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Determination of Mixderm® Cream: A Comparative Study Applied on Quaternary Mixture

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Abstract

A comparative study for the validation of two analytical approaches applied for the simultaneous determination of betamethasone valerate (BV), tolnaftate (TF), gentamicin (GM), and clioquinol (CQ) formulated in mixderm® cream. The first approach was TLC-spectrodensitometric method, which was advanced by separating the four components on TLC aluminum plates using chloroform-methanol-acetic acid-formic acid (6:1:0.15:0.25, v/v/v/v) as a mobile phase, then scanned at 254 nm. The detection limit is in the range 0.07–0.48 mg mL−1. The second approach was the chemometric method using two models: partial least squares (PLS) and principle component regression model (PCR). The proposed approacheswere validated according to ICH guidelines were applied for the determination of the quaternary mixtures in their analytical mixtures and pharmaceutical preparations.

Keywords: Betamethasone Valerate; Tolnaftate; Gentamicin; Clioquinol; Mixderm®; TLC-Spectrodensitometry

Introduction

Betamethasone valerate (BV) is a glucocorticoid used for the management of various disorders. Its lack of mineralocorticoid properties makes betamethasone particularly suitable for treating cerebral edema and congenital adrenal hyperplasia [1]. A review of the literature showed that methods reported for the determination of (BV) alone or in combinations were spectrophotometry [2], HPLC [3] and electrochemical methods [4]. Tolnaftate (TF) is a synthetic over-the-counter anti-fungal agent. It is used to treat jock itch, athlete's foot and ringworm [1]. Several methods were reported for (TF) alone or in combinations as spectrophotometry [5], spectrofluorometry [6], HPLC and HPTLC [7]. Gentamicin (GM) is a broad-spectrum antibiotics, but may cause ear and kidney damage [1]. GM was determined alone or in combinations by variety of methods as electrochemical methods [8], spectrophotometry [9] and HPLC [10]. Clioquinol (CQ) is an antifungal drug and antiprotozoal drug. The drug have been found to have activity against both viral and protozoal infections [1], determined alone or in combinations by methods such as spectrophotometry [11], spectrofluorometry [18], electrochemical methods [12], LC [13] and HPLC [14]. There is no reported method for the determination of the four drugs together either in their quaternary mixture or in the presence of their degradation products. Therefore, the objective of this work was to develop a validated and simple TLC-spectrodensitometric and chemometric methods for the determination of BV, TF, GM and CQ in bulk powders, laboratory prepared mixtures and pharmaceutical dosage form. The chemical structures of the cited drugs were displayed in Figure 1.

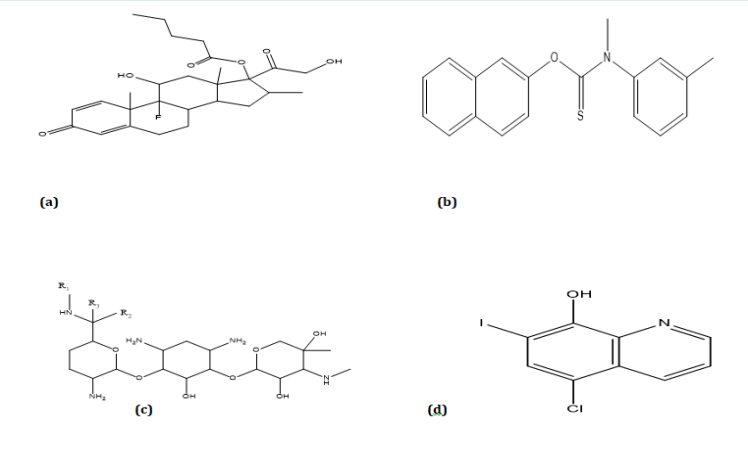


Figure 1: The chemical structures of (a) betamethasone valerate (BV), (b) tolnaftate (TF), (c) gentamicin (GM), and (d) clioquinol (CQ).

