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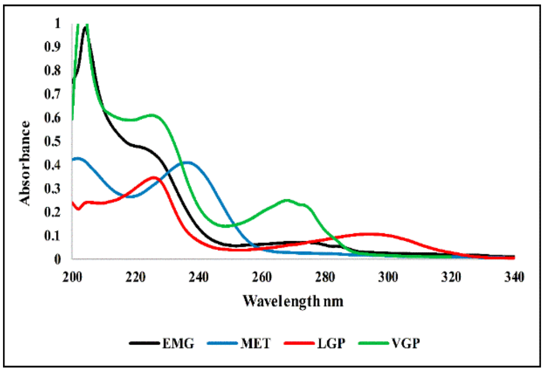

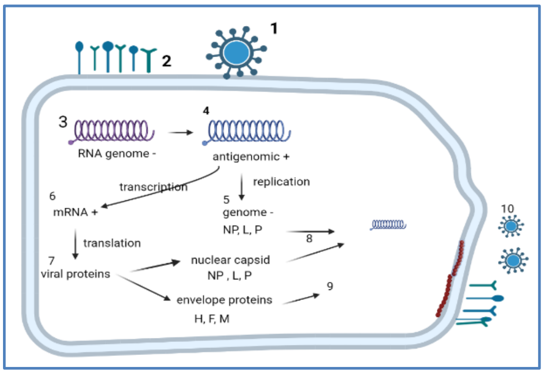

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Chemometric Assessment for Simultaneous Determination of the Quaternary Mixture

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Abstract

The current study aims to utilize two different chemometrics approaches as data assessment tools to analyze a quaternary combination in pure and pharmaceutical dosage forms including four antidiabetic medications, Metformin (MET), Empagliflozin (EMG), Vildagliptin (VGP), and Linagliptin (LGP). Both strategies were utilized to decide on the variables to extract crucial information and improve the accuracy of the procedures. The objective of this study is to determine the quantities of four components at the same time, both the Principle Component regression (PCR) and Partial Least Square (PLS-2) multivariate calibration techniques were effective. These methods eliminated the need for a prior separation step, making them ideal for pharmaceutical formulation analysis. The identification of the most advantageous spectral ranges and combinations was accomplished through careful consideration of various factors, including the minimization of the Root Mean Square Error of Calibration (RMSEC) values with the range of 0.0392 to 0.3366, the Root Mean Square Error of Prediction (RMSEP) values were within the range of 0.0419 to 0.3914, and the Relative Error of Prediction (REP) values were ranging from 0.2756 to 0.9591. These parameters were used to determine the optimal spectral regions that yielded the most accurate and precise results. No statistically significant variations in results were discovered between the suggested chemometric methodologies and the current official procedures. The provided methodologies offer a powerful tool for rapid and precise pharmaceutical formulation analysis, indicating their potential to improve quality control operations in the industry.

Introduction

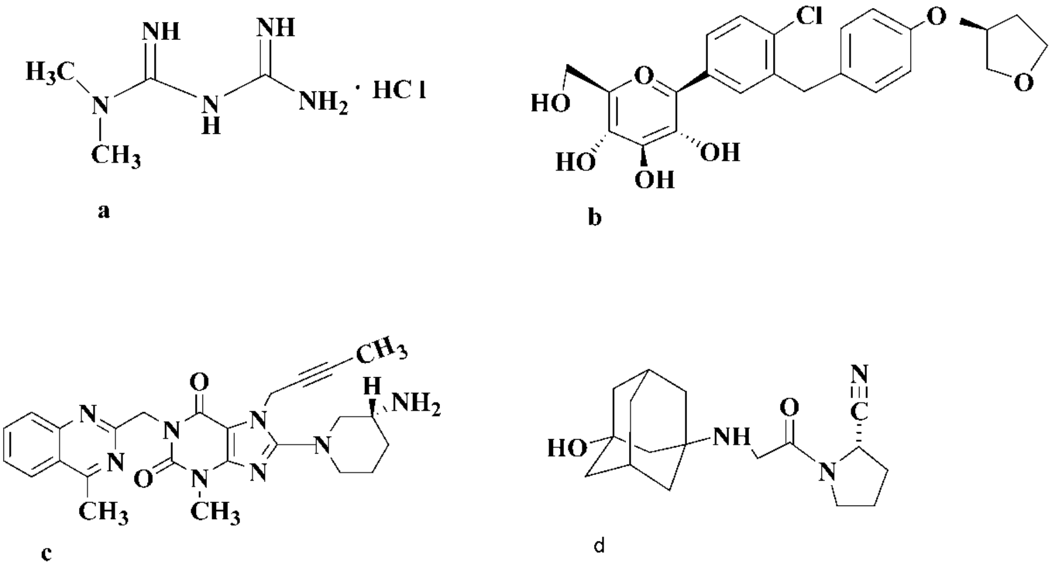
Type-2 Diabetes mellitus (T2DM) is a severe metabolic process condition with symptoms of high blood sugar levels triggered by reduced insulin production and elevated resistance to insulin. Despite the availability of a variety of oral anti-hyperglycemic medications and the efficacy of glucose-lowering treatment in the prevention and management of this disease and its associated issues [1].

