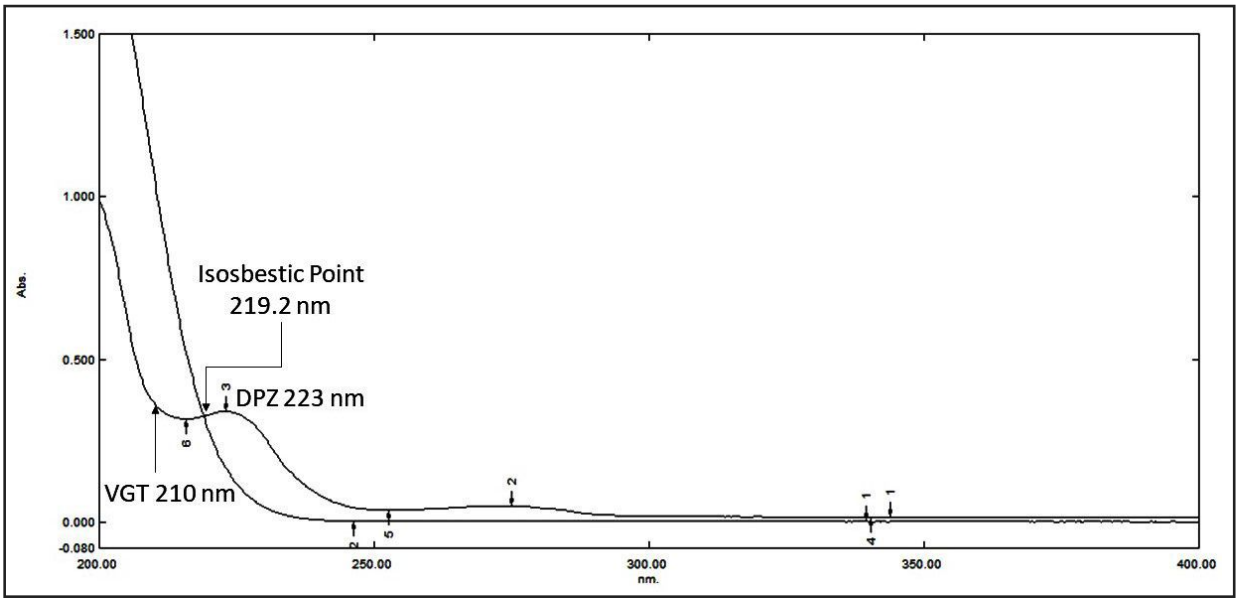

Figure 2. Overlapping UV spectra of DPZ (7.5 µg/ml) and VGT (75 µg/ml).

Table 1. Mean absorptivity values of DPZ and VGT at different wavelengths for SEM & ARM.

Drugs	SEM				ARM			
	DPZ		VGT		DPZ		VGT	
Wavelength (nm)	210	223	210	223	223	219.2	223	219.2
Avg. Absorptivity[a]	494.35 (*ax*1)	463.96 (*ax*2)	144.75 (*ay*1)	22.27 (*ay*2)	463.96 (*ax*1)	434.61 (*ax*2)	22.27 (*ay*1)	43.46 (*ay*2)

[a](n = 6) Mean of six determinations.

Second derivative zero-crossing method

Recorded UV responses of both DPZ and VGT were manipulated into their respective second derivative spectra so that they could be evaluated using the second derivative (Zero Crossing) method. Subsequently, an 8 nm wavelength interval (Δλ) and a scaling factor of 100 were used to trace the zero crossing points of DPZ and VGT, respectively, at 219.2 and 290.6 nm. Estimation was performed at 290.6 and 219.2 nm for DPZ and VGT, respectively, using their traced zero crossing points (Table 2). Using the amplitudes of the second derivative spectra and the concentration of the analytes, a further linear curve was built. The results were put through a regression analysis, with slope, intercept, and correlation coefficient values determined by the method of least squares (Puranik *et al*., 2006; Sen *et al*., 2016a, 2016b).

