GENETIC ALGORITHM WITH MODEL-UPDATING BASED PLS REGRESSION FOR THE SPECTROPHOTOMETRIC DETERMINATION OF CLOPIDOGREL, ATORVASTATIN, AND ASPIRIN IN PRESENCE OF ITS DEGRADATION PRODUCT **

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A novel spectrophotometric method is described, including multivariate regression/model updating, for the analysis of a quaternary mixture of clopidogrel, atorvastatin, aspirin, and its degradation product salicylic acid. The multivariate algorithms adopted are partial least squares with and without using a "Genetic Algorithm" for selecting variables. Upon updating both models, they could be effectively applied to determine the studied drugs in their pharmaceutical formulations. Similarly, clopidogrel and aspirin in their combined pharmaceutical preparations could be readily determined. Moreover, the proposed method could be extended to the determination of spiked salicylic acid as a minor component in aspirin raw material and dosage forms. The accuracy and precision of the proposed methods were approved through statistical comparison with the reported methods.

Keywords: *partial least squares, genetic algorithm, model-updating PLS, clopidogrel, atorvastatin, aspirin, salicylic acid.*

ГЕНЕТИЧЕСКИЙ АЛГОРИТМ С ОБНОВЛЕННОЙ РЕГРЕССИЕЙ ЧАСТНЫХ НАИМЕНЬШИХ КВАДРАТОВ ДЛЯ СПЕКТРОФОТОМЕТРИЧЕСКОГО ОПРЕДЕЛЕНИЯ КЛОПИДОГРЕЛА, АТОРВАСТАТИНА И АСПИРИНА В ПРИСУТСТВИИ ПРОДУКТОВ ИХ РАЗЛОЖЕНИЯ

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Предложен спектрофотометрический метод, включающий в себя множественную регрессию/обновление модели для анализа четверной смеси клопидогрела, аторвастатина, аспирина и продукта разложения — салициловой кислоты. Адаптированные многопараметрические алгоритмы представляют собой метод частных наименьших квадратов с использованием и без "генетического алгоритма" для выбора переменных. После обновления обе модели эффективно применены для определения исследуемых лекарственных средств в их фармацевтических составах. Аналогичным образом модели позволили легко определить клопидогрел и аспирин в их комбинированных фармацевтических препаратах. Метод может быть распространен на определение добавок салициловой кислоты как второстепенного компонента в аспириновом сырье и лекарственных формах. Точность и до-

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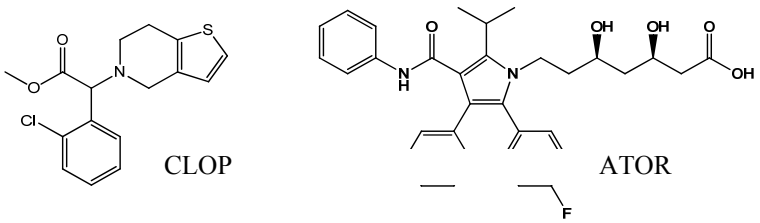
стоверность предложенных методов подтверждены статистическим сравнением с известными способами.

Ключевые слова: *метод частных наименьших квадратов, генетический алгоритм, обновление модели частных наименьших квадратов, клопидогрел, аторвастатин, аспирин, салициловая кислота.*

Introduction. Clopidogrel (CLOP) is a thienopyridine medication that is used to inhibit blood clot in various conditions, such as peripheral arterial diseases, coronary heart diseases, and cerebrovascular diseases. Its selective and irreversible action is due to inhibition of the adenosine diphosphate receptor [P2Y12] found in membranes of platelet cells [1]. Atorvastatin (ATOR) is an anti-hyperlipidemic drug that acts via inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. It acts predominantly in the liver, decreasing levels of hepatic cholesterol and plasma levels via decreasing hepatic cholesterol synthesis and increasing the catabolism of low-density lipoproteins (LDL) [2]. Aspirin (ASP) is a nonsteroidal anti-inflammatory drug. It is used to relief pain, treat hyperthermia, reduce inflammation, and prevent platelet aggregation. Its antithrombotic action is due to inhibition of the synthesis of thromboxane A_2 [3]. The ternary combination of the above three drugs is used for atherosclerotic patients suffering from various heart diseases. Clinical investigations showed that this combination therapy, when used in dyslipidemic patients with coronary heart diseases, decreased cardiovascular complications [4]. Salicylic acid (SAL) is the major degradation product of aspirin and is likely responsible for its anti-inflammatory properties via the suppression of COX genes [5].