Metformin (MET) (Fig.1.a), is a hypoglycemic drug belonging to the class called biguanide. It is chemically designated as 1,1-Dimethylbiguanide hydrochloride. It is commonly used for treating T_2DM with high efficiency in reducing fasting and postprandial blood glucose and decreasing glycosylated hemoglobin (HbA1c) in patients [2]. Patients with type 2 diabetes are advised to use metformin as their first-line medication, but the majority of them will eventually need additional treatments to keep their blood sugar levels under control. Early in the course of the illness process, maintaining strict glucose control may result in long-term advantages. Consequently, add-on combination therapy with two oral antidiabetic drugs may be helpful when metformin is unable to establish glycemic control [3].

Empagliflozin (EMG) is an efficient and selective sodium-glucose cotransporter 2 (SGLT2) inhibitor (Fig. 1b). (4-chloro-3-[4-[(3S)-tetrahydrofuran-3-yloxy]benzyl], is approved to treat type 2 diabetes. Empagliflozin lowers blood sugar levels in diabetes type 2 individuals in an insulin-independent manner by reducing kidney intake of glucose and raising glucose levels in urine secretion. [4,5].

Linagliptin (LGP) (Fig. 1c), 8-[(3R)-3-amino piperidine- 1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-ethylquinazolin-2-yl) methyl]-3,7-dihydro-1H-purine-2,6-dione]. Linagliptin promotes the release of insulin, suppresses the secretion of glucagon, and stops the inactivation of incretin peptides including glucagon-like peptide 1 (GLP-1) [6].

Vildagliptin, represented in Fig.1d, is chemically known as (S)-1-[2-(3-Hydroxyadamantan-1-ylamino) acetyl]pyrrolidine-2-carbonitrile. It serves as an orally administered, potent, and selective inhibitor of the enzyme dipeptidyl peptidase-4 (DPP-4). This compound plays a crucial role in enhancing the control of blood sugar levels in individuals with type 2 diabetes mellitus (T2DM). Vildagliptin's mode of action is its capacity to improve the function of both pancreatic alpha and beta islet cells. It accomplishes this by enhancing insulin secretion while simultaneously addressing the incorrect production of glucagon, which is common in T2DM patients. Vildagliptin improves glycemic control in type 2 diabetes patients by addressing these processes [7, 8].


a

b

c

d

Figure 1. Structure of (a) Metformin (MET), (b) Empagliflozin (EMG), (c) Linagliptin (LPG), and Vildagliptin (VGP).

Several techniques for evaluating metformin, empagliflozin, vildagliptin, and linagliptin in combination with other drugs or each other or alone were recorded in tablet pharmaceutical formulations, including spectrophotometry [9], different chromatographic techniques [10-21] in addition to derivative spectrophotometric [22-24], and different chemometric analysis [25, 26].