Ratio difference spectroscopic method

In the RDM, the ratio spectra of DPZ and VGT were acquired by dividing the absorption spectra of the combination (DPZ, 0.5–10 µg/ml, and VGT, 5–100 µg/ml) by those of VGT, 150 µg/ml, and DPZ, 15 µg/ml, separately. Ratio spectra were then obtained. The concentration-amplitude difference of ratio spectra at 236 and 242 nm was used to construct a calibration curve for DPZ, whereas the concentration-amplitude difference of ratio spectra at 208.4 and 215 nm was used to construct a calibration curve for VGT (Attimarad *et al*., 2019; Darwish *et al*., 2016; Lofty *et al*., 2013; Zaazaa *et al*., 2015).

First derivative of ratio spectra method

In the FDR approach, the ratio spectra of DPZ and VGT were acquired by dividing the absorption spectra of the mixture (DPZ 1–15 µg/ml and VGT 10–150 µg/ml) by those of the pure compounds (VGT 150 µg/ml and DPZ 15 µg/ml). Afterward, ratio spectra of the first derivatives were recorded. The quantity of DPZ was assessed using the first derivative signal at 226 nm. A similar method was used for VGT at 215.6 nm. After plotting the amplitudes of the first derivative signals versus their concentrations, regression equations were obtained (Attimarad *et al*., 2019; Darwish *et al*., 2016; Erk, 2001; Nakhla *et al*., 2021).

Analysis of sample solution

The procedure for making the sample solution and diluting it was described in the preceding section. Analyte concentrations were determined using standard absorptivity (Table 1) and the absorbances of test liquids at those wavelengths to solve the corresponding equations for SEM and ARM. Whereas peak amplitude was measured and analytes were quantified with unique regression equations in ^{2}DR, RDM, and FDR approach.

Table 2. Zero-crossing points and detection wavelengths for ^{2}DR method.

Drugs	Zero-crossing point (nm)	Detection wavelength (nm)
DPZ	219.2	290.6
VGT	290.6	219.2

Validation of spectroscopic methods

The established methodologies were endorsed as given in the recommendations of the ICH and several reported methods (Attimarad *et al*., 2019; Darwish *et al*., 2016; Erk, 2001; ICH, 2005; Lofty *et al*., 2013; Sen *et al*., 2023, 2022, 2016a).

Specificity

A specificity study was performed to examine the interaction between tablet formulation excipients and drug ingredients. All the commonly used excipients (European Medicines Agency, 2017; European Medicines Agency, 2012) like lactose (75 mg), microcrystalline cellulose (100 mg), sodium starch glycolate (10 mg), and magnesium stearate (5 mg) were mixed ratio wise (tablet weight was considered approximately 300 mg), diluted with water and passed through a Whatman filter paper no 41. Later, scanning of placebo and reference liquids was performed and analyzed in the UV region to determine the interference amongst formulation additives and medicines.

Linearity and range

Analysis of all the standard solutions consisting of DPZ and VGT in water was performed separately, for assessing the linearity and range of all five approaches. For SEM, absorbance was gauged at 223 and 210 nm, whereas the ARM used 223 and 219.2 nm for DPZ and VGT, respectively. For ^{2}DR, amplitude was measured at 290.6 and 219.2 nm for DPZ and VGT, sequentially. Differences in amplitude were measured at 236 and 242 nm for DPZ, 208.4 and 215 nm for VGT in RDM. However, in the FDR method, 226 and 215.6 nm were utilized for DPZ and VGT, respectively. Using absorbance versus concentration in SEM and ARM approach, amplitude difference versus concentration in RDM, the amplitude of second derivative spectra against concentration

in ^{2}DR, and amplitude versus concentration in FDR method, calibration graphs were created. Using the least-squares method, the slope, intercept, and correlation coefficient for DPZ and VGT at their respective wavelengths were calculated for regression equation.

Precision

Repeatability, intra-day and inter-day precision were measured and represented as percentage RSD for the acquired data so as to evaluate the precision of the procedures. Repeatability of measurement was performed at 5 and 50 µg/ml for DPZ and VGT, respectively six times for both the medicines and computing the percent RSD of the outcomes. Inside the linearity range, intra and inter-day precision experiments were conducted at 5 and 50 µg/ml for DPZ and VGT, respectively in triplicate on the same day and on three dissimilar days, correspondingly, and the % RSD of outcomes were estimated.