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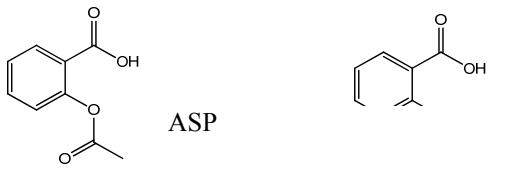

The literature review revealed several analytical methods for the simultaneous determination of CLOP, ATOR, and ASP in their ternary mixtures, such as spectrophotometry [6], HPTLC [7], and HPLC [8, 9].

To the best of our knowledge, no method has been yet reported for analyzing a quaternary mixture of CLOP, ATOR, and ASP together with its degradation product SAL.

Chemometrics is a chemical discipline that uses mathematical and statistical methods to overcome the problems of overlapping spectra of multicomponent mixtures. Chemometric techniques can improve the spectral information quality, resulting in a highly accurate and precise spectrophotometric technique. With this advantage; many analysts are encouraged regarding the use of different quantitative multivariate statistical techniques for the improvement of the analysis of different mixtures, mainly, principal component regression (PCR) and partial least squares (PLS). One of the major merits of the multivariate methods is the simplicity and rapid retrieval of data results, giving a much richer and realistic picture for analyzing a large number of samples in a short time. This contributes to increasing the importance of choosing the optimum technique for precise analysis practice [10, 11].

Simultaneous spectrophotometric analysis of the quaternary mixture containing CLOP, ATOR, ASP, and SAL is quite difficult to perform by classical spectrophotometric methods due to their severe spectral overlapping (Fig. 1).

Hence, the purpose of the proposed method is to create an accurate and precise multivariate regression algorithm for analyzing the studied drugs in their pharmaceutical preparations. In this work, the multivariate models are PLS and the genetic algorithm (GA) as an application of the variable selection procedure. Moreover, both models were updated to determine the studied drugs in their combined pharmaceutical formulations.

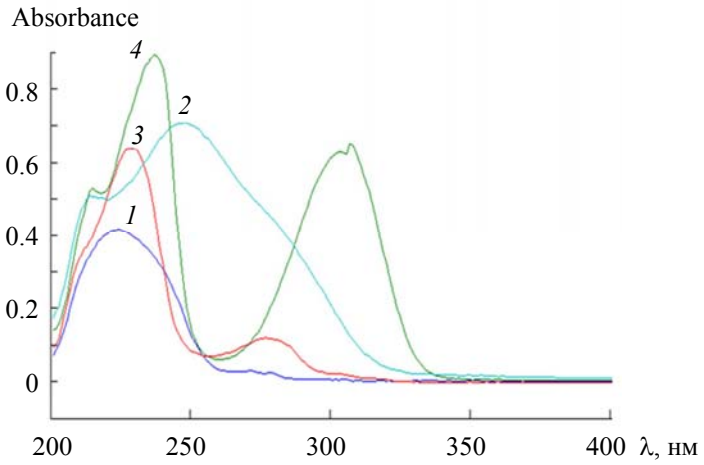

Fig. 1. Zero-order absorption spectra of (1) clopidogrel, (2) atorvastatin, (3) aspirin, and (4) salicylic acid in methanol (20 μg/mL each).

Experimental. Clopidogrel (Batch #:4901601001) was kindly supplied by Eva Parma Company, Cairo, Egypt. Atorvastatin (Batch #:V010217) was kindly supplied by Pfizer Egypt. Aspirin and salicylic acid as gift samples (Batch #:ER00003 and 156312474, respectively) were provided by Nile Company for Pharmaceutical and Chemical Industries, Cairo, Egypt. All drugs were used as received.

Plavix® tablets, claimed to contain 75 mg of clopidogrel per tablet (batch #: 6A919), is formulated by Sanofi Winthrop Industry, France.

Lipitor® tablets, claimed to contain 10 mg of atorvastatin per tablet (batch #: 6007), is produced by Pfizer Egypt, under license of Pfizer AG Switzerland, a subsidiary of Pfizer Inc., USA.

Aspirin Protect® tablets, tagged to contain 100 mg of aspirin per tablet (batch # BTAJG17 M), are produced by Bayer, Germany.

Myogrel Plus® tablets, tagged to contain 75 mg of (Clopidogrel and Aspirin) per tablet (batch #:1601272), is formulated by ADWIA Pharmaceutical Company, 10th of Ramadan City, Cairo, Egypt.

Methanol of HPLC grade was purchased from Sigma-Aldrich, Munich, Germany.

A UV-Visible dual beam spectrophotometer (1650, Shimadzu, Tokyo, Japan), loaded with UV-probe 2.1 software and matched quartz cells of constant bath length 10 mm, was used. Scanning occurred in the range of 200–400 nm with a sampling interval of 1 nm. An ultrasonic water bath (SS 101 H 230, USA) was used.