Chemometric methods have gained popularity due to their simplicity, lack of need for extensive extraction or separation steps, reduced time and chemical usage, and cost-effectiveness. These methods involve various mathematical and statistical techniques that enable the analysis of complex data sets, allowing the extraction of meaningful and relevant information. This has contributed to their increased adoption and applicability in various fields, including pharmaceutical analysis [27]. Multivariate calibration methods have evolved into an invaluable tool for analyzing data received from procedures. It is extremely beneficial for evaluating data that has a high range of variables. Multivariate calibration approaches, such as the Principle Component Regression (PCR) and Partial Least Square (PLS-2), have the benefit of being able to be used for complicated spectrum data without the need for separation [25, 26]. The use of multivariate calibration models for quality control of pharmaceutical formulations was demonstrated to be a viable alternative to univariate spectrophotometric and chromatographic approaches. The goal of this study is to develop devious chemometric approaches for detecting and quantifying MET, LGP, EMG, and VGP. According to the information presented, there are presently no documented procedures for detecting the concentrations of the four drugs in pure and dosage forms. This implies that the established approaches mentioned constitute a fresh and original strategy to concurrently analyse and quantify these four drugs in combination.

Theoretical background

PCR and PLS techniques

The PCR technique is similar to PLS in the way it accomplishes spectral destruction, but the mechanism is distinctive. In PCR, spectra are classified by the most significant deviation among spectral information, as well as concentration information is discarded; whereas, PLS models a combination of spectral and concentration data [28]. PLS is divided into two distinct categories: PLS-1 and PLS-2. The main distinction between PLS-1 and PLS-2 is in how signal handling and regression analysis take place. PLS-2 considers the whole set of factors for all components at the same time, and the model weights are updated accordingly. In contrast, PLS-1 computes the complete set of factors for one component at a time [28].

Experimental

Instrumental and software:

A UV-1800 PC double beam spectrophotometer (SHIMADZU, Japan) and Matlab with PLS toolbox v. 2.1 were used to perform chemometric calculations on the generated data.

Reagents and solutions

Prepared Samples

ApexBio Technology provided pure materials, including Linagliptin (purity >98% and CAS no. [668270-12-0]), empagliflozin (purity>98% and CAS no. [864070-44-0], metformin hydrochloride (purity >98% and CAS no. [1115-70-4]), and Vildagliptin (purity >98% and CAS no. [274901-16-5]). Pharmaceutical Glucophage® pills included 500 mg of metformin, Jardiance® tablets had 25 mg of empagliflozin, and Trajenta® tablets contained 5 mg of linagliptin, while Galvus tablets, each tablet contained 50 mg of vildagliptin. HPLC Grade Methanol was used.

Standard and Working Solutions

Four individual 100-mL volumetric flasks were fully loaded with 100 mg of each of the four chemicals MET, EMG, VGP, and LGP before being filled to the level with methanol to establish reference solution stocks of 1000 µgmL^{-1}. Working solutions were made from stock standard solutions by dilution processes in methanol to volumetric flasks of 50 mL containing (1.0-12, 2.0-30, 15-100, and 5.0-40 µgmL^{-1} MET, EMG, VGP, and LGP), and various ratios of laboratory-prepared mixtures were prepared from the corresponding stock standard solutions.

Procedures

Building of Calibration and Validation Set

For each of the four substances to be evaluated a five-level, four-factor calibration scheme [27] was implemented with five concentration limits. To test this concept, 25 different mixtures with varying MET, EMG, VGP, and LGP levels were constructed. Distribute varied portions of the individual standard reference solutions to several sets of 10 mL volumetric flasks filled with methanol to the desired volume. Fig. 2 illustrates zero-order absorption spectra acquired in the range of 200-350 nm.

Record the spectra of all calibration samples in the (210-320) nm range with a data interval of 1.0 nm and collect the absorbance spectrum data. To apply PCR and PLS to the data, the initial values of the calibrating data were then mean-centered with the statistical program SPSS [28], then leave-one-out was implemented as a cross-validating approach (LOO) [29]. Selecting an optimal number of variables using the proposed techniques is an important step to obtain accurate quantitation since collecting too many variables causes the data to become noisier. However, if the number of records preserved is excessively small, crucial information required for calibrating might get destroyed. To select optimal significant factors, Haaland and Thomas's criteria were selected [28]. The most prevalent method is LOO cross-validating, which ignores one pattern at a time. The whole process is repeated till every test sample is skipped just once. As indicated in Table 1, the ideal number of elements described by the generated models is 17 as a calibration set and 8.0 as a validation set for all components of PCr and PLS-2 approaches

Table 1. Composition of Calibration and Validation set of MTF, LGP, VGP, and EMG.