Accuracy

Recovery analysis was accomplished by means of the conventional standard addition procedure to crosscheck the viability and dependability of the anticipated methods. To a pre-examined sample solution (DPZ: 4 and VGT: 40 µg/ml), known concentrations of reference DPZ and VGT were supplemented at the 80, 100, and 120 percent levels, and the obtained solutions were re-examined using the proposed procedures and the percent recoveries were computed. Using the following formula, the accuracy of the proposed approaches was evaluated based on the proportion of standard DPZ and VGT retrieved from the formulation.

$$\% \text{ Recovery} = \frac{\begin{array}{c}(\text{Quantity of analyte found}\\ \text{after adding standard drug} -\\ \text{Quantity of analyte adding before}\\ \text{addition of standard drug})\end{array}}{\begin{array}{c}(\text{Quantity of standard}\\ \text{analyte added})\end{array}} \times 100$$

LOD and LOQ

For determining the sensitivity of the projected approaches in accordance with ICH suggestions, LOD and LOQ of DPZ and VGT were computed with the help of the below-mentioned equation.

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

$$\text{LOQ} = 10 \times \frac{\sigma}{S}$$

Where σ = The SD of the response, S = The slope of the linear graph.

Solution stability

By storing the solutions at ambient temperature and assessing them at regular time periods, the stability of the solutions were determined by monitoring any differences in absorbance/amplitude and spectral pattern as compared to newly created solutions.

Statistical comparison by one-way ANOVA

One-way ANOVA (Microsoft 365, Microsoft Corporation, USA) was utilized to draw comparisons between assay outcomes.

Comparison of proposed methods with reported methods

The proposed methods in terms of their name, range, limits of detection (LOD), limits of quantification (LOQ), specificity, solvents utilized, and their applications were compared (Table 7) with a recently published research paper (Attimarad *et al*., 2023) in order to establish the novelty of the projected methods.

RESULTS AND DISCUSSION

In quality control departments, where time and money are of the essence, the proposed spectrophotometric procedures are seen as a natural fit. Because of the simplicity, speed, low cost, and reproducibility of the results, UV spectroscopic methods are widely used for routine investigation of pharmaceutical preparation. These spectrophotometric methods are superior to other analytical methods and provide many benefits. Multi-component formulations with overlapping UV spectra make it difficult to analyze all analytes in a single run. The proposed work describes straightforward and inexpensive methods for analyzing DPZ and VGT simultaneously in binary mixture showing overlapping spectra.

Simultaneous equation method

This method was developed and demonstrated to be sensitive and selective enough for the evaluation of DPZ and VGT in tablet formulation. Substantial absorption was observed in the zero-order UV spectra of DPZ and VGT at 223 and 210 nm. As displayed in Figure 2, the UV spectra of DPZ and VGT show some overlap, allowing for simultaneous estimation of both components in the binary combination. Using a simultaneous equation, we determined how much active pharmaceutical ingredient was present in the formulation. Tables 1 and 3 display absorptivity values and the outcomes of method validation parameters, respectively.

Absorbance ratio method

In ARM, 223 and 219.2 nm were utilized for detecting and quantifying DPZ and VGT. The zero-order overlapping UV spectra exhibited substantial absorbance at 223 and 219.2 nm for DPZ and VGT, sequentially. Moreover, the UV spectra of DPZ and VGT exhibited isosbestic point at 219.2 nm which enables simultaneous evaluation of DPZ and VGT in the binary mixture by ARM (Fig. 2). Table 1 displays the absorptivity values and Table 3 shows the validation results of the proposed methods.

Derivatization of UV spectra

It is a well-established fact that the derivatization of UV spectra increases the specificity and selectivity of pharmaceuticals in combination preparation by enhancing the spectral resolution.

Table 3. A summary of the data obtained by linear regression as well as method validation for the proposed methodologies.