The Personal Spectroscopy UV-Probe 2.1 (Shimadzu) software was adopted in this study. Matlab R2013b (8.2.0.701) software was used for performing the wholly chemometric procedures. The PLS Toolbox software, version 2.1, was used for carrying out PLS and GA-PLS. Microsoft Excel, version 2010, were used for calculating the student's t-test and the variance ratio F-test.

Standard stock solutions of CLOP, ATOR, ASP, and SAL (100 μg/mL) were prepared in a 100-mL volumetric flask by dissolving 10.0 mg of the pure material in 60 mL of methanol added stepwise. This was sonicated for 15 min and then completed to the volume with methanol. Serial dilutions with methanol were applied for preparing the standard solutions for synthetic mixtures.

Procedures. Spectral features of the studied drugs. The zero-order absorption spectra of the studied drug solutions (20 μg/mL) were recorded against solvent blank over the wavelength range of 200–400 nm at an interval of 1 nm. The wavelengths selected were in the range of 230–320 nm, as shown in Fig. 1.

Experimental design for PLS and GA-PLS models. The calibration and validation sets were constructed based on multilevel multifactor design [12]. A calibration design of five levels (–2, –1, 0, +1, +2) and four factors was used to compute a total of 25 samples, which were prepared by applying serial dilution using a standard stock solution (100 μg/mL). The concentrations of each drug were selected relying on the range of linearity, the ratio of CLOP and ASP (1:1) in their pharmaceutical formulations, and the ICH guidelines regarding % of degradation product (SAL). The central (0) level of the experimental design was 21, 15, 21, and 5 μg/mL for CLOP, ATOR, ASP, and SAL, respectively, as shown in Table 1. The absorption spectra of the laboratory prepared mixtures were recorded over the wavelength range 230–320 nm with an interval of 1 nm, thus resulting in 91 data points. Pre-processing of the data showed the appearance of noise at wave-

lengths less than 230 nm, and wavelengths greater than 320 nm were rejected because the drugs do not have any absorbance in this spectral region.

TABLE 1. Concentrations of CLOP, ATOR, ASP, and SAL in the Calibration and Validation Sets

Mix. no.	CLOP	ATOR	ASP	SAL
1*	21	15	21	5
2	21	25	19	3
3	19	10	23	3
4	19	25	20	7
5*	23	15	23	4
6	20	10	21	7
7	23	10	20	5
8	21	20	20	4
9*	20	25	22	4
10*	20	20	23	6
11*	22	15	22	7
12	23	25	21	6
13*	22	25	23	5
14	21	5	23	7
15	23	20	19	7
16	23	5	22	3
17	19	15	19	6
18	22	20	21	3
19	19	20	22	5
20	21	10	22	6
21*	22	25	20	6
22	22	10	19	4
23	20	15	20	3
24	19	5	21	4
25*	20	5	19	5

The shaded rows* represent the validation set.

Data analysis was carried out by using the spectral data point and transferring this data to the Matlab® software to create PLS and GA-PLS. Seventeen mixtures were randomly chosen as a calibration set, and the remaining eight mixtures were used as a validation set to compute predictive benefits of the developed method.

Application to pharmaceutical preparations. Ten tablets were accurately weighed, finely powdered, and homogenously mixed; then an exactly weighed amount of the powder equivalent to 10.0 mg of the drug was transferred into a 100 mL conical flask, 60 mL of methanol was added, and the whole mixed, sonicated for 30 min, and filtered into a 100 mL volumetric flask and completed to the mark with methanol to produce stock solutions (100 μg/mL). Necessary serial dilutions of the stock solutions were made with methanol to get the corresponding different concentrations of the studied drugs covering the concentration range as shown in Table 1. Samples were analyzed using the proposed procedures mentioned before.

Results and discussion. The goal of this study was to create an easy, fast, and estimable chemometric assisted/spectrophotometric method for the estimation of the quaternary mixture of CLOP, ATOR, ASP, and SAL in their pharmaceutical formulations without previous tedious separation steps. Because their UV absorption spectra greatly overlapped (Fig. 1), the direct spectrophotometric analysis of this quaternary mixture is challenging; however, upon application of the multivariate techniques such as the PLS and GA-PLS models, the determination of the studied drugs in their quaternary mixture can be easily achieved. The evaluation and statistical comparison of the performance of the proposed method with the reported method was implemented [8].