Mix No.	**Concentration (µgmL^{-1})**			
	MET	EMG	VGP	LGP
1	1	2	15	6
2*	1	6	30	12
3	1	10	45	18
4	1	15	60	24
5*	1	20	80	30
6	3	2	30	18
7	3	6	45	24
8	3	10	60	30
9*	3	15	80	6
10	3	20	15	12
11	5	2	45	30
12*	5	6	60	6
13	5	10	80	12
14	5	15	15	18
15	5	20	30	24
16*	7	2	60	12
17	7	6	80	18
18	7	10	15	24
19	7	15	30	30
20*	7	20	45	6
21*	9	2	80	24
22	9	6	15	30
23	9	10	30	6
24	9	15	45	12
25*	9	20	60	18
* Validating set				

Pharmaceutical Assay:

Ten tablets of Trajent, Glucophage, Galvus, and Jardiance were powdered. From each powdered tablet, 20 mg was accurately weighed and dissolved in methanol, then transferred into separate 100 mL volumetric flasks. The solutions were filtered after sonication to eliminate any undissolved or insoluble particles, resulting in clear solutions. Serial dilutions were done on the filtered solutions to obtain solutions with various concentrations. These dilutions were meticulously made to obtain analytical concentrations. To generate working solutions for each constituent, 10.0 mL aliquots were obtained from the serially diluted solutions. The concentrations of MET, EMG, VGP, and LGP in these working solutions were (3.0, 5.0, 20, and 10 µgmL^{-1}), respectively. Standard addition procedures were used to check the correctness of the suggested approaches. Excessive amounts of the usual medications (MET, EMG, VGP, and LGP) were added to the mixture to test the accuracy and dependability of the established procedures and known quantities of the standards were spiked into the samples.

Results and Discussion

To facilitate a concurrent quantifiable evaluation of the MET, EMG, VGP, and LGP in their combined dose form, two Multivariate Modelling were constructed and tested. Fig. 2 depicts the UV absorption spectra of MET, EMG, VGP, and LGP. Given the difficulties faced by these medications' overlapping spectra in the 210-320 nm spectral region, multivariable spectral study approaches were developed to separate this complicated spectral overlap. This problem was satisfactorily resolved by the chemometric techniques used, particularly PCR and PLS, which eliminated the need for any preparatory separation processes. These PCR and PLS calibration models were carefully constructed, tested, and then used for predicting unknown samples. To facilitate accurate multivariate calibration, a multilevel multifactor design, as described in reference [28], was chosen for the calibration set. To enhance the calibration process, this design technique takes into account a variety of aspects and levels. In multivariate calibration, a well-thought-out experimental design for the standard composition of the calibration set is critical to producing the most exact and dependable prediction.