Parameters	SEM		ARM		^{2}DR		RDM		FDR	
	DPZ	VGT	DPZ	VGT	DPZ	VGT	DPZ	VGT	DPZ	VGT
Wavelengths (nm)	223	210	223	219.2	290.6	219.2	236–242	208.4–215	226	215.6
Linearity range (µg/ml)	0.5–10	5–100	0.5–10	5–100	0.5–10	5–100	0.5–10	5–100	1–15	10–150
Correlation coefficient	0.9997	0.9999	0.9997	0.9999	0.9996	0.9998	0.9999	1	0.9999	0.9995
Regression equation:	y = 0.0455x + 0.0025	y = 0.0142x + 0.0072	y = 0.0455x + 0.0025	y = 0.0043x + 0.0004	y = 0.0038x + 0.0004	y = 0.0084x + 0.0112	y = 0.099x + 0.0015	y = 0.0074x + 0.0021	y = 0.023x − 0.001	y = 0.0014x + 0.0019
LOD (µg/ml)	0.0422	0.9580	0.0422	0.5930	0.0861	0.4407	0.0273	0.5966	0.1404	1.9232
LOQ (µg/ml)	0.1278	2.9031	0.1278	1.7969	0.2610	1.3356	0.0828	1.8078	0.4254	5.8277
Specificity	No interferences									
Precision (% RSD) Repeatability of measurement (n = 6)[a]	0.7040	1.1807	0.7040	0.9036	1.2681	1.2447	0.9444	1.2345	1.3162	1.1775
Intra-day (n = 3)[a]	0.7232	0.9861	0.7232	1.1567	1.2811	0.8665	0.9015	1.3273	1.1225	0.9439
Inter-day (n = 3)[a]	0.9496	1.2374	0.9496	1.2512	1.1890	1.0486	0.9764	1.4644	0.7734	0.9821

[a]n = number of estimations, % RSD (% Relative SD).

In addition to eliminating the excipient effects, derivatization also enables us to calculate one analyte in the presence of another analyte. In order to obtain the ratio spectra, which are free of divisor analyte and excipient interventions, the mixture spectra are divided using one of the analyte spectra. Using a spectrum that has been optimized as a divisor is another way to reduce interference and errors made during investigations. Quantifications are conducted in proportion to the peaks, which makes them more exact, sensitive, and specific. This is another advantage of the ratio spectra approach, which also has a number of other advantages. As a direct result of this, ratio spectroscopic methods came into existence. These methods, when contrasted with other spectroscopic approaches, produce results that are of a higher quality.

Second derivative zero crossing method

Following the manipulation of the zero-order UV spectra of the analytes (DPZ and VGT) into their respective second derivative spectra, the second derivative signal at 290.6 and 219.2 nm was traced consecutively using 8 nm as the wavelength interval (Δλ), and 100 as the scaling factor. The second derivative UV spectra of DPZ and VGT which exhibit overlapping of spectra, zero-crossing point, and detection wavelength has been displayed in Figure 3, which enables simultaneous assessment of DPZ and VGT in the binary mixture. The number of analytes existing in the formulation was computed with the help of a regression equation.

Divisor and scaling factor optimization for the FDR spectra

In order to acquire the signal of the FDR spectra that is as excellent as it can possibly be, optimization of the many various choices of experimental parameters was done. Among all of these, the most crucial one was the optimization of the divisor and the scaling factor. Different concentrations of DPZ and VGT were experimented in order to find a divisor that had an appropriate level of concentration. In the end, a VGT concentration of 150 µg/ml and a DPZ concentration of 15 µg/ml were chosen as the appropriate divisors for the quantitative investigation of DPZ and VGT in their binary blend using the RDM and FDR methods. In addition, the scaling factor was set to one and optimized for this value because it was determined to be the most appropriate for accomplishing the goal of reaching the FDR spectra. In order to come up with the most accurate first derivative spectrum, a number of different wavelengths, including 2, 4, 8, and 10 nm were tried. Based on the findings, an optimal wavelength of 8 nm was found to be appropriate, and hence, this wavelength was selected and utilized with a scaling factor of 1.

