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Original Article

New Eco-friendly UV-spectroscopic Methods for Simultaneous Assessment of Dapagliflozin, Saxagliptin and Metformin in Ternary Mixture

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ABSTRACT

Background and Aim: Qternmet XR® consisting of dapagliflozin (10 mg), saxagliptin (5 mg), and metformin hydrochloride (1000 mg), is a fixed-dose combination (tablets) that improves glycemic control in individuals with diabetes mellitus (type 2). The projected work presents four spectrophotometric methods that are eco-friendly, quick, effortless, accurate and reproducible for the concurrent assessment of the ternary mixture. **Materials and Methods:** The 1st approach works on the notion of unravelling pre-existing equations (simultaneous) by measuring absorbance at 223, 212 and 232.6 nm for dapagliflozin, saxagliptin and metformin hydrochloride, sequentially. The second method namely ratio difference spectroscopy works by evaluating the variation in amplitude at two dissimilar wavelengths in the ratio spectra. Whereas the derivative ratio spectrum zero-crossing approach (third approach) relied on the utilization of the derivative ratio signals at zero-crossing locations. The fourth approach is the double divisor-ratio spectra derivative approach in which the first derivative of ratio spectrum was acquired and the concentrations of all 3 drugs in their combination were quantified. **Results and Discussion:** All the three drugs exhibited excellent linear correlation in the concentration series of 2-10 µg/mL for simultaneous equation method and 0.5-10 µg/mL for all the other methods with an exceptional correlation coefficient value. Furthermore, the projected approaches were authenticated in line with ICH strategies and which displayed suitable precision, accuracy and sensitivity. **Conclusion:** The developed spectrophotometric approaches when compared to other analytical procedures are regarded to be more cost-effective because they do not require expensive solvents or sophisticated instruments. Therefore, the projected methods could be effectively employed for the concurrent assessment of dapagliflozin, saxagliptin and metformin hydrochloride in ternary mixture.

Keywords: Dapagliflozin, Saxagliptin, Metformin, Simultaneous Equation Method, Ratio Difference Spectroscopic approach, Derivative Ratio Spectrum-Zero crossing approach, Double Divisor Ratio Spectra Derivative approach, Ternary mixture.

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INTRODUCTION

Diabetes affected 463 million individuals globally till 2019 and this figure is predicted to climb to 578 million by 2030. This figure is again expected to climb to 700 million by 2045. Those living in high-income countries are more likely to be affected, as are those living in metropolitan areas. One out of every two diabetics does not know they have the disease. According to current predictions, there will be 374 million people with impaired glucose tolerance in 2019, 454 million by 2030, and 548 million by 2045.[1] Several trials have been conducted to discover more effective anti-diabetic medications with better glucose control and fewer side effects. Sodium-glucose cotransporter-2 inhibitors (SGLT-2) have of late been licenced for the treatment of DMT2 either individually or in conjunction with existing diabetes medications.[2] Dapagliflozin (DPZ) having the chemical term (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl]-6-(hydroxymethyl)-oxane-3,4,5-triol has been classified as sodium-glucose co-transporter-2 (SGLT2) inhibitor. SGLT2 is the transporter that is principal factor for glucose being taken back up by the kidneys. It can be utilized to manage type 2 diabetes mellitus as an adjuvant to controlled eating habits and regular workout, frequently in conjunction with other medications. Saxagliptin (SGT) recognized as (1*S*,3*S*,5*S*)-2-[(2*S*)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile is a competitive,

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reversible DPP-4 inhibitor. GLP-1 and glucose reliant insulinotropic polypeptide (GIP) breakdown is delayed once this enzyme is inhibited. GLP-1 and GIP suppress liberation of glucagon from beta cells of pancreas thereby promoting release of insulin. Together, these processes inhibit glycogenolysis in the liver and enhance insulin liberation.[3,4] Metformin Hydrochloride (MTH) a biguanide antidiabetic is chemically identified as 1,1-dimethyl biguanide hydrochloride. It is used to treat diabetes mellitus (type 2) and is the medicine primarily used for the patients who are overweight. Instead of promoting insulin secretion, they exert anti-diabetic effect in presence of some insulin. The delay in glucose absorption from the GI tract, increased sensitivity to insulin, release of glucose into cells and suppression of gluconeogenesis in the liver are all possible mechanisms of action.[5-8] Chemical constructions of all three analytes are given in Figure 1.

Multiple medications are needed to effectively regulate blood sugar in diabetic patients. In 2019, the newer combination containing DPZ, SGT and MTH was approved by the USFDA research, the combination operates through 3 harmonious processes to assist regulate type 2 diabetes patients' blood sugar levels who could have advantage taking DPZ and SGT along with MTH as part of their treatment schedule.[3,4]

Qternmet XR® consisting of dapagliflozin (10 mg), saxagliptin (5 mg), and metformin hydrochloride (1000 mg) formulated as tablets (extended release) is a fixed-dose combination that improves the blood sugar level in individuals with diabetes mellitus (Type 2) if given in conjunction with controlled regime of diet and regular workout.[9-11] Literature survey disclosed numerous analytical approaches for the assessment of DPZ, SGT and MTH independently and in combined dosage form by means of UV-spectrophotometry,[12-16] HPLC[3-4,17-21] and HPTLC.[22,23] Moreover, few approaches were described for the assessment of DPZ, SGT and MTH in mixed formulation/synthetic mixture using HPLC[10] and HPTLC.[11] Nevertheless, the simultaneous estimation of DPZ, SGT and MTH in the ternary mixture has not been reported so far by anyone by UV-spectroscopic methods. Therefore, this paper is the first to detail the development and validation of four easy, reproducible, responsive, profitable, eco-friendly and exact UV-spectroscopic approaches for determining DPZ, SGT, and MTH in a ternary combination. The following are some of the advantages of the proposed methods: they provide a relatively easy standard and sample preparation approach, have a vast concentration range with high sensitivity, and have all been validated according to ICH recommendations. UV-spectroscopic approaches are considered as easy, quick, and cost-effective analytical procedures for assessing the quality of pharmaceuticals that are used on a regular basis. However, effect of multicomponent formulations and formulation additives are the major drawback of direct UV-spectroscopic approaches. Therefore, in addition to simultaneous equation method, derivative and ratio derivative UV-spectroscopic approaches were also developed to overcome these effects, and those were found to be appropriate for the concurrent estimation of DPZ, SGT and MTH lacking any interference.[24]