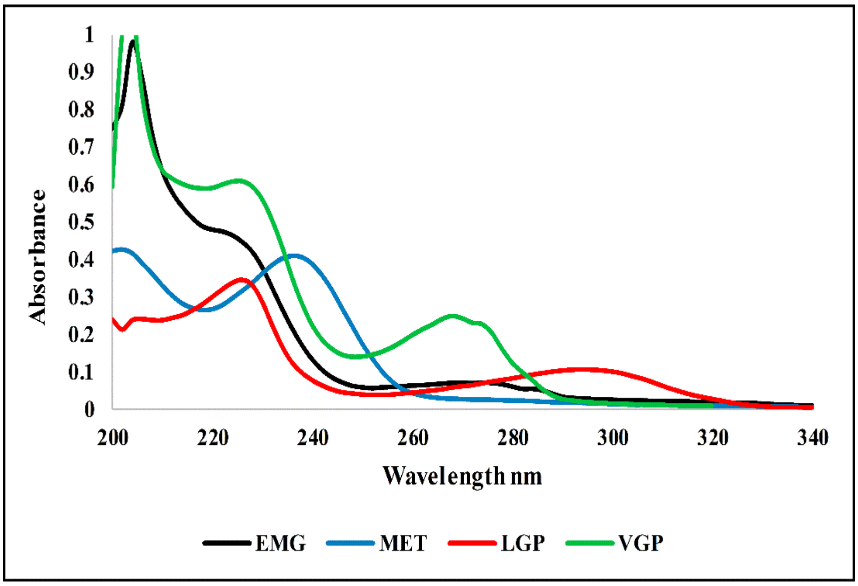


Figure 2. Zero-order absorption spectra of MET, EMG, LGP, and VGP

Optimization and Validation of PCR and PLS-2

The procedure to estimate the ideal amount of latent variables is critical since it aids in retaining only relevant and essential data for calibration before building PLS-2 and PCR models. There are several ways to determine the optimal number of latent variables [28, 29]. One such method is cross-validation, which is to eliminate one sample from the calibration set at a time [30]. Based on the Haaland and Thomas criteria [28], the optimal number of latent variables (LVs) contributing to data variance was determined to be six, as shown in Table 2. An external validation set was used to test the produced model for prediction, as illustrated in Table 3. The number of latent variables (LVs) in PCR and PLS-2 calibration models was improved using mean-centered data and left one-out cross-validation. The optimal number of LVs was 6 for both PCR and PLS-2 techniques, as illustrated in Figs 3. The produced model's prediction performance was assessed employing validation data specified by arranging real vs expected concentrations for each constituent. The root mean squares error of cross-validation (RMSECV) was determined, which displays the quality and reliability of expectations. Fig.3 depicts the relationship between RMSECV and variables. To validate the constructed calibration curves, an independent set of 25 mixture solutions was recorded depending on PCR and PLS-2, the measurements were constructed upon the zero-order absorption data in the spectrum of the wavelengths from 210-320 nm for the calibration set. The ideal number of variables was determined using cross-validation and 17 calibration spectra. The linear behaviors of the models were investigated at various levels of MET, EMG, VGP, and LGP throughout the combination solution between the ranges containing 1.0-12, 2.0-30, 15-100, and 5.0-40 µgmL^{-1}, correspondingly. The expected concentrations for each constituent were plotted against the true values to create calibration graphs, as shown in Figures 4 and 5. Table 2 shows the statistical characteristics of the two models, including the regression equations. The slope and intercept of each plot were all quite close to one. According to their correlation coefficients, each constituent showed a respectable linear connection, indicating an accurate linear correlation for all of the compounds. Table 2 highlights the findings from the statistical analysis (RMSEC, RMSEP, REP, R^2, intercept, and slope). The statistical variables used in the measurement step were examined and determined to be suitable for all of the suggested techniques.

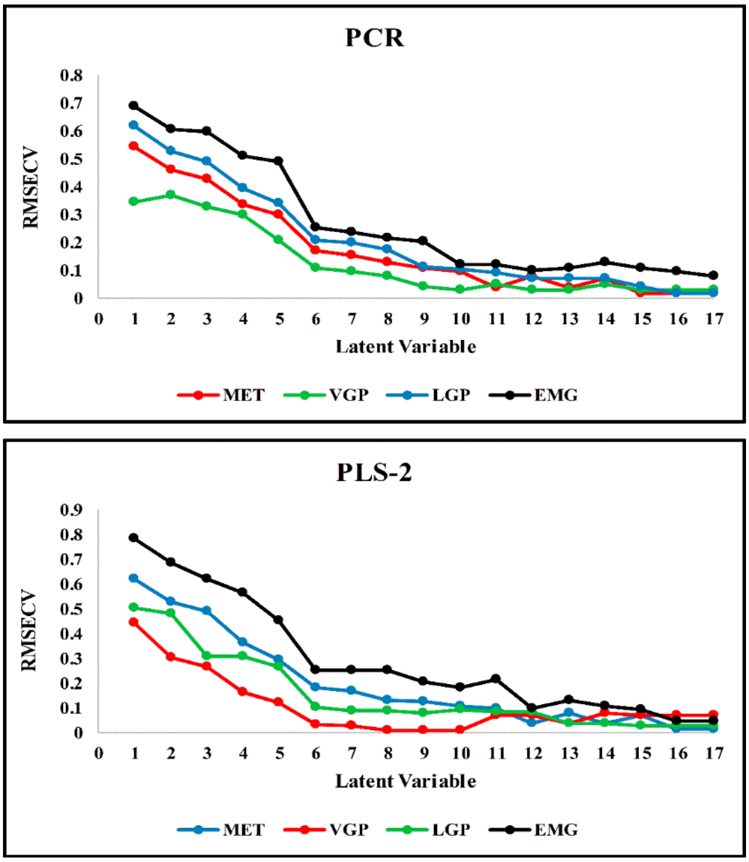


Figure 3. RMSECV plot of the cross-validation results of the calibration set as a function of the number of Latent Variables used for PCR and PLS-2 calibration.