Ratio difference spectroscopic method

The wavelengths chosen for this procedure were 236 and 242 nm for determining DPZ, whereas 208.4 and 215 nm for determining VGT. The amplitude difference at the given wavelength was then calculated for both DPZ and VGT. The amount of DPZ was estimated utilizing the linear regression equation derived by plotting the variation between the amplitude values at 236 and 242 nm ($\Delta P_{236–242}$) of the ratio spectra presented in Figure 4A against their respective concentrations. The amount of VGT was computed by means of linear regression equation achieved by plotting the variation in amplitude values at 208.4 and 215 nm ($\Delta P_{208.4–215}$) of the ratio spectra displayed in Figure 4B against their respective concentrations.

First derivative of ratio spectra method

DPZ was successfully determined using this method by dividing the spectra of mixed reference solutions by 150 µg/ml of VGT. Using one as a scaling factor and eight as Δλ, the resultant ratio spectra were manipulated to their first-order spectra. By means of the first derivative signal at 226 nm, the

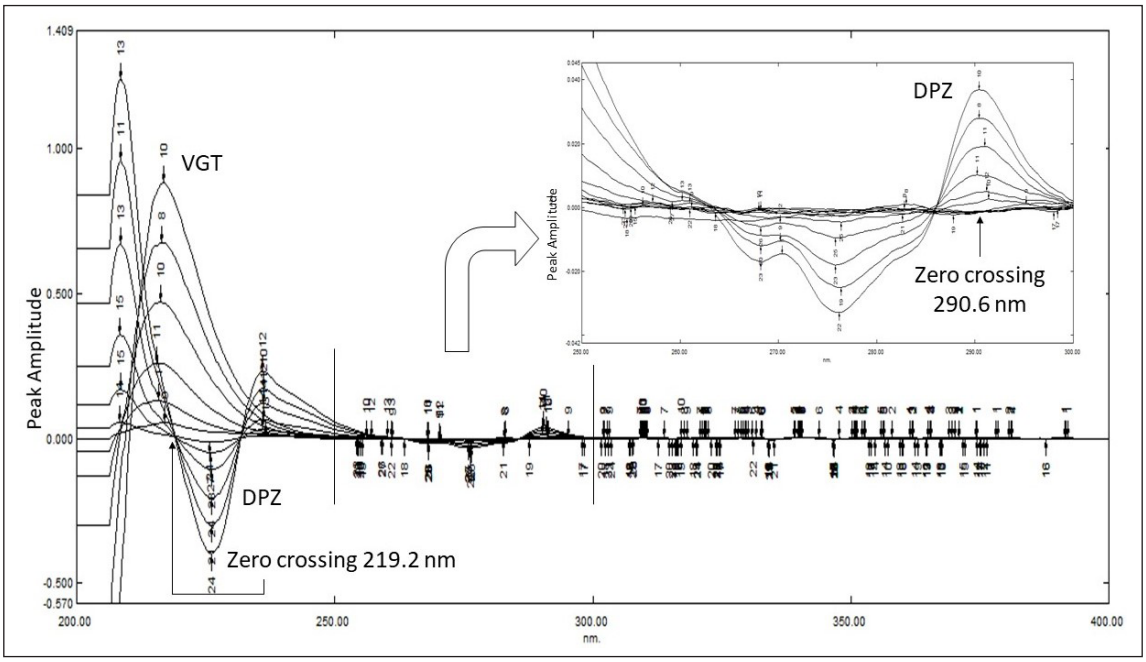

Figure 3. Overlain second derivative UV spectra of DPZ (0.5–10 µg/ml) and VGT (5–100 µg/ml) showing zero crossing points and detection wavelengths.