MATERIALS AND METHODS

Chemicals and Reagents

DPZ, SGT and MTH reference standard employed for the entire investigation was collected from Dalton PharmaChem, Vadodara, Gujarat, India. All other solvents, chemicals and excipients (specificity study) of AR grade used in the study was acquired from Loba Chemie Pvt. Ltd., Mumbai, India.

Instruments

UV-1800 with UVProbe (Shimadzu Corporation, Kyoto, Japan; Double beam UV-visible spectrophotometer) having equivalent sample compartment (Quartz; 1 cm) was employed for the entire investigation. Adventurer Pro AVG264C (Electronic balance, Ohaus Corporation, Pine Brook, NJ, USA) was utilized for the measurement of weight.

Preparation of Standard Solution

The preparation of stock solution of all three drugs (DPZ, SGT and MTH) were carried out by taking weight of 10 mg of standard analytes individually and shifting into a 100 mL standard flask singly. Concentration of standard analytes were lowered using water and capacity was filled up to 100 mL to attain the concentration of the analytes 100 µg/mL. Additionally, reduction in concentration was achieved by dilution employing water.

Preparation of Synthetic mixture

The synthetic admixture of DPZ, SGT and MTH was formulated in the proportion of 10:5:1000 w/w/w. Conventional excipients such as carboxymethyl cellulose sodium (0.060 gm), crospovidone (0.010 gm), lactose anhydrous (0.030 gm), magnesium stearate (0.015 gm), microcrystalline cellulose (0.3 gm), silicon dioxide (0.010 gm), talc (0.030 gm) was measured appropriately and transferred to a mortar together with DPZ, SGT and MTH pure drugs and mixed thoroughly. (Above mentioned calculation is for laboratory prepared synthetic mixture which is equivalent to 1 tablet).[25-27]

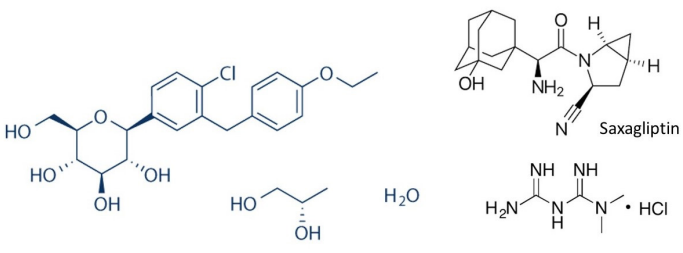

Figure 1: Chemical constructions of DPZ (Dapagliflozin); SGT (Saxagliptin) and MTH (Metformin hydrochloride).

Table 1: Average absorptivity values of DPZ, SGT and MTH at various wavelengths for SEM method.

Drugs	DPZ			SGT			MTH		
Wavelengths (nm)	223	212	232.6	223	212	232.6	223	212	232.6
Absorptivity*	773.76	777.54	449.86	164.59	204.23	82.67	588.29	564.26	748.94

*(n = 6) Average of six determinations.

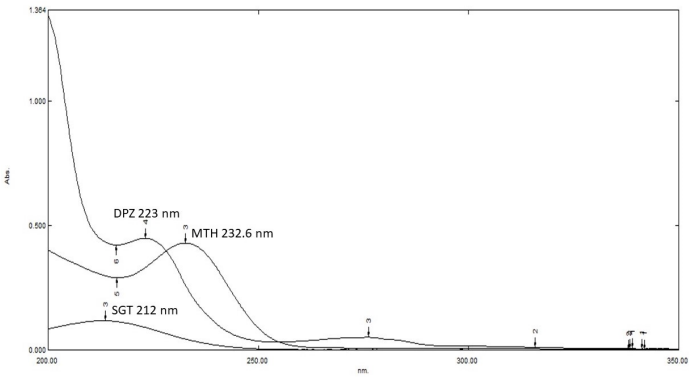

Figure 2: Overlain UV-spectra of DPZ, SGT and MTH (6 µg/mL).

Preparation of mixed standard solution

A series of the mixed standard solution was made by transferring a suitable volume of DPZ, SGT and MTH standard solutions (0.05-1 mL) into a sequence of 10 mL standard flask separately and level was filled with distilled water to reach desired concentration (0.5-10 µg/mL).

Preparation of sample solution

Equivalent quantities of laboratory-prepared synthetic mixture (DPZ: 2 mg, SGT: 1 mg and MTH: 200 mg) were weighed and put into a 100 mL standard bottle. In the 100 mL standard flask, pure DPZ (198 mg) and SGT (199 mg) were added. 50 mL water was poured to the standard bottle and mixed for 10 min. Water was used to make a volume of up to 100 mL, which was then passed across Whatman filter paper (41). 1 mL of the above-mentioned solution was shifted to a 100 mL standard bottle and water was used to make the capacity up to 100 mL. Afterwards, 2.5 mL of the subsequent solution was shifted to a standard bottle (10 mL) and the capacity was filled with water to achieve the desired concentration (DPZ, SGT, and MTH: 5 µg/mL each).

