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Chemometrics assisted UV spectrophotometry for the quantitative determination of metformin and sitagliptin in commercial sample compare to HPLC analysis

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Abstract

In this study, simple, quick, accurate, precise, and eco-friendly spectrophotometric methods were proposed for the simultaneous analysis of metformin (MET) and sitagliptin (STG) in prepared binary mixtures and their combined dosage form. These proposed methods were based on partial least squares (PLS) and continuous wavelet transform (CWT). The mean recovery and root mean square error (RMSE) of the PLS approach were 101.11%, 100.37% and 0.231, 0.963 for MET and STG, respectively. The CWT was over the concentration range of 6–12 and 5–160 μg/mL with a coefficient of determination (R^2) of 0.9987 and 0.9999 for MET and STG, respectively. The Daubechies (Db2) and Symlet (Sym3) wavelet families with wavelengths of 236 nm and 245 nm were considered the best families for MET and STG, respectively. The limit of detection (LOD) and limit of quantification (LOQ) of CWT were found to be 0.4081, 2.6094 μg/mL and 1.0612, 5.3023 μg/mL for MET and STG, respectively. The mean recovery percentage was 99.91 and 100.16, for MET and STG, respectively in mixtures. The analysis of variance (ANOVA) test confirmed that there are no significant differences between the proposed and high-performance liquid chromatography (HPLC) methods, which can be used for routine simultaneous determination of MET and STG in commercial tablets.

Keywords: *Spectrophotometry, Partial least squares, Continuous wavelet transform, Metformin, Sitagliptin*

1. Introduction

A chronic metabolic syndrome is diabetes mellitus (DM) and its characterization is hyperglycemia, which has become an epidemic all over the world. DM can lead to serious complications such as kidney failure, limb amputation, blindness, and cardiovascular disease [1]. Type 1 diabetes (T1D), type 2 diabetes (T2D), and gestational diabetes mellitus (GDM) are three main kinds of DM. Diabetes is defined as a metabolic change caused by a defect in insulin secretion or insulin function. Finally, it causes persistent hyperglycemia and various physiological changes [2].

There is insulin resistance in Type 2 diabetes that causes abnormally high blood sugar levels [3]. Disorders in pancreatic beta cells cause type 2 diabetes, which prevents a person from being able to use insulin [4]. Complications of type 2 diabetes involve hypertension and dyslipidemia, including low serum high-density lipoprotein concentrations and high serum low-density lipoprotein concentrations [5].

For the first-line treatment of type 2 diabetes, a biguanide named metformin (MET) can be used as monotherapy and in combination with other glucose-lowering drugs [6]. It decreases intestinal glucose absorption, peripheral glucose uptake is improved, fasting insulin levels reduce, as well as insulin sensitivity increases [7].

Sitagliptin (STG) is the other drug for the therapy of patients with type 2 diabetes, which is an orally active, powerful, and selective dipeptidyl peptidase-4 (DPP-4) inhibitor. The function of STG is associated with an increase in the concentration of the active incretin hormone. After a meal, the reduction of postprandial glucose concentration and fasting glucose concentration is the responsibility of incretins, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [8,9].

Several techniques such as ion-pair reversed-phase high-performance liquid chromatography (RP-HPLC) [10], hydrophilic interaction liquid chromatography (HILIC) [11], HILIC-tandem mass spectrometry (HILIC-MS/MS) [12], ultra-performance liquid chromatography (RP-UPLC) [13,14], UPLC-MS/MS [15], high-performance thin-layer chromatography (HPTLC) [16] have been implemented for the simultaneous analysis of MET and STG with each other or with other components. The limitations of these methods comprise time-consuming, costly, complex, and excessive use of polluting solvents [17,18]. In addition, the use of separation and preconcentration methods is necessary for the low concentration of drugs in complex biological matrices. Two common techniques are solid-phase extraction (SPE) and liquid-phase extraction (LLE), which have been used in this case. The mentioned approaches possess many disadvantages [19,20]. Contrary to the mentioned methods, there are many reports about spectrophotometry for the simultaneous determination of components due to the simplicity of the process, availability of instruments, low cost, accuracy, and precision [21]. However, the presence of spectral overlap related to the drugs is its restriction. Therefore, improvement of spectral data is considered [22]. In order to solve and overcome the problem of overlapping components without prior separation, chemometrics-assisted UV spectrophotometric methods such as derivative spectrophotometry [23], partial least squares (PLS), principal component regression (PCR) [24], continuous wavelet transform (CWT) [25], and so on has been applied.

The PLS approach is usually named component-based structural equation modeling. Geladi and Kowalski proposed this linear statistical method. Unlike other techniques such as PCA, which evaluate the hyperplanes of maximum variance between the input and output variables, PLS places the input and output variables into a new

space. Afterward, it can acquire a linear regression approach in the projected space. PLS is known as a non-parametric way. According to the size of the sample, it is powerful and does not need any data normalization [26].