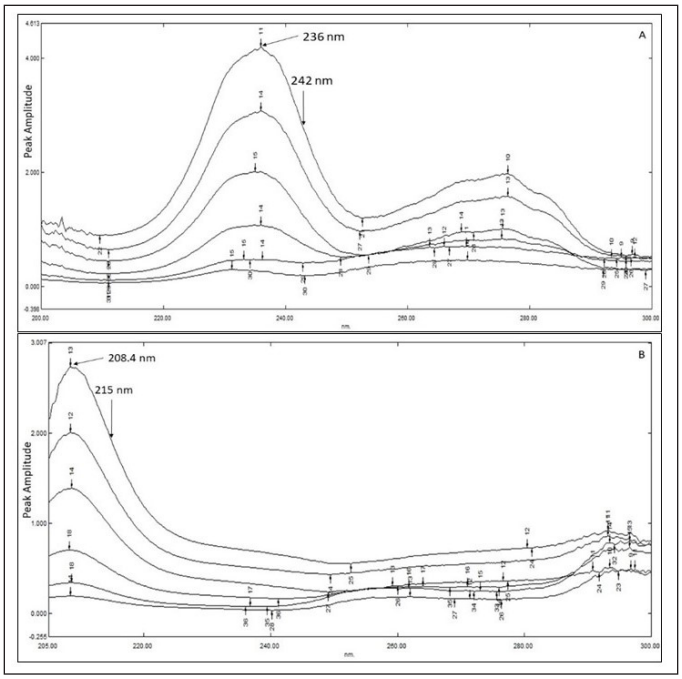

Figure 4. Overlapping ratio spectra of DPZ employing 150 µg/ml VGT as the divisor (A); Overlapping ratio spectra of VGT utilizing 15 µg/ml DPZ as the divisor (B).

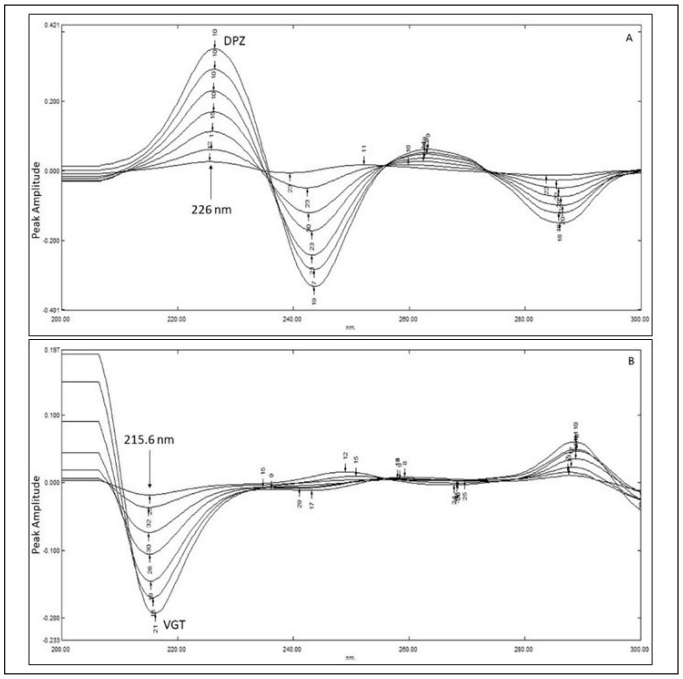

Figure 5. Overlain first derivative ratio spectra of DPZ employing 150 µg/ml VGT as the divisor (A) and Overlain ratio spectra of VGT utilizing 15 µg/ml DPZ as the divisor (B) at Δλ = 8.

quantity of DPZ was calculated. For VGT, a similar technique was utilized, with 15 µg/ml DPZ serving as the divisor, 1 as the scaling factor, and 8 as Δλ. The VGT concentration was then calculated by detecting the first derivative signal at 215.6 nm. The first derivative signal's amplitudes were put up against their respective concentrations, and regression equations were produced. Within the concentration series of 1–15 µg/ml for DPZ and 10–150 µg/ml for VGT a linear association was observed. Figure 5A and B depicts the overlaying FDR spectra of DPZ and VGT.

Table 4. Recovery data of the anticipated methodologies.

Drugs	Recovery level (%)	Recovery (%)[a]					RSD (%)				
		SEM	ARM	^{2}DR	RDM	FDR	SEM	ARM	^{2}DR	RDM	FDR
DPZ	80	97.39 ± 0.61	102.68 ± 0.24	102.30 ± 0.54	96.77 ± 0.52	101.13 ± 1.43	0.62	0.23	0.53	0.54	1.41
	100	97.51 ± 0.74	101.54 ± 1.47	101.47 ± 1.48	98.06 ± 1.02	101.23 ± 0.88	0.76	1.44	1.46	1.04	0.87
	120	97.58 ± 1.35	102.13 ± 0.58	100.86 ± 1.07	98.42 ± 1.53	101.75 ± 1.14	1.39	0.57	1.06	1.55	1.12
VGT	80	101.69 ± 1.58	97.81 ± 1.26	97.40 ± 0.74	101.73 ± 0.85	97.92 ± 1.10	1.55	1.29	0.76	0.83	1.13
	100	101.19 ± 1.28	97.89 ± 0.79	98.09 ± 1.03	101.41 ± 1.49	97.98 ± 1.34	1.26	0.81	1.05	1.47	1.37
	120	100.56 ± 1.36	98.35 ± 1.60	98.06 ± 0.97	101.84 ± 0.57	98.02 ± 0.96	1.35	1.63	0.99	0.56	0.98