PLS and GA-PLS models. PLS, as a multivariate chemometric model, is commonly used for the simultaneous determination of compounds showing severe overlapping in their absorption spectra [13], as it minimize the errors and improves the analytical power of the method. The measurement of each component oc-

curs at many data points in the wavelength range of the absorption spectra. PLS differs from classical least-squares (CLS) and (PCR) models in its capacity to use all spectral data information and analyte concentrations, resulting in a more accurate analysis process [14], while in PCR the absorbance matrix is only used. This means that PLS takes into account errors in both the concentration and the spectra, while PCR assumes that the concentration estimates are error free. GA are a natural and robust technique that can select the best subset of variables for our predictive model; they usually work better than traditional feature selection techniques; GA can manage data sets with many features and do not require specific knowledge of the problem under study. GA requires less time for some special application and permits more chances for getting optimal solutions. GA resolves the optimization problem by exploring all regions of potential solutions and utilizing confirming areas through mutation, crossover, and selection operations applied to individuals in populations. The problem of over-fitting can be avoided by applying a number of independent short trials, and the selected final model can be obtained based on the solutions of all trials.

Furthermore, the application of GA as a variable selection method allows selection of the most relevant variables and eliminates the irrelevant ones. It can improve the model quality [15–17]. Therefore, coupling of GA with PLS results in enhancement of the quality and model prediction capability. Moreover the enhancement of the predictive power of PLS model through data reduction and variable selection procedure was introduced [18]. The calibration model in PLS was designed between the component concentration and latent variables of data matrix. The linear combination was found between Latent variables formed using the concentration values and original ones. The selection of the number of factors is a critical issue in the PLS algorithm. It could be achieved by applying the cross validation (CV) method in which one sample was left out at a time using 17 samples as calibration set [19]. The PLS calibration was performed on 16 samples. By using this calibration, the concentration of the sample left out was predicted. This process was totally repeated for 17 times until leaving out was applied for each sample. The method created by Haal and Thomas [20] was applied to select the optimal number of factors. The model selection depends on choosing the smallest number of factors that result in an insignificant difference between the corresponding Root Mean Square Error of Cross-Validation (RMSECV) and the minimum RMSECV. The RMSECV was recalculated upon

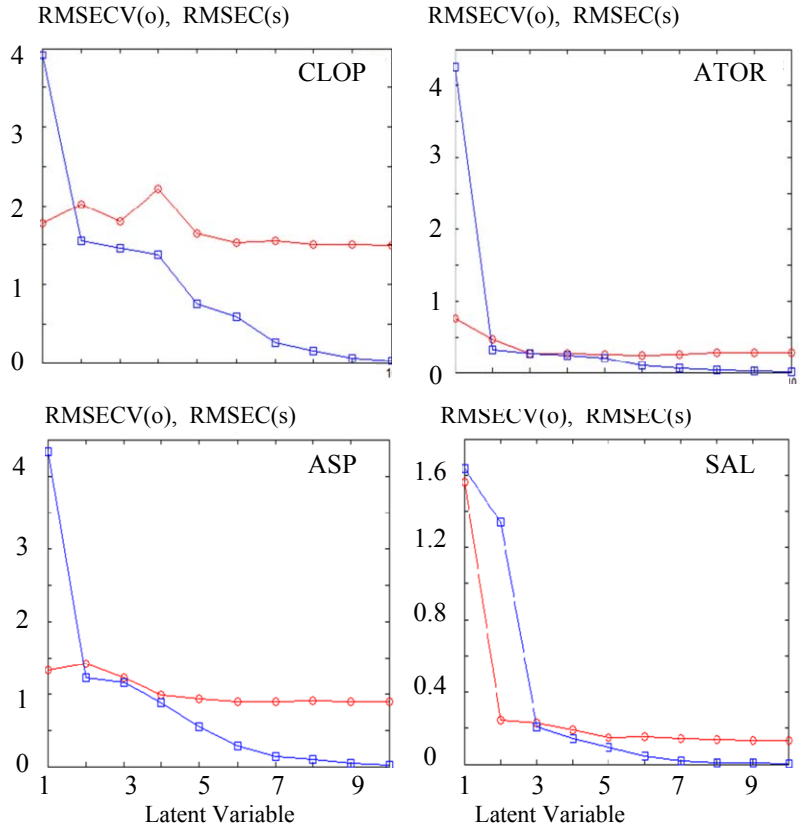

Fig. 2. RMSEC plot of the cross validation, results of the training set as a function of the number of principle components used to construct the PLS data.

addition of each new factor and checking the precision and the accuracy of the validation predictions. RMSECV values of different developed models were compared. The model with the smallest number of factors was chosen.

Six latent variables were found enough for PLS method of each of CLOP, ATOR, ASP and four for SAL to model the data as shown in Fig. 2.

The GA procedure allows improvement of the calibration quality. Selection of wavelengths was achieved in which irrelevant variables were excluded and the relevant one related to the studied component was selected [21].The parameters of GA was chosen and carried out on original data containing 91 variables for CLOP, ATOR, ASP, and SAL. Using a PLS with the maximum number of latent variables was determined by cross-validation on the model containing all the variables [22].The parameters of GA were studied and shown in Table 2.