Table 2 Statistical results for the optimized PCR and PLS-2 in the calibration step by using the cross-validation

Parameters	PCR				PLS-2			
	MET	**EMG**	**VGP**	**LGP**	**MET**	**EMG**	**VGP**	**LGP**
RMSEC	0.0505	0.0414	0.2679	0.0821	0.0438	0.0837	0.3661	0.0392
RMSEP	0.0540	0.0442	0.2864	0.0877	0.0468	0.0895	0.3914	0.0419
REP	0.9591	0.3632	0.4336	0.5746	0.8319	0.7347	0.5929	0.2756
R^2	0.9994	0.9999	0.9996	0.9999	1.0	0.9999	1.0	0.9999
Intercept(a)	0.0680	- 0.0759	-0.3478	0.0966	0.0034	-0.0318	0.0892	-0.0301
Slop(b)	0.9904	1.0080	1.0041	0.9953	0.9965	1.0054	0.9965	1.0011

Linearity ranges were contained (1.0-12, 2.0-30, 15-100, and 5.0-40 µgmL^{-1} MET, EMG, VGP, and LGP), respectively.
RMSEC: Root Mean Square Error of Calibration
RMSEP: Root Mean Square Error of Prediction
REP: Relative Error of Prediction

Table 3 Recovery results obtained for the determination of four components in the validation set using PCR and PLS-2 chemometric techniques.

Mixture (µgmL^{-1}) MET:EMG:VGP:LGP	Recovery (%)							
	PCR				PLS-2			
	MET	**EMG**	**VGP**	**LGP**	**MET**	**EMG**	**VGP**	**LGP**
1:6:30:12	101.00	101.66	99.34	99.83	101.70	99.95	100.03	100.86
1:20:80:30	99.30	100.35	100.12	99.60	99.83	100.85	99.49	99.96
3:15:80:6	103.30	100.67	99.99	99.81	100.37	100.01	99.88	98.16
5:6:60:6	100.32	99.80	100.31	102.70	100.32	99.82	99.72	101.00
7:2:60:12	98.57	99.50	100.32	99.97	100.42	99.50	99.83	99.75
7:20:45:6	99.95	100.20	99.96	101.85	99.95	99.60	99.82	97.84
9:2:80:24	100.20	95.45	99.99	100.42	99.90	99.45	99.94	99.63
9:20:60:18	100.12	100.54	98.34	99.972	100.13	100.95	99.75	100.56
Mean	100.35	99.78	99.79	100.52	100.32	100.02	99.80	99.72
SD	1.4049	1.8617	0.6688	1.1091	0.5993	0.5864	0.1604	1.1721
RMSEP	0.0540	0.0442	0.2864	0.0877	0.0468	0.0895	0.3914	0.0419

Pharmaceutical Assay

The validation findings indicated reliable estimations of MET, EMG, VGP, and LGP utilizing the PCR and PLS-2 procedures. The recoveries varied from 99.40 to 100.45 percent, while the RSD% readings were all less than 2%. The accuracy of the proposed PCR and PLS-2 techniques was evaluated using the conventional addition method. Known amounts of MET, EMG, VGP, and LGP standard solutions were added to the prescription formulation sample. This shows that the suggested methodologies for assessing the amounts of these chemicals in prescription pharmaceutical formulations are accurate and trustworthy. Furthermore, the lack of excipient interference confirms the procedures' accuracy, as illustrated in Table 4.

Statistical Analysis

Table 5 demonstrates that the proposed methods' results were statistically compared to the methods reported [20, 31], the computed student's and F- tests were obtained in terms of accuracy and precision; the results showed there is no significant difference discovered. The One-way ANOVA was used to compare the suggested techniques to the described method; the findings show that there was no significant difference, as shown in Table 6.