Procedure /Method

Simultaneous Equation Method (SEM)

DPZ, SGT, and MTH in a laboratory prepared synthetic combination were estimated using the simultaneous equation approach. The UV-spectrum of individual standard analytes under study was recorded in the UV region of 200-400 nm. The overlapping UV-spectra were utilized for the selection of appropriate wavelength for the determination of proposed analytes in a laboratory-prepared synthetic mixture. The overlain zero-order spectra of DPZ, SGT and MTH displayed λ_{max} at 223, 212, 232.6 nm for DPZ, SGT and MTH, respectively (Figure 2). Subsequently, the absorptivity of all three drugs were calculated and tabulated (Table 1). The quantity of drug present in the synthetic mixture was quantified using the formula.

$$Cx = \frac{\{A1(ay2az3 - az2ay3) - ay1(A2az3 - az2A3) + az1(A2ay3 - ay2A3)\}}{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - ay2ax3)}$$

$$Cy = \frac{\{ax1(A2az3 - az2A3) - A1(ax2az3 - az2ax3) + az1(ax2A3 - A2ax3)\}}{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - ay2ax3)}$$

$$Cz = \frac{\{ax1(ay2A3 - A2ay3) - ay1(ax2A3 - A2ax3) + A1(ax2ay3 - ay2ax3)\}}{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - ay2ax3)}$$

In the above equation, Cx, Cy and Cz are the amount of DPZ, SGT and MTH, sequentially in the synthetic mixture or the sample solutions.

A1, A2 and A3 are the absorbances of the sample at 223, 212, 232.6 nm. ax1, ax2 and ax3 are the absorptivity of DPZ at 223, 212, 232.6 nm, sequentially. ay1, ay2 and ay3 are the absorptivity of SGT at 223, 212, 232.6 nm, sequentially. az1, az2 and az3 are the absorptivity of MTH at 223, 212, 232.6 nm, respectively.[28,29]

Ratio Difference Spectroscopic Approach (RDS)

The ratio spectra were created by recording the UV absorption band of drug solutions made at various concentrations of DPZ and of the mixture of 3 drugs and dividing them by the total of the SGT and MTH (0.5 µg/mL of both the drugs in water) absorption spectra as the double divisor. Then the amplitude at 222 nm was deducted from the amplitude at 245 nm of the ratio spectrum. Further, a standard curve was constructed utilizing amplitude difference against concentration. The UV-spectra of analyte solutions generated with various concentrations of SGT and the ternary mixture were saved and divided by using DPZ and MTH (0.5 µg/mL separately in water) as a double divisor and ratio spectra were saved. Then the peak amplitude at 240 nm was deducted from the peak amplitude at 220 nm of the ratio spectrum. Further, a calibration curve was plotted using amplitude difference against concentration. To get the ratio spectra, absorption band of solutions generated at various concentrations of MTH and the ternary mixture were stored and divided by the total of the absorption band of DPZ and SGT (0.5 µg/mL separately in water) solutions as the double divisor. Then the peak amplitude at 241 nm was deducted from the peak amplitude at 220 nm of the ratio spectrum. Further, a calibration curve was plotted using amplitude difference against concentration.[30-36]

Table 2: Summary of linear regression and method validation data for the projected approaches.

Parameters/Drugs	SEM			RDS			DRZC			DDRS		
	DPZ	SGT	MTH	DPZ	SGT	MTH	DPZ	SGT	MTH	DPZ	SGT	MTH
Wavelength (nm)	223	212	232.6	222-245	220-240	220-241	286.4	219	228.6	252	252	249
Linearity range (µg/ml)	2-10											
Correlation coefficient	0.9975	0.9975	0.9995	0.9979	0.9987	0.9993	0.9974	0.9996	0.9995	0.9993	0.9992	0.9999
Regression equation	y = 0.069x + 0.0373	y = 0.0193x + 0.0045	y = 0.0671 + 0.0331	y = 0.7687x + 0.00128	y = 0.1169x + 0.0106	y = 1.6064x + 0.0524	y = 0.1211x - 0.0795	y = 0.0041x - 0.0014	y = 0.2369x + 0.062	y = 0.0697x + 0.0213	y = 0.0605x + 0.0178	y = 0.1948x + 0.0086
LOD (µg/ml)	0.0244	0.0829	0.0438	0.0212	0.0283	0.0330	0.1281	0.1185	0.0441	0.0991	0.1077	0.0873
LOQ (µg/ml)	0.0740	0.2512	0.1328	0.0643	0.0857	0.0999	0.3880	0.3590	0.1336	0.3002	0.3265	0.2645
Specificity	**Specific (No interference)**											
Precision (% RSD) Repeatability of measurement (n=6)*	1.1538	1.4025	1.2349	1.5797	1.5498	0.9322	1.3290	1.6518	1.6935	1.4456	1.3264	1.2689
Intra-day (n=3)*	1.1117	1.1731	1.0289	1.5402	1.4482	1.1132	1.1059	1.8139	1.2152	1.5391	1.4169	1.0409
Inter-day (n=3)*	1.3117	1.3399	1.1207	1.4559	1.4043	0.9940	1.1822	1.6663	1.3414	1.7797	1.4712	1.2688

*n = number of estimations, % RSD (% Relative standard deviation).

Derivative Ratio Spectrum-Zero crossing Method (DRZC)

Firstly, the UV absorption bands of DPZ, SGT and MTH and their combination (3 drugs) at different concentrations (linearity range) were documented. Later, the ratio spectra were plotted by dividing the absorption band of DPZ, MTH, and their ternary combination with SGT using a reference spectrum of 2 µg/mL of SGT. Subsequently, first order ratio spectra were produced by converting the ratio spectra into their first derivative. The first derivative ratio signals at 286.4 nm (zero-crossing spot for MTH) and 228.6 nm (zero-crossing spot for DPZ) in the first derivative of ratio spectra were proportional to the concentrations of DPZ and MTH in the ternary mixture, respectively. The amplitudes of first derivative ratio spectra were noted down against ascending concentrations of pure DPZ and pure MTH, with pure SGT as a divisor, to produce calibration graphs. The above-mentioned calibration graphs can be used to determine the contents of DPZ and MTH. Similarly, the saved spectra of DPZ, SGT, and their ternary mixture with MTH were divided by a reference spectrum of 2 µg/mL MTH to get the ratio spectra. Further, the first derivatives of the ratio spectra were recorded. Derivative ratio signals at 219 nm (zero-crossing spot for DPZ) and 286.6 nm (zero-crossing spot for SGT) in the first derivative of the ratio spectra were corresponds to SGT and DPZ respectively. The calibration graphs for DPZ and SGT were created by computing the first derivative ratio values in relation to ascending concentrations of standard DPZ and standard SGT and utilizing standard MTH as a divisor. DPZ and SGT can be determined using this method. By using both above-mentioned spectrophotometric procedures, the amount of DPZ in the ternary mixture can be found.[37-39]