There are different types of mathematical trans shapes in wavelet transforms, which can be utilized to map a spatial (or time) series into a space-frequency (or time-frequency) domain. CWT can perform spatial or time series analysis with smooth and continuous changes in the wavelet domain. Compared to the Windowed Fourier transform (WFT) with a fixed-width window, a more efficient and accurate localization of variability is provided by CWT [27]. The decomposition of the spectrum into wavelet coefficients (WFs) can be performed using the mother wavelet function in CWT [28]. Lotfy et al. reported the simultaneous determination of STG and MET in their binary mixture using ratio difference (RD), ratio subtraction (RS), and a novel approach of induced amplitude modulation (IAM) methods [29]. Shokouhi et al. studied the simultaneous determination of MET and STG in anti-diabetic commercial tablets using artificial neural network (ANN) and least squares support vector machine (LS-SVM) [30].

This is the first study for the simultaneous estimation of MET and STG using PLS and CWT-assisted UV techniques in anti-diabetic drugs. These suggested procedures are simple, quick, inexpensive, and without a prior separation process. Also, the comparison was performed between the proposed methods and HPLC via analysis of variance (ANOVA). The importance of this study is the simultaneous determination of antidiabetic drugs in the quality control laboratories of pharmaceutical factories, which is cheaper and faster than the reference chromatography methods and does not require additional solvents that pollute the environment. This method can provide accurate results and according to the ANOVA test results, it is comparable to the reference method (HPLC).

2. Experimental

2.1. Chemicals

Pure MET and STG were obtained from Mahban and Murpen pharmaceutical companies, respectively. Zipmet tablets containing 500 mg MET and 50 mg STG were purchased from Abidi Co.