Materials and Methods

Apparatus and Software

- The TLC-spectrodensitometric system: CAMAG TLC scanner 3 S/ N 130319 operated with winCATS software, Linomat 5 autosampler (CAMAG, Muttenz, Switzerland), CAMAG microsyringe (100 µL). TLC aluminum sheets (20x20 cm) pre-coated with silica gel 60 F_{254} (Merck KgaA, Darmstad, Germany) were used. Calculations were performed using the Excel program.
- Ultraviolet/Visible spectrophotometer (Spectronic Genesys® with WINPEC® application software) with1 cm quartz cell, Spectronic, (USA).
- All calculations and statistics were carried out on computer using MATLAB® program version 7.9.

Chemicals and Reagents

- **Pure Samples**

Standard (BV), (TF), (GM), and (CQ) were kindly donated bySIGMA Pharma Co., Quesna, Egypt. Their purity was found to be 100.13±0.84, 99.30±0.55, 100.02±0.30 and 99.80±0.50 %, respectively.

- **Market Sample**

Mixderm® cream (SIGMA pharmaceutical industries Co., Quesna city, Egypt), labeled to contain 0.6 mg (BV), 10 mg (TF), 1 mg (GM) and 10 mg (CQ) per one gm cream (batch No. 61688) were purchased from the Egyptian local market.

- **Solvents**

Methanol and chloroform (Analar grade), formic acid and acetic acid solutions were supplied from (Adwic, El Nasr pharmaceutical Chemicals Co., Egypt).

Standard Solutions

- **Stock Solutions**

Solutions were prepared in methanol of concentrations: 2 mg mL^{-1} BV, 2 mg mL^{-1} TF, and 1 mg mL^{-1} of CQ; and in methanol and water of concentration 4 mg mL^{-1} GM.

- **Working Solutions**

Working solutions were freshly prepared by further dilution of suitable volumes from each stock solutions with methanol to get solutions of final concentration for TLC-spectrodensitometric method, 0.5 mg mL^{-1} BV, 0.5 mg mL^{-1} TF, 2 mg mL^{-1} GM, and 0.6 mg mL^{-1} of CQ; for chemometric method, 100 µg mL^{-1} BM, 100 µg mL^{-1} TF, 2000 µg mL^{-1} GM, and 6 µg mL^{-1} of CQ.

Procedure

- **For TLC–Densitometric Method**

- **Chromatographic Conditions**

TLC aluminum sheets 20 x 20 cm pre-coated with 0.25 mm silica gel 60 F_{254}were used. The prepared samples were tested as bands (bandwidth: 6 mm, bands were dispersed 1 cm apart from each other and 1.5 cm from the bottom of the plate). The developing system used was chloroform-methanol-acetic acid-formic acid (6:1:0.15:0.25, v/v/v/v) as a mobile phase of total volume approximately 10 milliliters. Linear ascending expansion was completed in a chromatographic tank previously saturated with the developing system for 15 min. at room temperature (25 ± 2 °C) to a distance of approximately 8 cm from the lower edge (approximately 10 min). The developed plates were dried in air for approximately 5 min. and scanned at 254 nm. The detection was done using Camage TLC scanner 3 operated

in the reflectance-absorbance mode. The slit dimension was kept at 3 mm x 0.45 mm and the scanning speed was 20 mm/s. All measurements were performed by winCATS software.

➢ **Application to Pharmaceutical Preparation**

A four-grams portion of cream was conveyed to a 50-mL volumetric flask, tending to bypass catching cream to the walls of the volumetric flask. A 30- mL portion of methanol and 10 mL portion of water was added to the flask, and the cream was granted to melt by warming at 60°C in a water bath with shaking. The solution was granted to cool to room temperature. The volume was made up to the mark with methanol and mixed. The solution was centrifuged at 10000 rpm for 10 min, and a clear supernatant solution was obtained. A portion of the supernatant was diluted with methanol to obtain a final concentration 24 µg mL^{-1} of BV, 400 µg mL^{-1} of TF, 40 µg mL^{-1} of GM and 400 µg mL^{-1} of CQ.

➢ **Linearity and construction of calibration curves**

Aliquot volumes (1-7), (6-18), (1-6) and (8-18) µg $band^{-1}$ of BV, TF, GM and CQ were separately conveyed from their working solutions into 10 mL volumetric flasks and diluted to volume with methanol. Aliquot of 10 µL of each solution was applied to the TLC plate using a 100 µL syringe. The chromatographic conditions were tested and the chromatograms were recorded. The calibration curves were constructed by plotting the recorded peak area versus the corresponding drug concentrations, from which the regression equations were calculated.The calibration curveswere made from the average of three experiments.