Double Divisor Ratio Spectra Derivative Method (DDRS)

The ratio spectra were generated by recording the absorption band of drug solutions made at various concentrations of DPZ and of the mixture (3 drugs) and dividing them by the total of the absorption band of SGT and MTH (0.5 µg/mL separately in water) as the divisor. Subsequently, the first derivatives of the ratio spectra were constructed. The quantity of DPZ was then estimated by taking the amplitude at 252 nm equivalent to maxima or minima in the spectral region chosen. The UV-spectra of solutions generated with various concentrations of SGT and the ternary mixture were saved and divided by using DPZ and MTH (0.5 µg/mL separately in water) as divisor and ratio spectra were saved. Subsequently, the 1st derivative of ratio spectra was recorded and their signals at 252 nm corresponding to the maxima or minima were measured for the estimation of SGT. To get the ratio spectra, UV-spectra of solutions generated at various concentrations of MTH and the ternary mixture were saved and divided by

the total of the absorption spectra of DPZ and SGT (0.5 µg/mL separately in water) solutions as the divisor and subsequently, their first derivative spectra were plotted. The amount of MTH was calculated by taking the first derivative of ratio spectra and detecting signals at 249 nm, which correspond to maxima or minima.[40-44]

Analysis of sample solution

Sample solution was prepared and diluted as discussed in the previous section. For SEM, amount of analyte was calculated by solving simultaneous equation using standard absorptivity values (Table 1) and absorbances of sample solutions at their respective wavelengths. Whereas, for the other three methods (RDS, DRZC, DDRS), peak amplitude was measured, and the quantification of analytes was done using the regression equations.

Validation of Spectroscopic Methods

The projected approaches were authenticated using the regulations of the "International Conference on Harmonization".[28,37,39-41,43-48]

Specificity

Tests were undertaken to see if the tablet excipients used in the formulation interacted with the active ingredient in the medication. All of the tablet excipients (in accordance with the marketed formulation) were combined proportionately and diluted with water before being filtered with the help of Whatman filter paper (41). Further, all dummy and standard solutions were compared by scanning in the UV zone in order to examine the chemical interaction (if any) between excipients and analytes.

Linearity and Range

Linear correlation of all the four approaches and their range were assessed by evaluating all the standard solutions individually, consisting of DPZ, SGT and MTH in water and absorbances were recorded at 223, 212 and 232.6 nm in SEM method; whereas, amplitude difference (DPZ: 222-245 nm; SGT; 240-220 nm; MTH; 241-220 nm) was measured in RDS method. However, the amplitude was measured in DRZC (DPZ: 286.4; SGT: 219; MTH: 228.6 nm) and DDRS (DPZ: 252; SGT: 252; MTH: 249 nm) approach. Calibration plots of absorbances of standard analyte solutions against concentration in SEM approach; amplitude difference of reference analyte solutions against concentration in RDS approach; peak amplitude versus concentration in DRZC and DDRS method were constructed. Regression assessment was done applying least square approach to get the correlation coefficient, slope and intercept values. Each response was the average of six determinations.

Precision

Repeatability, intra-day and inter-day precision were conducted to assess the method precision. The repeatability of proposed approaches was assessed by analyzing sample solutions (DPZ, SGT, and MTH: 2 and 8 µg/mL) six times and calculating the percent RSD by measuring the responses of all the drugs at various wavelengths depending on the method. Inside the operating range, intra-day precision was assessed by testing sample solutions in triplicate at two dissimilar concentrations (DPZ, SGT, and MTH: 2 and 8 µg/mL) on the same day for three times. Whereas, Inter-day precision was checked on three different days taking two dissimilar concentrations (DPZ, SGT and MTH: 2 and 8 µg/mL) of sample solutions inside the concentration range and calculating the percentage RSD.

Accuracy

In order to perform recovery studies and thereby confirm the suitability and reliability of the projected approaches, standard addition procedure was utilized. To a pre-analyzed sample solution (DPZ, SGT and MTH: 2, 4 and 6 µg/mL), having an equivalent amount, standard DPZ, SGT and MTH of known concentration at 50, 100 and 150% level was added and reanalyzed by projected approaches and thereafter % recoveries were computed. The accuracy study outcomes were analysed on the basis of percentage of reference DPZ, SGT and MTH recuperated from the pharmaceutical preparation by making use of the below mentioned formula:

% Recovery = (Quantity of analytes after adding of standard analytes- Quantity of analytes before adding of standard analytes) / (Quantity of standard analytes added) × 100

LOD and LOQ

The assessment of sensitivity of the recommended methodologies was performed by measuring LOD and LOQ. The below mentioned formula as given in ICH guidelines was utilized for the measurement of LOD and LOQ of the analytes under study

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

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

In the above equation σ = The SD of the response, S = Mean of the slope (standard graph)

Stability of the Solution

The solution stability was assessed by retaining the drug solutions at ambient temperature and in refrigerated conditions (6°C) and observing changes in absorbance and shape of the spectra in comparison with fresh solutions and analyzing them at recurrent intervals.