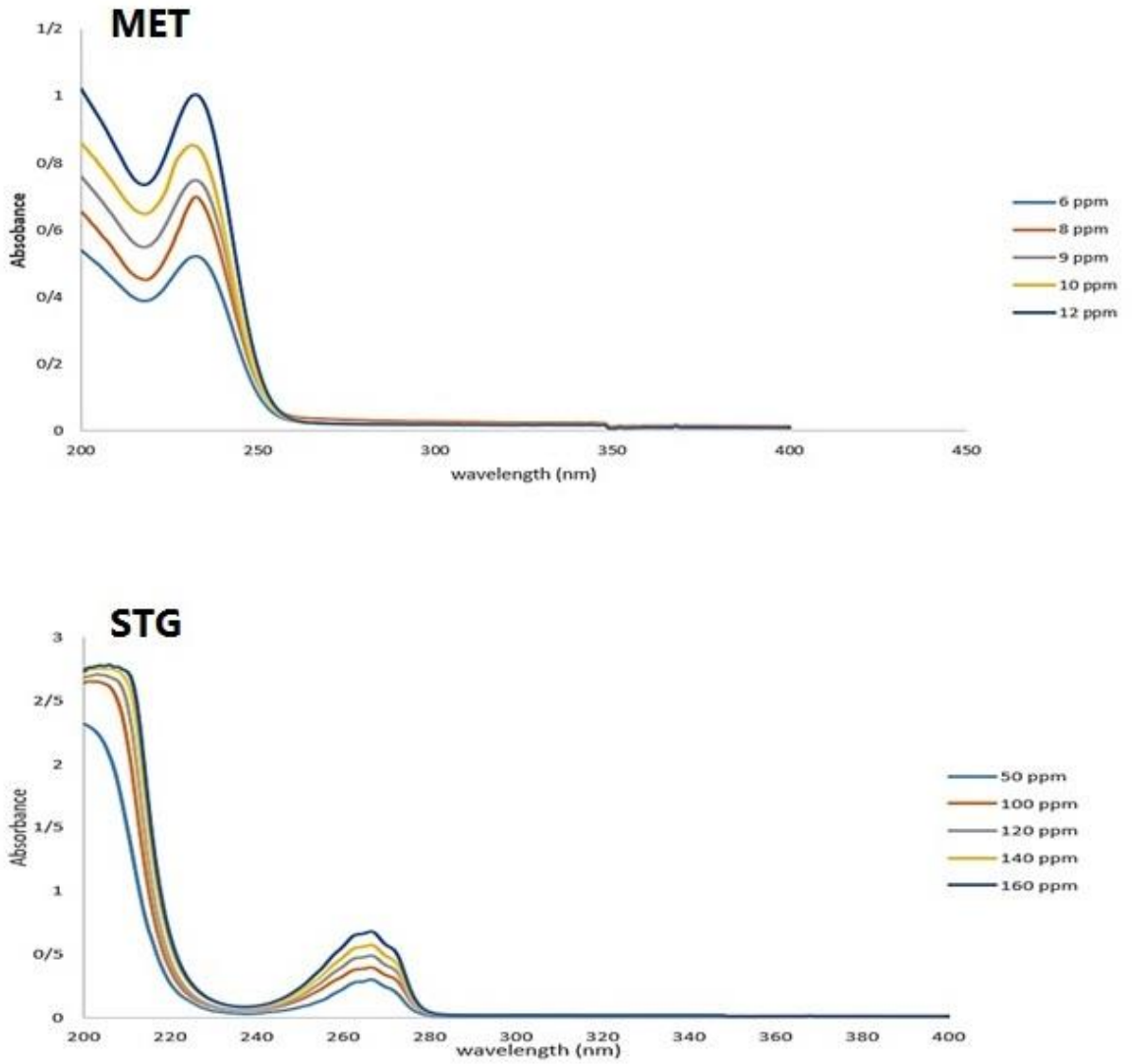
2.2. Instrument and software

A chromatographic system (Agilent 1260, USA) equipped with a mass detector was used. The stationary phase was the C18 column (250 mm, 4.6 mm, 5 μm). The mobile phase consisted of acetate buffer (pH=5) and acetonitrile (35: 65, v/v/v) at a flow rate of 1 mL/min. Measurement was carried out at a wavelength of 245 nm. An Optizen 3220 UV double beam UV–Vis spectrophotometer (South Korea) was utilized to record the absorption of solutions. MATLAB (Ver 8.6.0) (R2018b) was applied to write the program of PLS, as well as the usage of the CWT toolbox.

2.3. Preparation of standard solutions

At first, stock solutions of MET (100 μg/mL) and STG (200 μg/mL) were separately prepared. Then, standard solutions of MET and STG were made from stock solutions in the range of 6-12 and 50-160 μg/mL, respectively. Eventually, the absorption spectra of standard solutions related to both components were recorded by UV-Vis in

the range of 200-400 nm. The spectra UV-Vis related to the MET and STG at different concentrations are exhibited in Scheme 1.



Scheme 1. Spectra of different concentrations of MET and STG

2.4. Preparation of laboratory mixtures

Various concentrations of MET (6-11 µg/mL) and STG (50-90 µg/mL) from stock solutions were mixed together. These mixtures were used for modeling and validation. Their absorption was recorded by UV-Vis.

2.5. Assay of MET and STG in pharmaceutical formulation

Ten tablets of Zipmet were accurately weighed and finely powdered. An amount of this powder equivalent to one tablet was transferred into a 100 mL volumetric flask and 50 mL distilled water was added. Then, the solution was

stirred for 30 min at a rate of 50 rpm. After complete dissolution, the volume of the sample was reached to the mark with distilled water. Finally, dilution was performed to be within the appropriate absorption range. The absorbance corresponding to 3 repetitions was recorded by UV-Vis. The absorptions related to 3 repetitions of the real sample (Zipmet tablet containing MET and STG) analysis were entered into the MATLAB environment and the PLS and CWT methods were implemented separately on them for the prediction of MET and STG values.

2.6. CWT theory

The mother wavelet ψ starts and a family $\psi_{\tau,s}$ related to the "wavelet daughters" can be achieved through scaling and translating ψ (Eq 1).

$$\Psi_{\tau,s}(t) = \frac{1}{\sqrt{|s|}}\Psi\left(\frac{t-\tau}{s}\right), \qquad s,\tau \in R, s \neq 0 \tag{1}$$

Where s denotes a scaling or dilation factor, which adjusts the wavelet width and τ represents a translation parameter and its location control by this parameter. Scaling a wavelet is described by expanding it (if |s| > 1) or compacting it (if |s| < 1). On the other hand, translating indicates shifting its position in time.

Based on a time series x(t) ∈ L^2 (R) and wavelet ψ, its CWT is a function of two variables, $W_{x;\psi}$ (τ, s) (Eq 2).

$$W_{x;\psi}(\tau,s) = \int_{-\infty}^{\infty} x(t)\frac{1}{\sqrt{|s|}}\psi^*\left(\frac{t-\tau}{s}\right)dt \tag{2}$$

The position of the wavelet in the time domain and frequency domain is stated by τ and s, respectively. The wavelet transform can map the original series into a function of τ and s, which simultaneously express information of time and frequency. In the Fourier case, there is no time localization parameter, as well as cosine and sine functions instead of a wavelet function can be observed. These are essential differences between the wavelet transform and the Fourier transform [31].

3. Results and discussion

3.1. UV spectra

The spectra standard solutions of MET and STG are exhibited in Fig 1. The highly overlapped spectra can be observed, which prevents their direct simultaneous determination of components via different univariate spectrophotometric techniques. Therefore, chemometrics tools, including PLS and CWT were used for the quantification determination of the MET and STG. The previous study used CWT and LS-SVM to estimate trimethoprim (TMP) and sulfamethoxazole (SMZ) in Co-trimoxazole tablets simultaneously. The problem of UV–vis spectrophotometry was spectral overlap in determining TMP and SMZ. Hence, the chemometric-assisted spectrophotometric method was used to solve this problem [25]. In the other study, multivariate calibration methods, including PLS and principal component regression (PCR) along with spectrophotometry were proposed for the simultaneous determination of salmeterol and fluticasone in Inhalation Spray due to the specific overlap of components [32].