➢ **For Chemometric Method**

➢ **Construction of Calibration Set**

Multilevel partial factorial design [15] was used for the development of the calibration and validation sets. Fifteen mixtures were needed for construction the calibration model. The laboratory-prepared mixtures of BV, TF, GM and CQ were abled in their concentration ranges. The spectra of the abled mixtures were recorded in the range of 200-400 nm and conveyed to Matlab® for subsequent data manipulation.

➢ **Application to Validation Set**

Into sets of 10-mL volumetric flask, aliquots of each ingredient were conveyed from their working solutions to prepare five mixtures of different ratios of the studied drugs. The absorption spectra of the abled solutions from 200 to 400 nm were recorded and conveyed to Matlab®. The concentration of each ingredient was calculated using the constructed model.

➢ **Application to Pharmaceutical Preparation**

As described before, then the solution was centrifuged at 10000 rpm for 10 min, and a clear supernatant solution was obtained. Further dilution was done to obtain a final concentration 24 µg mL^{-1} of BV, 400 µg mL^{-1} of TF, 40 µg mL^{-1} of GM and 400 µg mL^{-1} of CQ. The concentration of each ingredient was calculated using the constructed PLS and PCR models. When applying the standard addition technique, contrasting known concentrations of the standard of each ingredient were added to the dosage form before proceeding in the previously mentioned procedure.

Results and Discussion

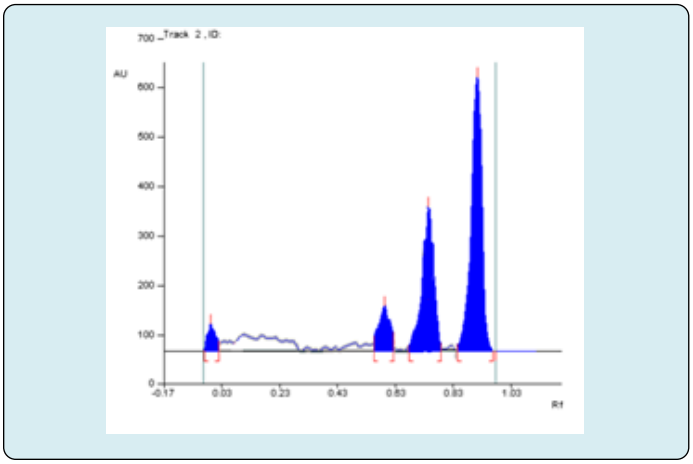
This work was aimed to develop, and validate simple, accurate, selective, and precise analytical approaches which were TLC-spectrodensitometric and chemometric methods, for the simultaneous assessment of the quaternary mixture of BV, TF, GM and CQ in their pure form and pharmaceutical dosage form.

TLC–Densitometry

This approach offers a simple manner for quantification directly on TLC plate by calculating the optical density of the separated bands. The amounts of ingredients are detected by the comparison between them and the standard curve from reference materials chromatographed simultaneously under the same conditions.

➢ **Optimization of the Method**

To optimize the approach conditions, it was necessary to check the effect of various variables. To separate the three drugs from each other's, several ratios of different developing systems were investigated. Certainly, it was established that the best separation of the cited drugs was achieved by applying the developing system using chloroform-methanol-aceticacid-formicacid (6:1:0.15:0.25, v/v/v/v). R_f for BV, TF, GM and CQ were 0.59± 0.01, 0.91±0.02, 0.05±0.01, and 0.74±0.01, respectively. Different scanning wavelengths were tried; on using 245 nm where the separated peaks were sharp and symmetrical with minimum noise, as shown in Figure 2.