Statistical comparison by one-way ANOVA

Assay results were compared using one-way ANOVA, Microsoft 365, Microsoft Corporation, USA.

RESULTS AND DISCUSSION

The projected spectrophotometric approaches are thought to be ideal for usage in quality control sections where cost and speed of assessment are critical. UV-spectroscopic approaches are broadly utilized for regular investigation of pharmaceutical preparation owing to their easy, rapid, cheap and reproducible results. As compared to other analytical methods, these spectrophotometric approaches are superior and offer numerous advantages. However, analyzing all analytes without previous separation in the case of multi component formulations which have overlapping UV-spectra is tough. The proposed work formulates some simple and cost-effective approaches to the concurrent analysis of DPZ, SGT and MTH in ternary mixtures having overlapping spectra.

Simultaneous Equation Method (SEM)

A simultaneous equation approach was established and validated for the assessment of DPZ, SGT and MTH in a laboratory formulated synthetic mixture with satisfactory sensitivity and selectivity. The zero-order UV-spectra showed highest absorbance at 223, 212 and 232.6 nm for DPZ, SGT and MTH, respectively (Figure 2). The UV-spectra of DPZ, SGT and MTH exhibited overlapping of spectra as shown in Figure 2 which facilitates simultaneous estimation of DPZ, SGT and MTH in the ternary mixture. The amount of drugs present in the mixture was calculated using a simultaneous equation. Absorptivity values and outcome of method validation parameters are shown in Table 1 and 2, respectively.

Ratio Difference Spectroscopic method (RDS)

Derivatization of UV-spectra results in increase of specificity and selectivity of drugs in combined formulation by improving the resolution of spectra is a well-established fact. Derivatization also allows us to compute one analyte coexisting with other analyte and eradicates the excipient effects. The basis of the ratio spectroscopic method is to divide the mixture spectrum using one of the spectrums of analyte to get the ratio spectrum, which is devoid of divisor analyte and excipient interferences. Additionally, using an optimized spectrum as a divisor lowers noise and investigational mistakes. A further benefit of the ratio spectra approach is that measurements are taken in relation to the peaks, making them more precise, sensitive, and specific. Therefore, ratio spectroscopic methods were developed which gives better results compared to other spectroscopic techniques. In order to select a double divisor of suitable concentration, different concentrations of DPZ, SGT, and MTH were tried. Finally, SGT and MTH (0.5 µg/mL separately in water), DPZ and SGT (0.5 µg/mL separately in water), DPZ and MTH (0.5 µg/mL separately in water) was chosen for the assessment of DPZ, SGT and MTH in their ternary mixture for this method. Ratio difference spectroscopic method was performed by generating ratio spectra by recording the absorption band of solutions made at various concentrations of DPZ and of the ternary mixture and dividing them by the total of the UV-spectra of solutions of SGT and MTH (0.5 µg/mL separately in water) as the double divisor, as shown in Figure 3(A). Then the peak amplitude at 222 nm was deducted from the amplitude at 245 nm of the ratio spectrum. Subsequently, correlation coefficients and regression equations were calculated from linearity graphs plotted taking amplitude difference values and the corresponding amount of DPZ. The UV-spectra of solutions generated with various concentrations of SGT and the ternary mixture were saved and divided by using DPZ and MTH (0.5 µg/mL separately in water) as divisor and ratio spectra were saved, as shown in Figure 3(B). Then the peak amplitude at 240 nm was deducted from the amplitude at 220 nm of the ratio spectrum. Subsequently, correlation coefficients and regression equations were calculated from linearity graphs plotted taking amplitude difference values and the equivalent quantity of SGT. To get the ratio spectra, absorption band of solutions produced at various concentrations of MTH and the ternary mixture were saved and divided by the total of the absorption band of DPZ and SGT (0.5 µg/mL separately in water) solutions as the divisor, as shown in Figure 3(C). Then the amplitude at 241 nm was deducted from the peak amplitude at 220 nm of the ratio spectrum. Subsequently, correlation coefficients and regression equations were calculated from linearity graphs plotted taking amplitude difference values and the respective amount of MTH.

Optimization of divisor and scaling factor for first derivative of ratio spectra

To get the best possible curve of the 1st derivative of ratio spectra, optimization of various settings of instrumental parameters were carried out. Optimization of the divisor and scaling factor were the most important among them. In order to select a divisor of suitable concentration, dissimilar concentrations of DPZ, SGT, and MTH were tried. Finally, 2 µg/mL of SGT as divisor was selected for the quantification of MTH and DPZ. Similarly, 2 µg/mL of MTH was chosen for the assessment of DPZ and MTH in their ternary mixture for DRZC method. Furthermore, the scaling factor was fixed /optimized at four as it was found most suitable for achieving the first derivative of the ratio spectra. Various wavelengths (2, 4, 8, 10 nm) were investigated for the first derivative spectra in order to find the best one. The results indicated that an optimum wavelength of 8 nm was suitable, and this wavelength was chosen and employed with a scaling factor of 4.

Derivative Ratio Spectrum-Zero crossing Approach (DRZC)

An analytical approach namely the Derivative ratio spectrum zero-crossing approach was developed and validated. In this method, solutions of various concentrations of DPZ and MTH in linearity range was scanned and saved between 200–400 nm.

Subsequently, the recorded spectra were divided by using the spectrum of the reference solution of 2 µg/mL SGT. The ratio spectra thus achieved were converted to their first derivative spectra. The amounts of DPZ and MTH in the ternary mixture were estimated by tracing the analytical signals in the 1st derivative spectra of the ratio spectra at 286.4 nm for DPZ and 228.6 nm for MTH, as shown in Figure 4. In the similar manner, the absorption spectra of DPZ and SGT were divided by the spectrum of a reference solution of 2 µg/mL MTH, yielding ratio spectra in the 200–300 nm range. Ratio spectra obtained by this procedure was then transformed into their first derivative using Δλ as 8 and scaling factor as 4. The concentrations of SGT and DPZ in the ternary mixture were estimated by tracing the signals in the first derivative ratio spectra at 219 for SGT and 286.6 nm for DPZ, as displayed in Figure 5.