TABLE 2. GA Parameters used for the Variable Selection Applied to CLOP, ATOR, ASP, and SAL Raw Data

Parameter	CLOP	ATOR	ASP	SAL
Population size	36.00	36.00	36.00	36.0
Maximum generations	59.00	97.00	100.00	100.00
Mutation rate	0.005	0.005	0.005	0.005
The number of variables in a window (window width)	1.00	2.00	1.00	3.00
Percent of population the same at convergence	80.00	50.00	80.00	80.00
Wavelengths used at initiation, %	50.00	10.00	30.00	15.00
Crossover type	Double	Double	Double	Double
Maximum number of latent variables	6.00	6.00	5.00	4.00
Cross validation	Random	Random	Random	Random
Number of subsets to divide data into for cross validation	10.0	4.00	10.00	10.00
Number of iterations for cross validation at each generation	2.00	2.00	2.00	2.00

TABLE 3. % Recoveries, Mean, %RSD, RMSEC, and RMSECV for CLOP, ATOR, ASP, and SAL in the Calibration Set by PLS and GA-PLS Models

SAL		ASP		ATOR		CLOP		Calibration
GA-PLS	PLS	GA-PLS	PLS	GA-PLS	PLS	GA -PLS	PLS	sets
102.46	101.31	100.07	101.67	100.51	100.37	98.36	98.01	1
97.88	99.64	99.67	98.75	101.79	100.57	102.56	103.71	2
97.5	96.92	100.39	98.77	99.82	100.24	98.61	101.45	3
98.63	97.23	100.15	98.95	99.52	99.79	100.72	101.68	4
98.99	97.61	99.92	100.3	101.26	101.53	100.89	100.46	5
99.21	97.33	100.16	99.34	98.85	99.72	98.11	99.86	6
100.25	101.49	99.65	100.62	100.58	100.02	102.67	102.83	7
101.51	102.31	99.75	99.92	98.15	100.54	97.59	98.5	8
99.97	100.78	99.54	102.87	99.54	98.98	98.09	98.00	9
99.5	101.05	100.11	101.77	97.21	100.08	97.97	99.94	10
99.76	99.78	100.49	99.59	100.99	100.99	99.88	102.51	11
103.51	100.59	100.4	101.35	99.26	99.21	98.83	98.19	12
101.86	102.95	100.01	98.18	99.50	100.13	102.53	102.77	13
103.56	102.75	99.87	99.94	101.02	99.95	100.70	101.1	14
99.17	98.24	100.2	100.36	100.99	100.19	98.33	98.42	15
98.45	99.46	99.26	97.3	99.97	100.08	102.12	102.85	16
97.66	98.56	100.49	100.69	99.39	96.74	98.61	98.37	17
99.99±1.94	99.88±1.98	100.01±0.4	100.02±1.4	99.90±1.18	99.95±1.02	99.8±1.8	100.51±1.9	Mean±%RSD
0.0539 [b]	0.1434 [a]	0.0693 [b]	0.2812 [a]	0.1221 [b]	0.0971 [a]	0.5093[b]	0.5906[a]	RMSEC
0.1476 [d]	0.1905 [c]	0.1681 [d]	0.8829 [c]	0.2384 [d]	0.2465 [c]	0.5146 [d]	1.5242 [c]	RMSECV

[a, b] Root mean square error of calibration (RMSEC) of PLS and GA-PLS, respectively.
[c, d] Root mean square error of cross-validation (RMSECV) of PLS and GA-PLS, respectively.

Applying GA reduces the absorbance data matrix to about 25, 15, 21, and 18% of the initial matrix for each of CLOP, ATOR, ASP, and SAL, respectively (23 variables for CLOP, 14 variables for ATOR, 19 variables for ASP, and 16 variables for SAL). These chosen variables were further used for carrying out the PLS model. The obtained results, including different statistical parameters of both models, are summarized in Tables 3 and 4.

The residuals of all samples were randomly scattered around zero for both models (PLS and GA-PLS) when the residual concentrations were plotted against the actual concentrations of the sample mixtures. A comparative study of both models indicated that the results of GA-PLS are better than PLS, as GA-PLS produces lower RMSEP and RMSECV values for CLOP, ATOR, ASP, and SAL, as well as decrease data complexity. This is possibly due to the fact that the irrelevant wavelengths have been excluded.

Validation of the developed models. An external validation step was used to examine the predictive capability of each model when utilized for the analysis of the studied drug samples. Table 4 shows the statistical treatment result, including root mean squares of the prediction error (RMSEP), and percent and mean recovery values, which proves the validity of the method. The RMSEP is calculated as follows:

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(y - y')^2}{n}},$$

where n is the validation sample number, y is the actual sample concentration, and y' is the predictable sample concentration in the validation or calibration set, respectively.