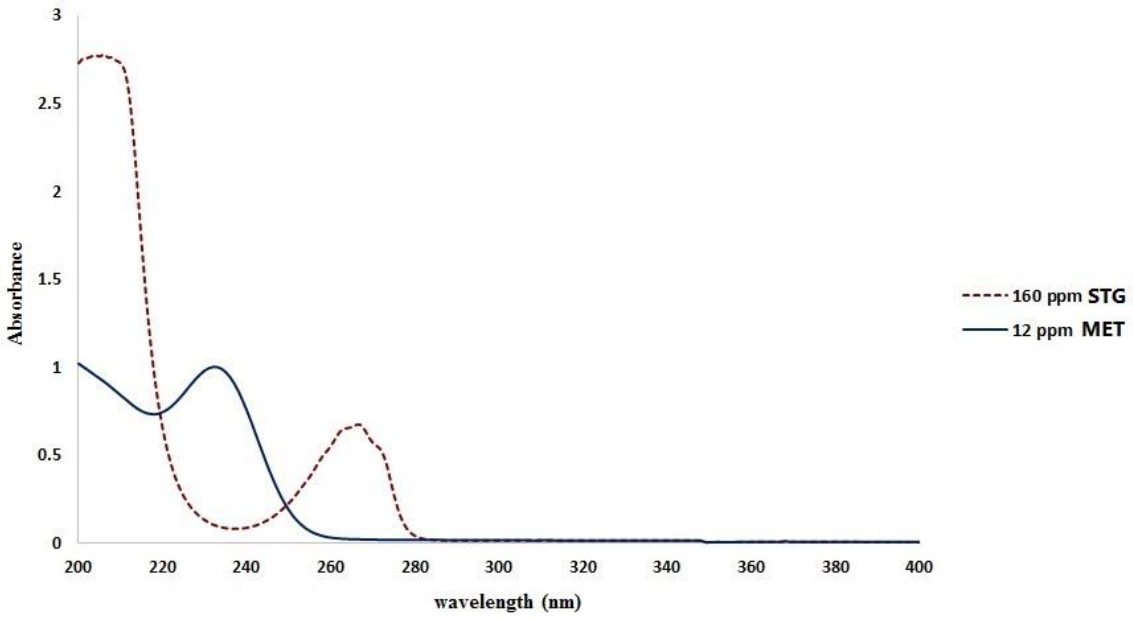


Fig 1. The absorption spectra of MET and STG

3.2. PLS method

Multivariate spectral calibrations are standard methods that are used for performing quantitative spectral analysis [33]. In the PLS model, it is assumed that a smaller set of latent variables that allow the elimination of undesirable alignment effects and optimize the content of the model information affect the experimental responses. First, the measured variables are normalized to eliminate the measured data units and to make the range of measurement changes uniform. Then, focus on the correlation between y and X in one p1 direction. Finally, the estimation problem can be resolved [34]. In order to perform the PLS method, the absorption of the mixtures was selected as the input matrix (X), which represents the absorption of 23 mixtures and 201 wavelengths. The target matrix (y) for both components represents the actual concentrations of each component (23×1). To determine the optimum number of components, the cross-validation (k-fold Cross-Validation) method was used. In k-fold cross-validation, the sample is randomly divided into k equal-sized subsamples, which are named "folds". From these k subsamples, a single subsample is maintained as the validation data for testing the model, and the k−1 remaining subsamples can be used for training set. Then, this process is repeated k times, with each of the k subsamples used once as the validation data. The optimum values were obtained by plotting the mean square error of prediction (MSEP) in terms of the number of components for each component (Fig 2 in supplement file). As shown in Fig 2S, the lowest errors were found to be 0.2655 and 19.3281 for MET and STG with the number of components of 6 and 2, respectively. To simultaneously determine MET and STG with this method, 14 mixtures were considered as training sets (65%) and 9 mixtures as test sets (35%) for the validity of the method. The predictive ability of this method was evaluated using these 9 mixtures by calculating the recovery percentage, mean recovery percentage, and root mean square error (RMSE) (Table 1 in Supplement file). Mean recovery was obtained in the range of 99.70% to 103.07% and 99.04% to 101.80% for MET and STG, respectively. Also, RMSE was lower than 1 for both components. These results indicate the acceptability of recoveries and errors for the cited drugs. The coefficient of determination (R^2) of 0.9966 (MET) and 0.9908 (STG) was obtained by plotting the predicted

values against the actual values, indicating the proximity of these values to each other (Fig 3). The non-scattering of points and their placement on a straight line confirms this subject. Also, the standard error of prediction (SEP) was found to be 0.088 and 0.590 for MET and STG, respectively.