Double Divisor Ratio Spectra Derivative Method (DDRS)

Firstly, different concentrations of ternary mixtures of DPZ, SGT and MTH were made and scanning was performed at UV range (200-400 nm). Subsequently, these spectra were divided by 0.5 µg/mL MTH and 0.5 µg/mL SGT taken as divisor to get their respective ratio spectra. Further, the first derivative of ratio spectra was attained using Δλ= 8 and scaling factor as 4, as shown in Figure 6(A) and (B). The amplitude of the first derivative ratio spectra at 252 nm was measured and the presence of DPZ was estimated from the calibration graph plotted using these amplitude values against concentration at 252 nm. Similarly, a second set of ratio spectra were acquired by dividing the ternary mixtures of DPZ, SGT and MTH with 0.5 µg/mL of DPZ and 0.5 µg/mL MTH taken as the double divisor. Further, utilizing Δλ as 8 and scaling factor as 4, the first derivative of ratio spectra was acquired, as displayed in Figure 7(A) and (B). The amplitude of the first derivative of ratio spectra at 252 nm were measured and the presence of SGT was estimated from the calibration graph plotted using these amplitude values against concentration at 252 nm. The final set of ratio spectra were acquired by dividing the ternary mixtures of DPZ, SGT and MTH with 0.5 µg/mL of DPZ and 0.5 µg/mL SGT taken as the double divisor. The first derivative of ratio spectra was then acquired utilizing Δλ as 8 and scaling factor as 4, as shown in Figure 8(A) and (B). The amplitude of the first derivative of ratio spectra at 249 nm were measured and the amount of MTH was determined from the calibration graph plotted using these amplitude values against concentration at 249 nm.

Method Validation

Validity of all the proposed procedures were assessed as per "International Conference on Harmonization" strategies. Outcome of various validation parameters are discussed in the following section.

Specificity

Overlain spectra of placebo (mixture of common excipients used in marketed formulations, as indicated in the previous section) and drug solutions indicate that excipients and standard pharmaceuticals did not interact.

Linearity and Range

Linear correlation and range were assessed by measuring absorbance at specified wavelengths for SEM method; whereas, amplitude difference was measured in RDS method. However, peak amplitude was measured in DRZC and DDRS method. Linear correlation was observed for all three drugs between 2-10 µg/mL for SEM approach and 0.5-10 µg/mL for RDS, DRZC and DDRS method. The value of correlation coefficient advocates the linearity of all the developed method (Table 2). Each response was the average of six determination.

Precision

Outcome of precision experiments (repeatability, intra and inter-day) stated in % RSD ensure ICH recommendation limits

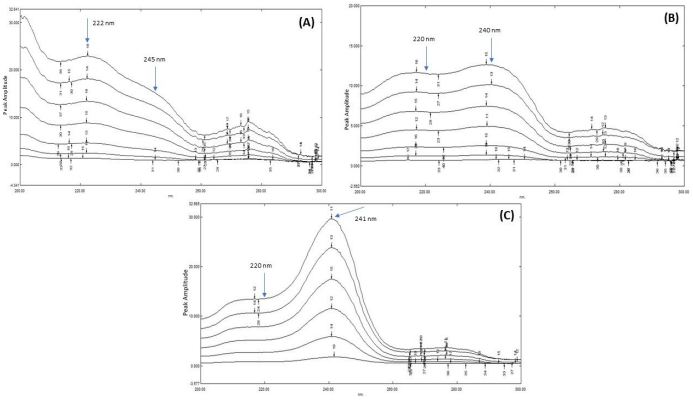

Figure 3: (A) Overlain ratio spectra of DPZ utilizing 0.5 µg/mL (SGT + MTH) as divisor; (B) Overlain ratio spectra of SGT utilizing 0.5 µg/mL (DPZ + MTH) as divisor; (C) Overlain ratio spectra of MTH taking 0.5 µg/mL (SGT + DPZ) as divisor.

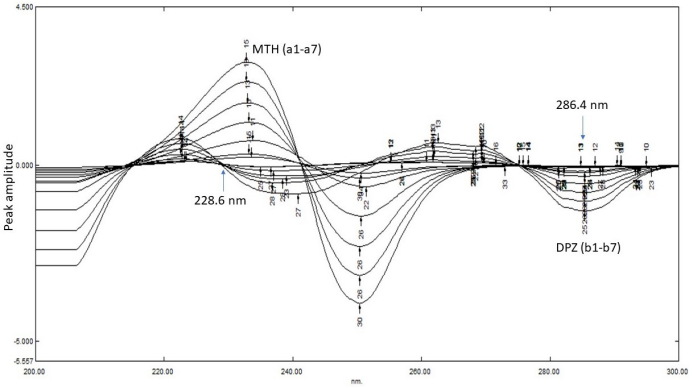

Figure 4: First derivative ratio spectra of MTH (a1) 0.5, (a2) 1, (a3), 2 (a4) 4, (a5) 6, (a6) 8, (a7)10 and of DPZ b1(0.5), b2(1), b3(2), b4(4), b5(6), b6(8), b7(10) (2 µg/mL SGT as divisor (Δλ =8 nm).