[a]Mean ± SD (n = 3), SD (Standard deviation), % RSD (% Relative SD).

Table 5. Outcomes of formulation investigation employing various approaches.

Drugs	Labeled Amount (mg/tab)	Amount Found (mg/tab)					Amount Found (%)[a]					RSD (%)				
		SEM	ARM	^{2}DR	RDM	FDR	SEM	ARM	^{2}DR	RDM	FDR	SEM	ARM	^{2}DR	RDM	FDR
DPZ	10	9.87	9.86	9.87	9.89	9.93	98.67 ± 0.53	98.58 ± 0.48	98.71 ± 0.51	98.87 ± 0.49	99.25 ± 0.87	0.54	0.48	0.52	0.49	0.88
VGT	100	98.79	99.29	99.28	99.78	98.88	98.79 ± 0.44	99.29 ± 0.52	99.28 ± 0.53	99.78 ± 1.31	98.88 ± 0.42	0.44	0.52	0.53	1.31	0.42

[a]Mean ± SD (n = 6), SD (Standard deviation), % RSD (% Relative SD).

Table 6. Statistical comparison of assay outcomes utilizing one-way ANOVA.

Groups	Methods	Mean[a]	Variance	*F*	*F crit*	*p*-value
DPZ	SEM	98.67	0.28	1.1771	2.7587	0.3449
	ARM	98.58	0.23			
	^{2}DR	98.71	0.26			
	RDM	98.87	0.24			
	FDR	99.25	0.76			
VGT	SEM	98.79	0.19	1.5364	2.7587	0.2222
	ARM	99.29	0.27			
	^{2}DR	99.28	0.28			
	RDM	99.61	1.31			
	FDR	98.88	0.17			

[a]n = 6 (Number of determination); p value (significant if $p < 0.05$)

Method validation

All of the proposed methods were evaluated for their validity in agreement with ICH regulations. The subsequent part talks about the results of different validation parameters.

Specificity

It was found that there was no interaction between excipients and analytes by looking at the overlapping spectra of drug solutions and placebos. A placebo is a mixture of familiar excipients that are used in the marketed formulation. This information was presented in the preceding section.

Linearity and range

Evaluating linear correlation and range requires taking absorbance readings at predetermined wavelengths for SEM and ARM approach, amplitude difference of ratio spectra in RDM and amplitude of second derivative spectra in ^{2}DR and amplitude of first derivative in FDR method. Linear correlation was observed for DPZ and VGT between 0.5–10 and 5–100 µg/ml, respectively for SEM, ARM, ^{2}DR, and RDM methods. However, in the case of FDR method, DPZ and VGT were observed to be linear between 1–15 and 10–150 µg/ml, correspondingly. Using the least-squares method, the slope, intercept, and correlation coefficient for DPZ and VGT at their respective wavelengths were calculated for regression investigation. The correlation coefficient values argue in favor of the linearity of every method that was created (Table 3). Each response reflected an average of the results of six separate investigations.

Precision

The results of the precision trials, which were expressed as a percentage RSD and ensured that ICH recommended limits, were met (<2). The outcome demonstrates that all of the proposed methods had great repeatability and reduced intra-and inter-day changeability (Table 3).

Accuracy

The proposed procedures' accuracy was determined using the standard addition method for recovering analytes. For each medicine, recovery rates in the experiments ranged from 96% to 103%, proving the efficacy of the established protocols (Table 4).

LOD and LOQ

Table 3 shows that the predicted procedures are highly sensitive because the LOD and LOQ amounts were observed as very small for all five approaches.