Model updating. The models can be simply updated by expanding the calibration set. In order for multivariate models to ignore uninformative variables, they must be samples having new variations in the calibration model, i.e., adding new samples (X_{new}) to the old calibration set (X):

$$\mathbf{X}_{\mathrm{upd}} = \begin{bmatrix} X \\ X_{\mathrm{new}} \end{bmatrix}, \quad \mathbf{Y}_{\mathrm{upd}} = \begin{bmatrix} Y \\ Y_{\mathrm{new}} \end{bmatrix}$$

The additional added number of samples can be too small for these samples to have sufficient weight comparable to the initial calibration set [23, 24]. First, we built a calibration model for the quaternary mixture of CLOP, ATOR, ASP, and SAL raw materials to determine all drugs in their pure form using the PLS-1 and GA-PLS models.

TABLE 4. % Recoveries, Mean, % RSD and RMSEP for CLOP, ATOR, ASP, and SAL in the Validation Set by PLS and GA-PLS

SAL		ASP		ATOR		CLOP		Validation
GA-PLS	PLS	GA- PLS	PLS	GA- PLS	PLS	GA-PLS	PLS	sets
98.14	96.91	101.07	100.79	100.26	101.68	101.11	102.28	1
100.27	99.27	99.92	99.04	100.96	101.36	99.72	102.02	2
101.38	101.04	97.58	97.97	100.05	100.29	102.95	103.23	3
100.84	100.2	97.73	96.93	99.16	100.15	99.25	100.14	4
100.73	99.61	101.33	99.13	99.87	102.93	102.85	103.34	5
99.02	99.41	99.07	95.04	100.16	99.67	97.99	99.69	6
101.93	102.42	97.78	100.51	99.24	99.13	99.22	98.55	7
98.08	98.4	102.66	102.3	97.85	98.65	98.51	98.58	8
100.05±1.46	99.66±1.66	99.64±1.9	99.10±2.00	99.69±0.94	100.48±1.4	100.2±1.9	100.98±1.96	Mean±%RSD
0.0725 [b]	0.0834 [a]	0.3856 [b]	0.4748 [a]	0.1122 [b]	0.2131 [a]	0.3767 [b]	0.4458 [a]	RMSEP

[a, b] Root mean square error of prediction (RMSEP) of PLS and GA-PLS, respectively.

When applying this calibration models to determine different pharmaceutical preparations, which include a single and co-formulated drug, and because of changes in the matrix during the determination of different pharmaceuticals, we need to update the calibration model to remove this effect and also eliminate interference of tablet excipients. The updating of the regression model was achieved by adding new variables to the calibration set. Thus, the model update produced satisfactory recoveries for determining CLOP, ATOR, ASP, and SAL in their pharmaceutical preparations and eliminates interference of excipients with high reproducibility, robustness, and minimal manipulation steps. The minimum number of samples required to efficiently update the developed models should be accurately determined. The influence of the number of samples added to the calibration set on RMSEP was determined for every developed multivariate calibration

model, as presented in Fig. 3. One to five samples consisting of 19–23 µg for CLOP, 5–25 µg for ATOR, 19–23 µg for ASP, and 3–7 µg for SAL in methanol were added to PLS. The predictive ability of the updated model was validated by external validation samples. Three samples were necessary to construct an efficient update of the model (Fig. 3). RMSEP is a diagnostic tool for examining errors in the predicted concentrations; it indicates both precision and accuracy.

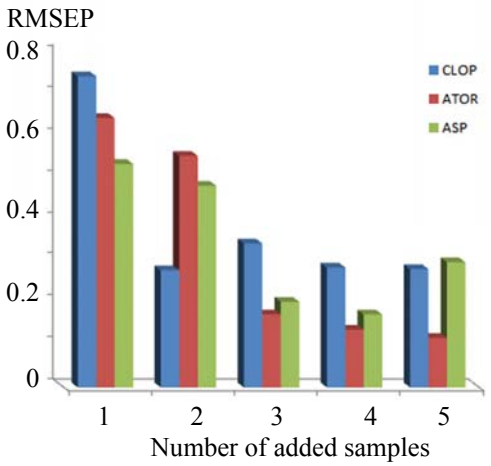

Fig. 3. Bar chart illustrating the influence of the number of samples added to the calibration set on RMSEP for each developed multivariate calibration model.

Pharmaceutical applications. The updated models were successfully used to determine CLOP, ATOR, and ASP in their pharmaceutical formulations, as well as for the determination of CLOP and ASP in their co-formulated tablets. The results showed that there was no interference from common tablet excipients such as talc powder, avisil, magnesium stearate, starch, gelatin, etc. The comparison method [8] was applied for both raw materials and pharmaceutical preparations, and the results obtained statistically proved the accuracy and precision of the proposed method [25], as shown in Tables 5 and 6. Also, the method was successfully applied for the determination of spiked SAL samples as a minor component in pure and dosage forms of ASP, as shown in Table 7.