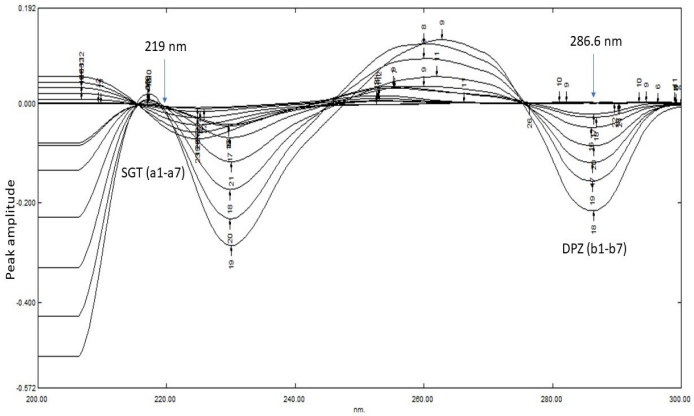

Figure 5: First derivative ratio spectra of SGT (a1) 0.5 (a2) 1 (a3) 2 (a4) 4 (a5) 6 (a6) 8 (a7) 10 and of DPZ b1(0.5), b2(1), b3(2), b4(4), b5(6), b6(8), b7 (2 µg/mL MTH as divisor (Δλ=8 nm).

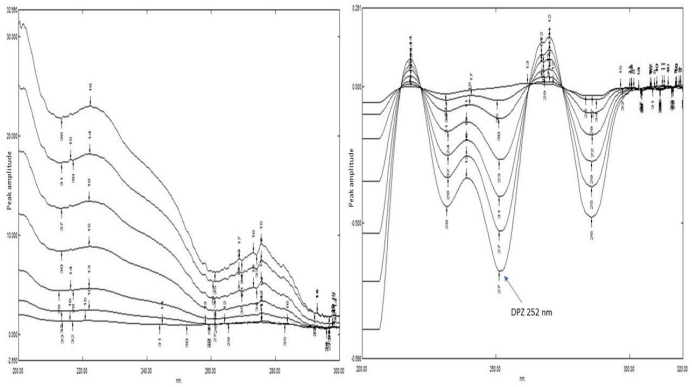

Figure 6: (A) Ratio spectra and (B) 1st derivative ratio spectra of DPZ using 0.5 µg/mL SGT+ 0.5 µg/mL of MTH as divisor at Δλ=8.

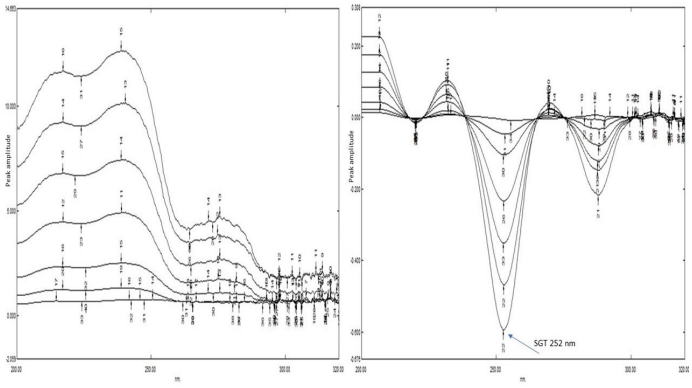

Figure 7: (A) Ratio spectra and (B) 1st derivative ratio spectra of SGT using 0.5 µg/mL DPZ+ 0.5 µg/mL of MTH as divisor at Δλ=8.

(< 2), which confirms the excellent repeatability, less intra and inter-day changeability of all the projected approaches (Table 2).

Accuracy

Accuracy of the projected procedures were calculated based on the retrieval of analytes by standard addition approach. The outcome of recovery studies was in the range of 97-103% for each drug displaying the correctness of all the developed procedures (Table 3).

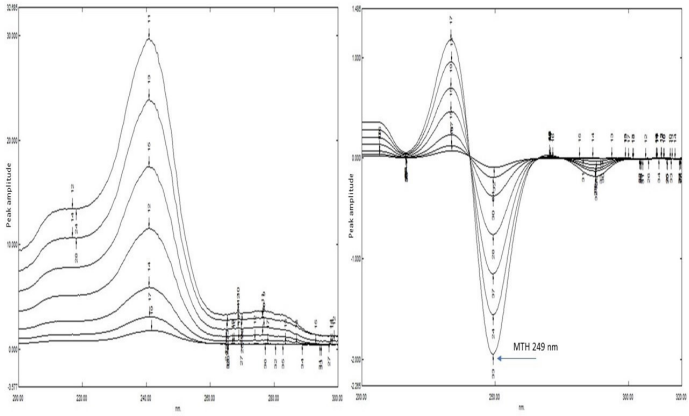

Figure 8: (A) Ratio spectra and (B) 1st derivative ratio spectra of MTH using 0.5 µg/mL SGT+ 0.5 µg/mL of DPZ as divisor at Δλ=8.

LOD and LOQ

The values of LOD and LOQ for all the four methods was proved to be very less which demonstrates the extent of sensitivity of the projected procedures (Table 2).

Stability of the Solution

Solution stability was carried out at ambient environment and refrigerated condition (6°C) and it was discovered to be unaltered up to 2 days at ambient environment and 10 days at cooled condition.

Determination of DPZ, SGT and MTH in ternary mixture

The projected approaches were effectively employed for the assessment of DPZ, SGT and MTH. Six replicate determinations have been carried out to achieve statistically proven data set and which was between 97 and 102% for all three analytes. Hence, the established methods can be employed for the concurrent evaluation of DPZ, SGT and MTH in ternary mixture (Table 4).

Statistical comparison by one-way ANOVA

An examination of the data from the assays was done using statistical methods to determine the impact of each of the four approaches that were projected. One-way ANOVA was used to compare the statistical significance of the four different approaches. The significance level for all tests was set at $p<0.05$. Table 5 demonstrates the outcomes of one-way ANOVA and it was found that the created procedures differed little from each other.

Table 3: Recovery information of the projected approaches.