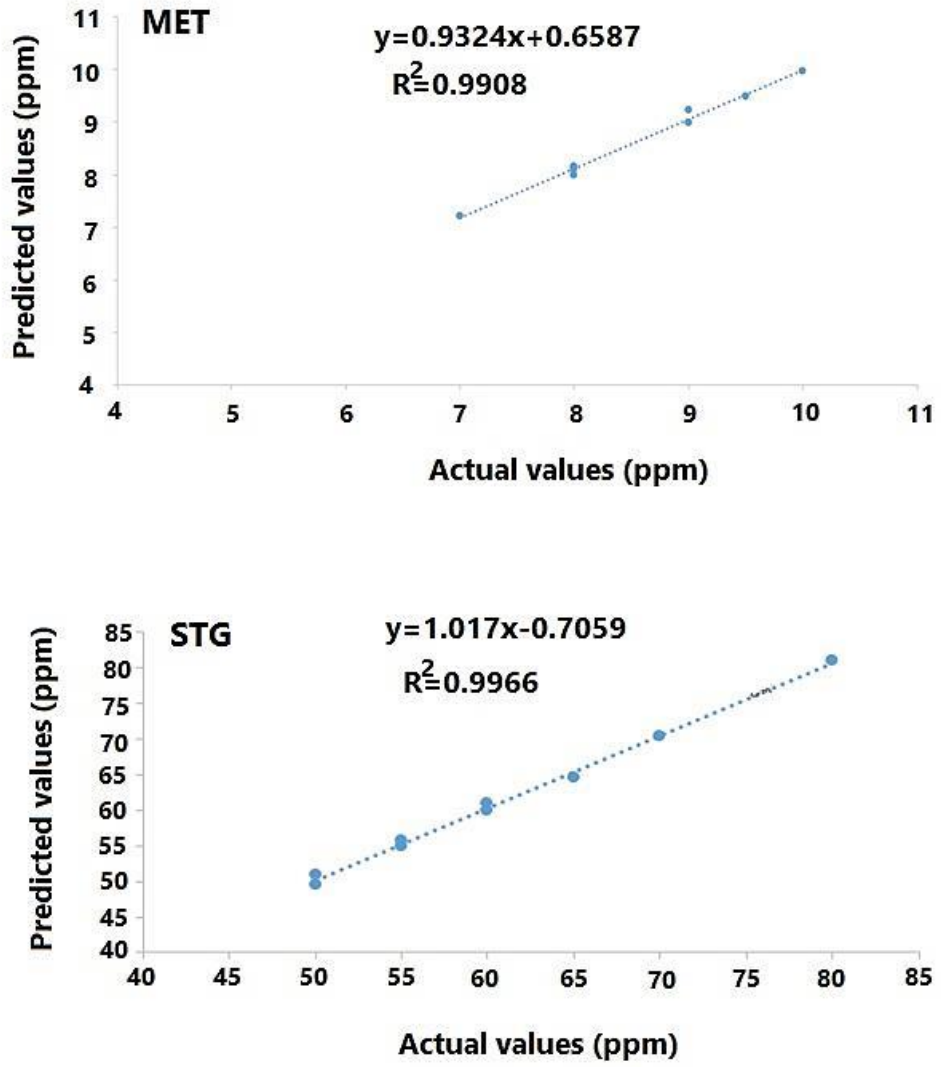


Fig 3. Actual concentrations versus predicted concentrations of MET and STG by PLS method

3.3. CWT method

The Excel file of absorption data obtained from the UV-Vis spectrophotometer related to the standard solutions was separately moved to the wavelet toolbox in MATLAB software and continuous wavelet 1-D section. In order to find the proper zero point of two components, various wavelet families were surveyed. Then, one mother wavelet family named Daubechies wavelet of the second-order (db2) was selected for MET with a high concentration of the standard (12 µg/mL). Afterward, this wavelet family was used for the STG as the other component with a high concentration of the standard (160 µg/mL). Eventually, 64 scales were considered to choose the best zero-crossing point. These steps were repeated for all other concentrations of standard solutions. The selection of the peak related to the MET when STG is the zero point or near zero is the basis of this method.

These steps were accomplished with the other family (Symlet (Sym3)) for STG. Afterward, by plotting the calibration curve of standard solutions in selected wavelet families, the R^2 of each component was investigated (Fig 4). The R^2 values of MET related to the Daubechies (Db2) with the scale parameter of 63 at 236 nm were 0.9987. Also, the values of R^2 corresponding to the Symlet (Sym3) family with a scale parameter of 49 at 245 nm were 0.9999 for STG. Figures of merit, including R^2, r, the limit of detection (LOD), and the limit of quantification (LOQ) for both components are given in Table 2. The LOD and LOQ values lower than 2.7 and 5.5 μg/mL, as well as the closeness of R^2 values to one, indicate the suitability of these families for analysis of MET and STG. Determination of LOD and LOQ parameters was accomplished using Eqs (3) and (4).

$$LOD = y_B + 3s_B \quad (3)$$

$$LOQ = y_B + 10s_B \quad (4)$$

Where y_B and s_B are the blank signal and standard deviations of the blank signal, respectively [35].

The initial spectrum of each standard solution was analyzed and resulting wavelet coefficients versus the wavelength were plotted. Therefore, a CWT spectrum of MET and STG was achieved (Fig 5 in Supplement file).