TABLE 5. Determination of CLOP, ASP, and ATOR in Dosage Forms by the Proposed and Comparison Methods

Preparation	PLS	GA-PLS	Comparison method [8]
	Recovery, %	Recovery, %	Recovery, %
Plavix® tablets (CLOP 75 mg/tab.)	100.78 100.74 103.33 100.97 101.15	100.79 100.32 99.89 102.47 102.11	101.17 98.32 100.65
Mean ± S.D. % RSE *t* *F*	101.39 1.09 0.48 1.50(2.45)* 1.94(6.94)*	101.12 1.13 0.50 1.19(2.45)* 1.81(6.94)*	100.05 1.52
Aspirin Protect® tablets (ASP 100 mg/tab.)	100.87 99.04 99.48 97.10 96.84	99.8 99.63 99.86 97.15 96.79	99.04 101.33 99.49

Continue Table 5

Preparation	PLS	GA-PLS	Comparison method [8]
	Recovery, %	Recovery, %	Recovery, %
Mean	98.67	98.65	99.95
± S.D.	1.69	1.54	1.21
% RSE	0.76	0.70	
t	1.15(2.45)*	1.26(2.45)*	
F	1.95(19.25)*	1.62(19.25)*	
Lipitor® tablets (ATOR 10 mg/tab)	100.03	99.77	
	100.43	101.83	100.87
	101.42	98.36	98.90
	103.12	98.65	100.41
	102.20	103.02	
Mean	101.44	100.33	100.06
± S.D.	1.27	2.03	1.03
% RSE	0.56	0.90	
t	1.59(2.45)*	0.21(2.45)*	
F	1.52(19.25)*	3.88(19.25)*	

N o t e. Each result is the average of three determinations.
*The values in parentheses are the tabulated *t* and *F* values at $P = 0.05$ [25].

TABLE 6. Determination of CLOP and ASP in Co-formulated Tablet by the Proposed and Comparison Methods

Preparation	PLS	GA-PLS	Comparison method [8]
	Recovery, %	Recovery, %	Recovery, %
Myogrel Plus® (CLOP 75 mg /tab.)	98.59	98.85	
	100.57	99.48	101.02
	99.62	100.52	98.44
	96.74	98.58	99.92
	98.63	97.02	
Mean	98.83	98.89	99.79
± S.D.	1.42	1.28	1.29
% RSE	0.64	0.58	
t	0.99(2.45)*	1.04(2.45)*	
F	1.21(19.25)	1.02(6.94)	
Myogrel plus® (ASP 75 mg /tab.)	101.03	100.21	
	100.57	98.44	102.02
	100.26	100.47	100.44
	98.93	98.90	100.12
	100.87	98.83	
Mean	100.33	99.37	100.86
± S.D.	0.84	0.91	1.02
% RSE	0.37	0.41	
t	0.76(2.45)*	2.09(2.45)*	
F	1.47(6.94)*	1.26(6.94)*	

N o t e. Each result is the average of three determinations.
*The values in parentheses are the tabulated t and F values at $P = 0.05$ [25].

TABLE 7. Determination of Spiked SAL in ASP Raw Material and Dosage Form by PLS and GA-PLS Models

Conc. of ASP	Conc. of SAL	% ratio of SAL:ASP	% Recovery of pure ASP		% Recovery of SAL in ASP pure		% Recovery of ASP dosage		% Recovery of SAL in ASP dosage*	
			PLS	GA-PLS	PLS	GA-PLS	PLS	GA-PLS	PLS	GA-PLS
19.0	3.0	15.79	100.70	103.48	99.43	102.61	100.23	103.61	98.01	97.72
20.0	3.0	15.00	99.82	99.88	97.04	100.53	101.57	99.89	98.08	100.99
21.0	3.0	14.29	99.60	100.35	102.1	101.49	98.24	100.35	102.39	99.49
22.0	3.0	13.64	97.99	99.39	98.23	99.48	97.97	99.39	101.04	101.55
23.0	3.0	13.04	99.74	99.23	99.61	97.72	97.75	98.64	100.75	101.85
		Mean	99.57	100.47	99.28	100.37	99.15	100.38	100.05	100.32
		SD	0.98	1.74	1.88	1.88	1.67	1.92	1.94	1.71
		% RSD	0.99	1.73	1.9	1.87	1.69	1.91	1.93	1.71
		% RSE	0.44	0.77	0.85	0.84	0.76	0.85	0.86	0.76

*Dosage form is Aspirin Protect® tablets.