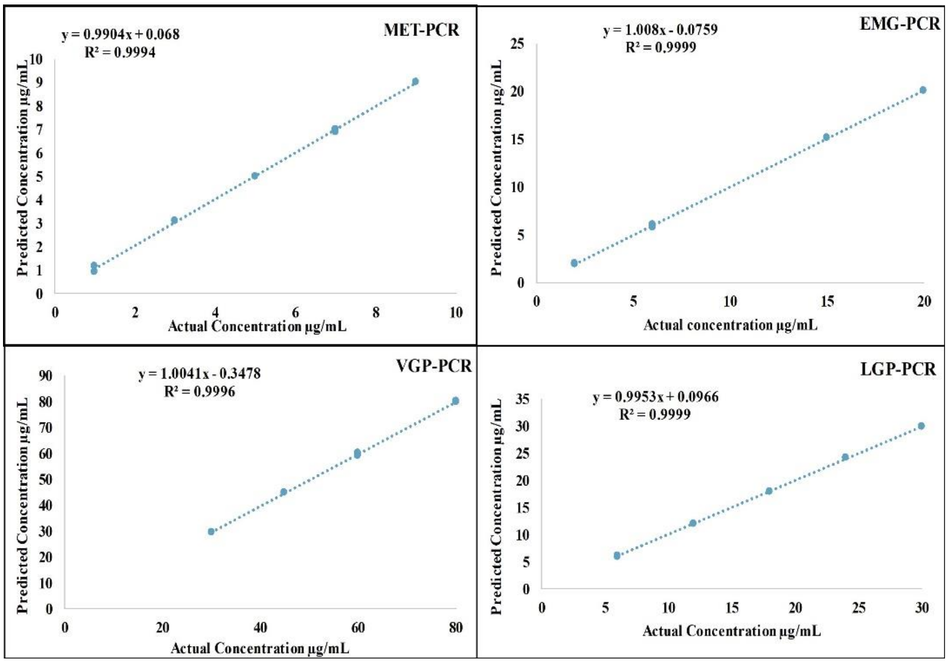


Figure 4. Calibration Curve of MET, EMG, VGP and LGP by PCR technique

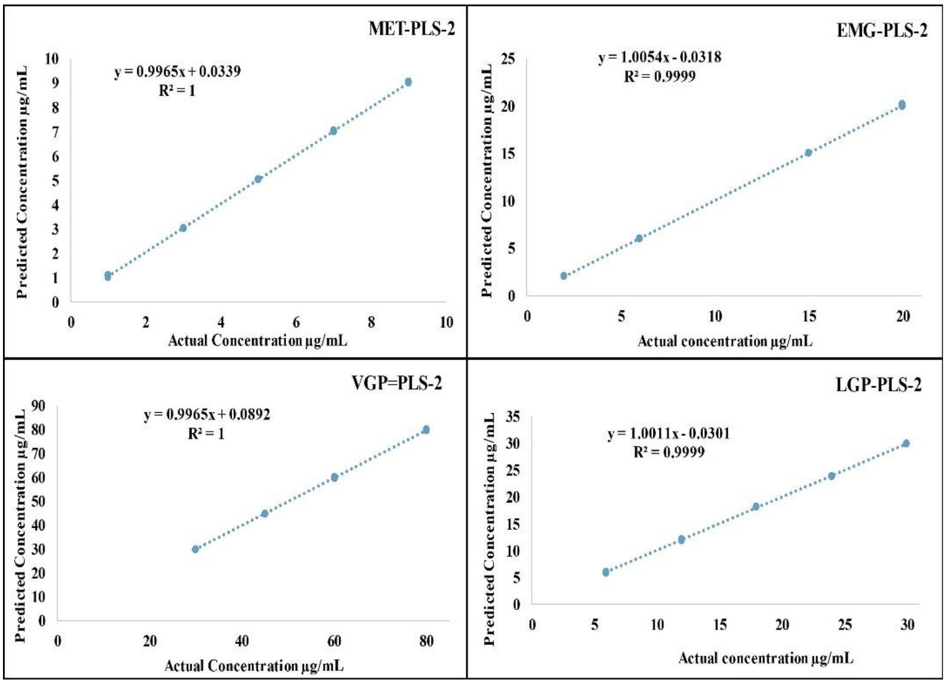


Figure 4. Calibration Curve of MET, EMG, VGP and LGP by PLS-2technique

Table 4 Determination of MET, EMG, VGP, and LGP in pharmaceutical formulation by PCR and PLS-2.