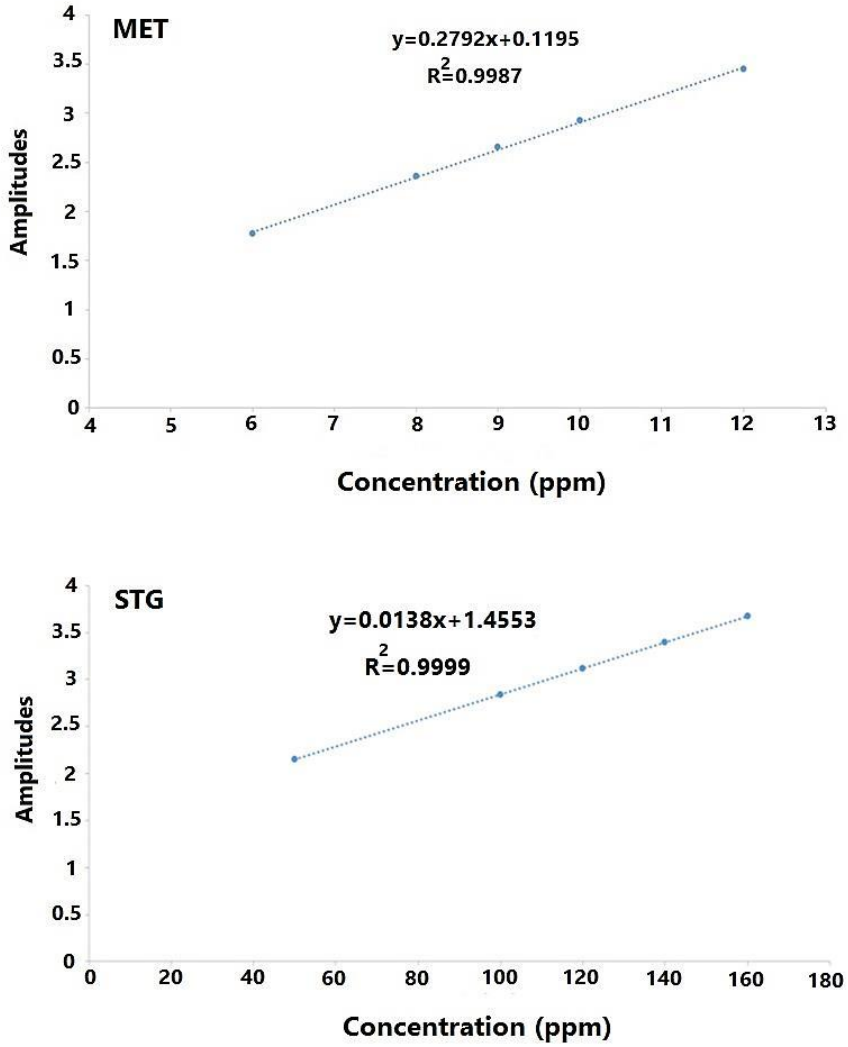


Fig 4. Amplitudes versus concentrations for MET and STG in CWT method

Table 2. The obtained statistical results of calibration standard solutions graphs by the CWT method

Parameters	MET	STG
Wavelength (nm)	236	245
Linear range (µg/mL)	6-12	5-160
Slope (a)	0.2792	0.0138
Intercept (b)	0.1195	1.4553
Regression Coefficient (R^2)	0.9987	0.9999
Correlation Coefficient (r)	0.9993	0.9999
LOD (µg/mL)	0.4081	2.6094
LOQ (µg/mL)	1.0612	5.3023

The validity of this approach was evaluated by 23 mixtures with various concentrations of MET and STG. Hence, the recovery percentage as an accuracy parameter, mean recovery, and RMSE values were calculated (Table 3). Acceptable recovery percentages of MET and STG were in the range of 99.17% to 100.94% and 98.72% to 101.54%, respectively. In addition, the appropriate efficiency of this method can be specified by the low RMSE values (MET=0.9932 and STG=0.4773). The R^2 values of mixtures corresponding to both components were studied by drawing predicted concentrations versus the actual concentrations (Fig 6). The proximity of values to one (MET=0.999, STG=0.9985) denotes the high model capability for the prediction of concentrations. Similar to this research, Ashrafi et al. proposed the determination of tamsulosin (TAM) and solifenacin (SOL) in synthetic mixtures, commercial formulations, and a biological sample by CWT and PLS [36].

Table 3. Obtained recovery, mean recovery, and RMSE by application of the CWT method in 23 synthetic mixtures

Added (µg/mL)		**Found (µg/mL)**		**Recovery (%)**	
STG	**MET**	**STG**	**MET**	**STG**	**MET**
50	11	50.37	10.99	100.74	99.90
50	10	50.42	10.03	100.84	100.30
50	9.5	50.53	9.48	101.06	99.86
50	9	50.77	8.93	101.54	99.22
50	8	50.77	8.02	101.54	100.28
55	10	54.29	10.02	98.72	100.29
55	9	54.4	8.95	98.90	99.44
55	8	54.51	7.99	99.10	99.91
60	10	59.51	10.02	99.18	100.29
60	9	59.56	8.99	99.26	99.91
60	8	59.67	3.24	99.45	99.77
60	7	59.77	7.06	99.61	100.94
60	6	59.88	5.98	99.80	99.66
65	9	64.94	8.99	99.90	99.98
65	8	65.15	8.04	100.23	100.56
65	7	65.26	6.97	100.40	99.57
70	9	70.45	8.93	100.64	99.22
70	8	70.53	7.95	100.75	99.48
70	7	70.63	6.94	100.91	99.17

80	8	80.58	8.01	100.72	100.24
80	7	80.63	7.01	100.79	100.14
90	7	89.88	7.01	99.86	100.25
90	6	89.94	5.99	99.93	99.83
Mean Recovery (%)				100.16	99.91
RMSE				0.4773	0.9932