Drugs	Level (%)	Recovery (%)*				RSD (%)			
		SEM	RDS	DRZC	DDRS	SEM	RDS	DRZC	DDRS
DPZ	50	98.41 ± 0.95	99.42 ± 1.40	99.15 ± 0.58	99.26 ± 1.60	0.97	1.41	0.58	1.61
	100	98.61 ± 0.84	99.81 ± 1.38	99.82 ± 1.47	99.44 ± 0.73	0.85	1.38	1.48	0.73
	150	99.59 ± 0.89	100.89 ± 2.01	99.01 ± 0.78	100.21 ± 1.66	0.90	1.99	0.79	1.66
SGT	50	98.82 ± 0.93	99.35 ± 1.64	98.73 ± 1.72	98.61 ± 0.53	0.94	1.65	1.74	0.53
	100	99.8 ± 1.81	100.10 ± 1.78	99.21 ± 0.90	100.00 ± 1.87	1.81	1.78	0.90	1.87
	150	99.85 ± 1.52	100.31 ± 1.48	99.08 ± 0.68	99.99 ± 1.94	1.52	1.48	0.68	1.94
MTH	50	99.83 ± 1.76	97.14 ± 0.47	100.09 ± 0.54	99.2 ± 1.21	1.76	0.48	0.54	1.22
	100	100.29 ± 1.31	98.27 ± 0.96	100.97 ± 1.72	100.15 ± 1.64	1.30	0.98	1.71	1.64
	150	99.42 ± 1.47	99.64 ± 1.75	99.31 ± 1.05	99.23 ± 1.86	1.48	1.75	1.06	1.77

*Mean ± SD (n = 3), SD (Standard deviation), % RSD (% Relative standard deviation).

Table 4: Outcomes of formulation evaluation by various approaches.

Drugs	Labelled Amount (mg/tab)	Amount Estimated (mg/tab)				Amount Estimated (%) *				RSD (%)			
		SEM	RDS	DRZC	DDRS	SEM	RDS	DRZC	DDRS	SEM	RDS	DRZC	DDRS
DPZ	10	9.85	9.95	9.94	9.98	98.45 ± 1.27	99.52 ± 1.76	99.40 ± 1.27	99.75 ± 1.62	1.29	1.77	1.12	1.63
SGT	5	4.95	4.97	4.97	4.94	99.03 ± 1.75	99.30 ± 1.94	99.30 ± 1.37	98.73 ± 0.95	1.77	1.95	1.38	0.97
MTH	1000	998.31	1000.38	998.05	1000.47	99.83 ± 1.60	100.04 ± 1.07	99.81 ± 1.97	100.35 ± 1.33	1.60	1.07	1.98	1.33

*Mean ± SD (n = 6), SD (Standard deviation), % RSD (% Relative standard deviation).

Table 5: Statistical comparison of assay results utilizing one way ANOVA.

Groups	Methods	Mean*	Variance	*F*	*F crit*	*p*-value
DPZ	SEM	99.75	1.67	0.0742	3.0984	0.9732
	RDS	99.53	3.07			
	DRZC	99.42	1.23			
	DDRS	99.74	2.63			
SGT	SEM	99.03	2.36	0.1223	3.0984	0.9459
	RDS	99.28	3.76			
	DRZC	99.21	1.85			
	DDRS	98.80	0.94			
MTH	SEM	99.84	2.47	0.1096	3.0984	0.9535
	RDS	100.10	1.14			
	DRZC	99.60	3.90			
	DDRS	99.77	1.74			

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

CONCLUSION

Four different methods namely SEM, RDS, DRZC and DDRS spectroscopic approaches were proposed for simultaneous assessment of DPZ, SGT and MTH in the ternary mixture. The approaches developed were authenticated in agreement with ICH recommendations. The proposed methods were accurate, easy, responsive, reproducible, and profitable. Furthermore, all of the established UV-spectrophotometric approaches necessitate less sample preparation steps and offer an extended range of concentration with good sensitivity. The projected spectrophotometric approaches are assumed to be valid in QC (quality control) section wherever the cost as well as speed of assessment stands critical. Moreover, when compared to other analytical procedures, these approaches are regarded to be more cost-effective because they do not require expensive solvents or sophisticated instruments. As a result, all of the established methods may be utilised for conventional quality control investigation of DPZ, SGT, and MTH in synthetic mixtures and mixed dosage forms (tablet) successfully.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DPZ: Dapagliflozin; **SGT:** Saxagliptin; **MTH:** Metformin hydrochloride; **$SGLT_2$:** Sodium-glucose co-transporter-2; **DPP-4:** Dipeptidyl peptidase-4; **USFDA:** United State Food and Drug Administration; **ICH:** International Conference on Harmonization; **SEM:** Simultaneous Equation Method; **RDS:** Ratio Difference Spectroscopic method; **DRZC:** Derivative Ratio Spectrum-Zero crossing Method; **DDRS:** Double Divisor Ratio Spectra Derivative Method.

SUMMARY

Qternmet XR®, consisting of dapagliflozin (10 mg), saxagliptin (5 mg), and metformin hydrochloride (1000 mg), improves glycemic management in diabetics (type 2). This paper proposes four quick, easy, accurate, and reproducible spectrophotometric procedures for assessing ternary mixtures simultaneously. Firstly, measured absorbance at 223, 212, and 232.6 nm for dapagliflozin, saxagliptin, and metformin hydrochloride in simultaneous equation approach. The second method, ratio difference spectroscopy, measures the amplitude difference at two different wavelengths. Third approach uses derivative ratio signals at zero-crossing sites. The fourth methodology is the double divisor-ratio spectra derivative approach, which quantifies the concentrations of all 3 medicines in combination using amplitudes at first derivative of ratio spectra. All three medications showed excellent linear correlation in concentration series of 2-10 µg/mL for simultaneous equation approach and 0.5-10 µg/mL for all other approaches. The proposed methodologies were authenticated according to ICH strategies and showed good precision, accuracy, and sensitivity. Because they do not require expensive solvents or specialized instruments, the new spectrophotometric techniques are considered cheaper than conventional analytical procedures. Thus, the proposed approaches might be used to assess dapagliflozin, saxagliptin, and metformin hydrochloride in a ternary mixture.

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