Drug	Taken µgmL^{-1}	*Recovery% ± RSD%		Standard Addition Technique ** Recovery% ± RSD% PCR	 PLS-2
MET	**3.0**	PCR	100.56±1.041	100.14±1.25	99.82±0.99
		PLS-2	99.56±1.21		
EMG	**5.0**	PCR	99.55±1.53	99.45±1.43	100.12±1.48
		PLS-2	99.62±1.41		
VGP	**20**	PCR	100.06±0.54	100.19±0.44	100.71±0.41
		PLS-2	99.40±0.91		
LGP	**10**	PCR	100.34±0.97	100.49±1.08	100.25±0.51
		PLS-2	99.83±1.61		

*average of five replication

**Average of three replication

Standard added (5.0 µg/mL, 3.0 µg/mL, and 5.0 µg/mL), (4.0 µg/mL, 8.0 µg/mL, and 12.0 µg/mL), (5.0 µg/mL, 10 µg/mL, and 20 µg/mL), and (5.0 µg/mL, 10 µg/mL, and 25 µg/mL) for, MET, EMG, VGP, and LGP respectively.

Table 5 Statistical comparison of the PCR and PLS-2 techniques with the reported determination of MET, EMG, VGP, and LGP in pharmaceutical formulations.

							Reported methods			
Drugs	**Techniques**	**Mean Recovery**	**RSD%**	**n**	**Student t-test (2.306)***	**F-value (6.39)***	**Mean Recovery**	**RSD%**	**n**	**Ref.**
MET	**PCR**	100.56	1.041	5	0.53	0.35	99.39	0.86	5	[20][a]
	PLS-2	99.56	1.21		2.01	0.55				
EMG	**PCR**	99.55	1.53	5	0.066	0.57	98.70	0.831	5	[20][a]
	PLS-2	99.62	1.41		0.82	0.27				
VGP	**PCR**	100.06	0.54	5	1.81	0.29	100.01	1.75	5	[31][b]
	PLS-2	99.40	0.91		0.98	0.26				
LGP	**PCR**	100.34	0.97	5	0.17	0.16	100.58	1.33	5	[20][a]
	PLS-2	99.83	1.61		0.26	0.73				

*Theoretical values of t and F at (P=0.05)

[a] Chromatographic separation was achieved on a Symmetry® AcclaimTM RSLC 120 C18 column (100 mm × 2.1 mm, 2.2 µm) applying an isocratic elution based on potassium dihydrogen phosphate buffer pH (4) - methanol (50:50, v/v) as a mobile phase. The ultraviolet detector was operated at 225 nm.

[b] In the first mixture, isocratic elution using a mobile phase of potassium dihydrogen phosphate buffer pH (4.6) - acetonitrile - methanol (30:50:20, v/v/v) at a flow rate of 1 mL min−1 with UV detection at 220 nm was performed. In the second method, isocratic elution based on potassium dihydrogen phosphate buffer pH (4.6) - acetonitrile (60:40, v/v) at a flow rate of 1 mL min−1 with UV detection at 210 nm was performed.

Table 6 Using one-way ANOVA to compare both PCR and PLS-2 techniques with the reported methods for determination of MET, EMG, VGP, and LGP in pharmaceutical formulations.

Parameters		**ss**	**df**	**ms**	**F-value *(3.88)**
MET	**Between-group**	0.8752	2	0.4376	0.4198
	Within group	12.5073	12	1.0422	
	Total	13.3825	14		
EMG	**Between-group**	1.4019	2	0.7009	0.4551
	Within group	18.4832	12	1.5402	
	Total	19.8885	14		
VGP	**Between-group**	1.4114	2	0.7057	0.4984
	Within group	16.9894	12	1.4157	
	Total	18.4008	14		
LGP	**Between-group**	0.1520	2	0.0760	0.1354
	Within group	6.7321	12	0.5610	
	Total	6.8841	14		

Conclusions

The suggested techniques have the advantage of being able to assess MTF, EMG, VGP, and LGP in quaternary mixes and medication dosage forms comprising them with no interference with each other or with other excipients. Multifunctional assessment algorithms were constructed utilizing spectral and determination matrix data, and the results were validated using a validation set of mixes of the four substances. As a result, these approaches have the potential to be used for routine quality monitoring of dosage forms for medicines incorporating substances.

Conflict of Interest

The authors declare no conflicts of interest regarding this manuscript's publication and/or funding.

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