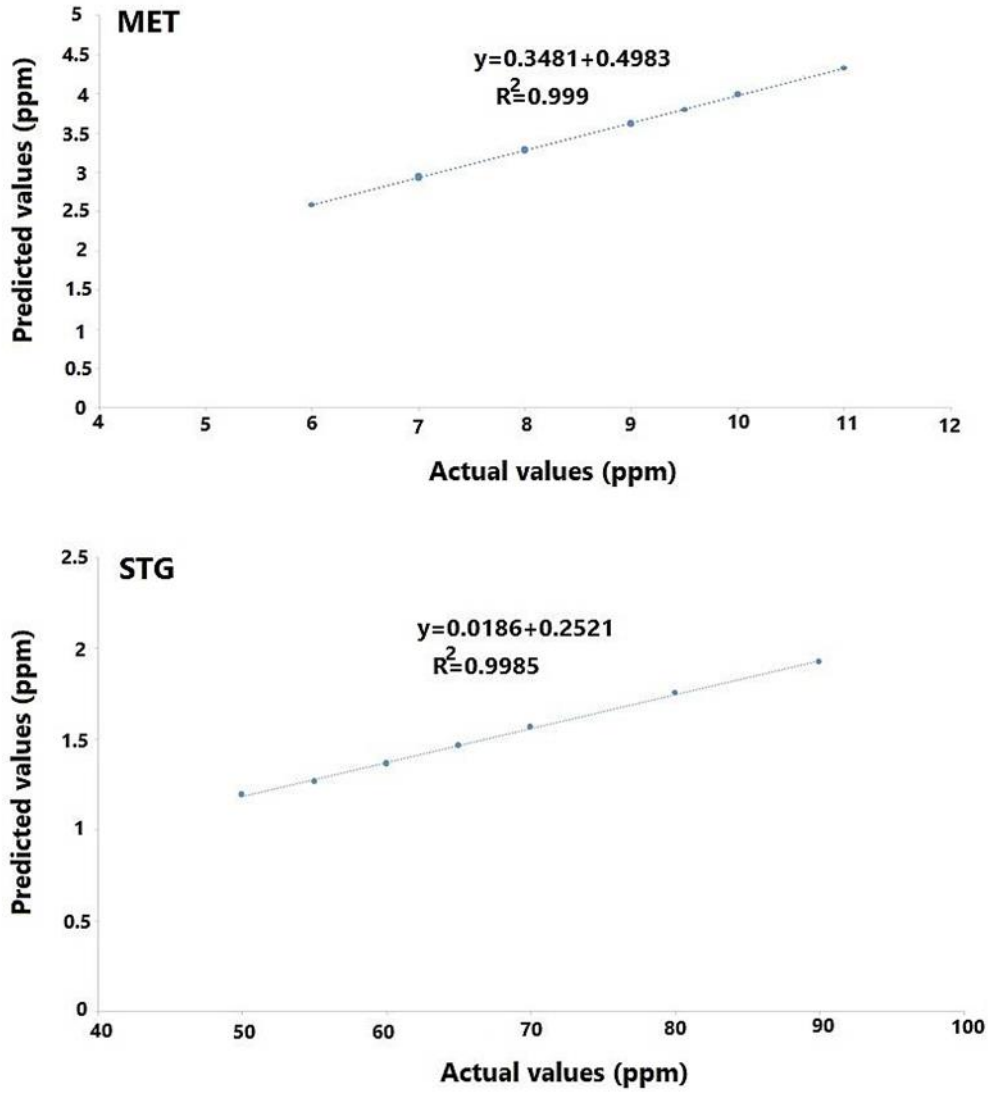


Fig 6. Predicted concentrations versus actual concentrations for MET and STG in CWT method

3.4. Tablet analysis using HPLC

Tablet (Zipmet) analysis was performed using HPLC as a reference technique. The sharp peaks and clear baseline separation are the properties of this chromatogram. As shown in its chromatogram (Fig 7), MET and STG retention times (RT) were 1.893 and 4.851 min, respectively. The excipients present in the Zipmet tablet did not have any interference with MET and STG peaks because no excipient peaks appeared in the chromatogram of the pharmaceutical formulation [37].