Table 7. Comparison of proposed methods with reported methods.

Methods compared	Drugs	Name of method	Range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Specificity	Solvent used	Application/Limitation
Proposed methods	DPZ	SEM	0.5–10	0.0422	0.1278	Specific	Water	Marketed formulation
		ARM		0.0422	0.1278			
		^{2}DR		0.0861	0.2610			
		RDM		0.0273	0.0828			
		FDR	1–15	0.1404	0.4254			
	VGT	SEM	5–100	0.9580	2.9031			
		ARM		0.5930	1.7969			
		^{2}DR		0.4407	1.3356			
		RDM		0.5966	1.8078			
		FDR	10–150	1.9232	5.8277			
Reported UV spectroscopy (Attimarad *et al*., 2023)	DPZ	RSM	1–15	0.300	0.911	Specific	Ethanol and water	Marketed formulation and laboratory-prepared solution
		DRS		0.189	0.573			
		CSM		Not mentioned	Not mentioned			
	VGT	RSM	7.5–75	2.323	7.040			
		DRS		1.226	3.715			
		CSM		1.885	5.714			

Stability of the solution

Testing the stability of the solution found that it remained unchanged for 2 days at room temperature and for 10 days in the refrigerator (6°C).

Determination of DPZ and VGT in tablet formulation

The planned methods for evaluating DPZ and VGT were successfully implemented. Results were between 98% and 102% for both the analytes, as determined by six repeat determinations used to create a statistically reliable data set. Therefore, we can use the proposed methodologies to evaluate DPZ and VGT together on tablets (Table 5).

Statistical comparison using one-way ANOVA

The effects of each of the five planned procedures were examined using statistical techniques on the data from the assays. The one-way ANOVA in Microsoft 365, Microsoft Corporation, USA was used to relate the statistical implication of the five distinct strategies. All tests had a significance level of $p < 0.05$. The outcomes of the one-way ANOVA are shown in Table 6, and it was discovered that the processes developed differed little from one another.

Comparison of proposed methods with reported methods

Proposed methods were compared (Table 7) with recently published research article (Attimarad *et al*., 2023) in terms of their name of methods, range, LOD, LOQ, specificity, solvents used, and application. Results of the comparison suggest that the proposed methods are quite similar in terms of various outcomes. However, the proposed methods stand out against the reported methods in terms of range, sensitivity, and solvent used. Furthermore, the reported approaches have certain shortcomings, such as the absence of validation results for DPZ in the CSM method (Table 4). Additionally, there are instances where the method name does not correspond accurately in certain places. All of the presented methods have been validated in line with ICH standards and they each have their own set of advantages, including broad concentration range, great sensitivity, and a low barrier in terms of reference and sample preparation. Therefore, the proposed methods are found to be comparable with the reported methods and can cover up the shortcomings of reported methods and thus can be utilized as alternative methods along with reported methods for the simultaneous assessment of DPZ and VGT in the combined formulation.

CONCLUSION

Five distinct spectroscopic approaches, SEM, ARM, ^{2}DR, RDM, and FDR were developed for the synchronized evaluation of DPZ and VGT in a combined marketed formulation. All approaches were validated in accordance with ICH recommendations. It was determined that the proposed approaches are economical, easy, sensitive, precise, and accurate. In addition, the UV-spectrophotometric approaches developed need minimal sample preparation and have a broad concentration series and good sensitivity. There is no statistically significant variation among the five approaches. Therefore, all the described methodologies are suitable for regular quality control examination of DPZ and VGT in the binary mixture or tablet dosage form.

LIST OF ABBREVIATIONS

ARM, Absorbance ratio method; CSM, Constant ratio substation coupled with multiplication with divisor spectrum method; DPZ, Dapagliflozin propanediol; ^{2}DR, second Derivative

zero crossing method; DRS, Derivative ratio spectroscopic technique; FDR, First derivative of ratio spectra method; ICH, International Conference on Harmonization; RDM, Ratio difference method; RSM, Ratio difference spectroscopic method; SGLT2, Sodium-glucose Co-transporter-2; SEM, Simultaneous equation method; UV, Ultra violet; VGT, Vildagliptin.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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