Conclusions. PLS is a robust multivariate calibration technique. A model built by using the whole spectrum may contain some irrelevant variables that may raise the chance of over-fitting and complexity of the model. A variable selection procedure, such as a genetic algorithm, has been applied to select the most informative variables and reject uninformative ones, which reduced the complexity of the method without affecting the predicting ability. Two chemometric models (PLS and GA-PLS) have been developed as effective methods to resolve quaternary mixtures of CLOP, ATOR, ASP, and SAL and its synthetic mixtures and pharmaceutical preparations. These methods can be operated using a simple spectrophotometer that is inexpensive, readily available, and needing no previous separation steps; hence, they reduce analysis time. When updating models, they can be successfully applied to determine the studied drugs in their pharmaceutical formulations. The results of the proposed and reported methods were statistically compared, confirming that no significant difference exists between them regarding accuracy and precision.

Conflict of interest. The authors confirm that there is no conflict of interest between them.

REFERENCES

1. M. J. Quinn, D. J. Fitzgerald, *Circulation*, **100**, 1667–1672 (1999).
2. K. Sharma, S. S. Hallan, B. Lal, A. Bhardwaj, N. Mishra, *Artif Cell. Nanomed. Biotechnol*., **44**, 1448–1456 (2016).
3. C. Battle, H. Hutchings, O. Bouamra, P. A. Evans, *PloS One*, **9**, e91284 (2014).
4. P. Wenaweser, S. Windecker, M. Billinger, S. Cook, M. Togni, B. Meier, A. Haeberli, O. M. Hess, *Am. J. Cardiol*., **99**, 353–356 (2007).
5. R. Amann, B. A. Peskar, *Eur. J. Pharmacol*., **447**, 1–9 (2002).
6. S. Singh, N. Dubey, D. Jain, *Asian J. Chem*., **3**, 885–887 (2010).
7. S. Londhe, S. Mulgund, R. Deshmukh, K. Jain, *Acta Chromatogr*., **22**, 297–305 (2010).
8. S. V. Londhe, R. S. Deshmukh, S. V. Mulgund, K. S. Jain, *Indian J. Pharm. Sci*., **73**, 23–29 (2010).
9. H. Kaila, M. Ambasana, A. Shah, *Int. J. Chem. Technol. Res*., **3**, 456–465 (2011).
10. P. Geladi, *Spectrochim. Acta, B, At. Spectrosc*., **58**, 767–782 (2003).
11. H. Khajehsharifi, Z. Eskandari, N. Sareban, *Arab. J. Chem*., **10**, S141–S147 (2017).
12. R. G. Brereton, *Analyst*, **122**, 1521–1529 (1997).
13. A. Abbaspour, R. Mirzajani, *J. Pharm. Biomed. Anal*., **38**, 420–427 (2005).
14. A. G. Frenich, D. Jouan-Rimbaud, D. Massart, S. Kuttatharmmakul, M. M. Galera, J. M. Vidal, *Analyst*, **120**, 2787–2792 (1995).
15. K. A. Attia, M. W. Nassar, M. B. El-Zeiny, A. Serag, *Spectrochim. Acta, A: Mol. Biomol. Spectrosc*., **170**, 117–123 (2017).
16. K. A. Attia, M. W. Nassar, M. B. El-Zeiny, A. Serag, *Spectrochim. Acta, A: Mol. Biomol. Spectrosc*., **156**, 54–62 (2016).
17. K. A. Attia, N. M. El-Abasawi, A. El-Olemy, A. H. Abdelazim, *Spectrochim. Acta, A: Mol. Biomol. Spectrosc*., **189**, 154–160 (2018).
18. R. Leardi, L. Nørgaard, *J. Chemom*., **18**, 486–497 (2004).

19. H. W. Darwish, S. A. Hassan, M. Y. Salem, B. A. El-Zeany, *Spectrochim. Acta, A: Mol. Biomol. Spectrosc*., **122**, 744–750 (2014).
20. D. M. Haaland, E. V. Thomas, *Anal. Chem*., **60**, 1193–1202 (1988).
21. T. Mahmood, K. H. Liland, L. Snipen, S. Sæbø, *Chemom. Intell. Lab. Syst*., **118**, 62–69 (2012).
22. R. Leardi, *Chemometrics*, **14**, 643–655 (2000).
23. N. E. Wagieh, M. A. Hegazy, M. Abdelkawy, E. A. Abdelaleem, *Talanta*, **80**, 2007–2010 (2010).
24. X. Capron, B. Walczak, O. De Noord, D. Massart, *Chemom. Intell. Lab. Syst*., **76**, 205–214 (2005).
25. J. C. Miller, J. N. Miller, *Statistics and Chemometrics for Analytical Chemistry*, Harlow, England, Pearson Education Ltd.; 39–73, 107–149, 256 (2005)