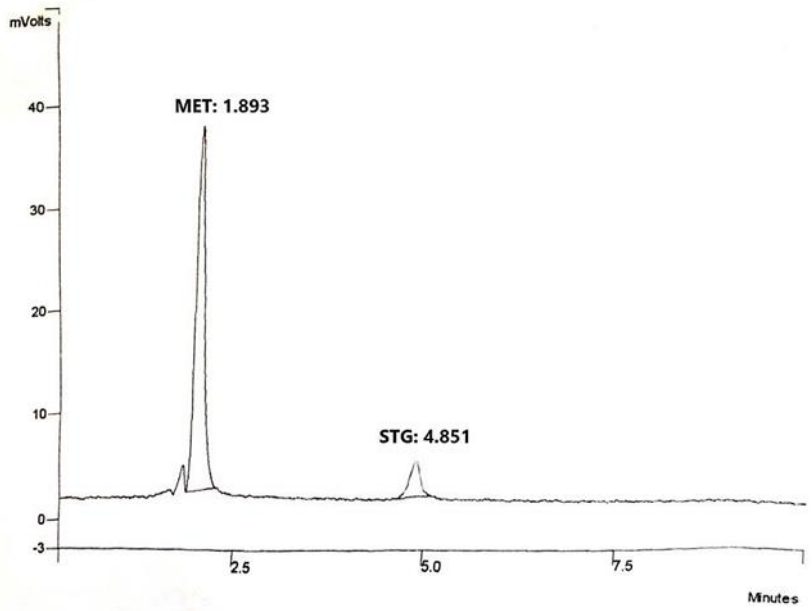


Fig 7. Chromatogram of the Zipmet tablet containing MET and STG

3.5. Comparison of spectrophotometry and chromatography

Analysis of Zipmet sample was done three times by spectrophotometric method and then chemometrics approaches (PLS, CWT) were implemented. Calculation of the mean recovery percentage and relative standard deviation (RSD%) for both components revealed the high performance of the proposed methods (Table 4). RSD values of PLS and CWT were obtained <0.9. The mean recovery of PLS was found to be 99.93% and 98.59% for MET and STG, respectively. In the CWT, this parameter was 99.36% and 97.23% for MET and STG, respectively. It can be seen that the results of chromatography and chemometrics are comparable with each other. Comparison between chemometric methods and HPLC reveals good coincidence. Although the HPLC technique is more specific than the chemometrics coupled with spectrophotometry it needs high-cost equipment and materials like HPLC-grade solvents and columns. On the other hand, chemometric approaches are less expensive and they do not need complicated instrumentation and any prior separation procedure. These methods require software for the resolution and determination of the drugs of the mixture [38].

Table 4. Results of analyzing Zipmet tablet by the proposed and reference methods.

500 mg MET and 50 mg STG in tablet formulation

	MET		STG	
Parameters	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
PLS	99.93	0.855	98.59	0.690
CWT	99.36	0.549	97.23	0.136
HPLC	101.10	0.494	96.86	0.412

The two methods were compared by ANOVA test to determine if there was a significant difference between the methods. Table 5 shows that the calculated values are smaller than the critical values, which confirms the absence of significant differences.

Table 5. Statistical analysis of the PLS, CWT, and HPLC methods using an ANOVA test

Source of variation	SS	df *	MS	F Calculated	F Critical
Between groups					
MET	0.0155555	2	0.0077777	0.1076923	5.1432528
STG	0.0822222	2	0.0411111	0.4683544	5.1432528
Within groups					
MET	0.4333333	6	0.0722222		
STG	0.5266666	6	0.0877777		
Total					
MET	0.4488888	8			
STG	0.6088888	8			

SS, sum of squares; df, degree of freedom; MS, mean squares.

* Degree of freedom for between groups: h-1; Within Groups: h (n-1); Total: hn-1; h, number of methods; n, number of samples of each method.

3.6. Comparison with other methods

The proposed methods (PLS and CWT) were compared to the other techniques in terms of recovery percentage, relative standard deviation (RSD%), and linear range (Table 6). It can be stated that the accuracy and precision of the suggested approaches are close to the other techniques. Also, the analytical instrumentations of the chromatographic reported methods are more complex. On the other hand, the spectroscopy method is fast and inexpensive.

Table 6. Comparison between suggested methods and other techniques

Method	Recovery (%)		RSD (%)		Linear range (μg/mL)		Ref.
	MET	STG	MET	STG	MET	STG	
HPLC	98.70	98.83	1.363	1.069	5-200	10-300	[9]
RP-HPLC	99.84	---	0.280	---	2.5-62.5	---	[10]
RP-UPLC	---	100.2	---	0.600	---	3.75-	[13]
UV-Vis+PLS	99.93	98.59	0.855	0.690	6-12	22.5	Present
UV-Vis+CWT	99.36	97.23	0.549	0.136	6-12	5-160	study
						5-160	Present study

3.7. Selectivity of the methods

As shown in Table 7, the effect of foreign species containing several ion species was studied to estimate both components. The results revealed that there was no significant measurable effect via the mentioned ionic species. The allowed concentrations of these interfering substances are higher than the concentrations of MET and STG, which represent a good selectivity between drugs and other species. Mentioned ions can be allowed at relatively high concentrations [39].

Table 7. Interfering effect of several species for the determination of MET and STG

Species	Tolerance limit [X]/ [MET]	Tolerance limit [X]/ [STG]
Li^{+}, Na^{+}, Mg^{2+}	300	500
Ca^{2+}, K^{+}	400	600
Cu^{2+}	250	170
Fe^{2+}	500	360
Fe^{3+}	200	80
NO^{3-}, SO_4^{2-}, CO_3^{2-}	100	400

4. Conclusion

The suggested methods (PLS, CWT) are simple, fast, inexpensive, and accurate. These allow the determination of MET and STG in laboratory-prepared mixtures and tablet samples with good accuracy and precision without initial separation. PLS and CWT possess many benefits over separation methods such as HPLC which are costly and require more sophisticated tools and treatment of data. In addition, the developed procedures are non-polluting to the environment compared to the reference method. ANOVA test did not show a significant difference between the reference method and chemometrics methods. The suggested methods are proper for routine quality control analysis of MET and STG in pharmaceutical preparations.

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