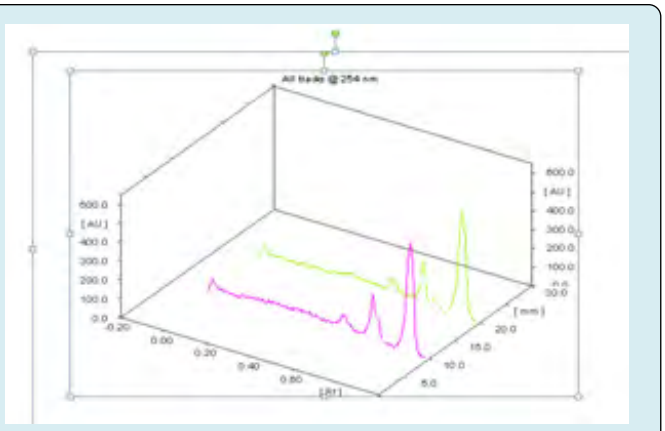


Figure 2: (A) 2D-TLC chromatogram; (B) 3D-TLC chromatogram of (a) GM, (b) BV, (c) CQ and (d) TF, using chloroform-methanol-aceticacid-formicacid (6:1:0.15:0.25, v/v/v/v) as the developing system.

➢ **Method Validation**

Method validation was completed according to the International Conference on Harmonization (ICH) guidelines [16] regarding linearity, range, precision, accuracy, limit of detection and limit of quantitation.

- **Range and Linearity**

The linearity of the suggested method was assessed by preparing different calibration curves. Analysis was carried out on a series of standard drug solutions, the calibration curves were constructed between AUC and corresponding concentrations of bands. Linear regression analysis was applied and analytical parameters were calculated. The linear concentration ranges and other statistical parameters for the proposed method were listed in Table 1.

- **Limits of Detection and Quantitation**

The limit of detection (LOD) and limit of quantitation (LOQ) of the proposed method were calculated (Table 1).

Parameter	BV	TF	GM	CQ
Concentration range (µg mL^{-1})	01-Jul	Jun-18	01-Jun	Aug-18
Slope	19.89	12.52	12.329	20.029
Standard deviation of the slope (SD_b)	0.156	0.084	0.068	0.218
Intercept	100.86	224.79	87.77	379.96
Standard deviation of the intercept (SD_a)	0.6962	1.064	1.1764	2.927
Standard deviation of the residuals (SDy/x)	0.824	0.89	0.282	1.822
Number of determinations	7	7	6	6
Correlation coefficient (r)	0.9998	0.9999	0.9999	0.9997
Determination coefficient (r^2)	0.9997	0.9998	0.9999	0.9995
Limit of detection, LOD (µg mL^{-1})	0.1155	0.28	0.3149	0.482
Limit of quantitation, LOQ (µg mL^{-1})	0.35	0.8498	0.9542	1.46

Table 1: Analytical parameters and method validation sheet obtained by applying the proposed TLC-spectrodensitometric method for determination of BV, TF, GM and CQ in quaternary mixture.

- **Accuracy**

For the study of the accuracy of the suggested method, repeated analysis (three times) of different concentrations of BV, TF, GM and CQ within the linearity range were performed (Table 1). By applying standard addition technique to the pharmaceutical formulation, the conflict of excipients was studied. The acceptable accuracy demonstrated that the excipients in the pharmaceutical formulation did not interferein the analysis of these compounds in the pharmaceutical formulation as shown in Table 2.

Drug	TLC–densitometry			PLS		
	Taken µg mL^{-1}	Found µg mL^{-1}	Recovery%* ±SD	Taken µg mL^{-1}	Found µg mL^{-1}	Recovery%* ±SD
BV	4	4.02	100.43±0.93	30	30.04	100.13±0.96
TF	12	12.07	100.57±0.91	35	34.97	99.91±0.93
GM	4	4.03	100.67±0.88	90	90	100.00±0.85
CQ	14	14.15	101.08±1.00	4	3.99	99.75±0.75

*Average of three experiments.

Table 2: Application of standard addition technique to the analysis of Mixderm® cream by applying the proposed methods.

- **Precision**

The inter-day and intra-day precision of the proposed method were determined. The results expressed as percentage recoveries and RSD are shown in Table 3.

	Drug	Conc. Level (µg mL-1)	% Recovery* ± SD	%RSD
Inter-day	BV	3	98.95±0.50	0.51
		4	99.77±0.48	0.48
		5	99.06±0.55	0.56
	TF	10	100.45±0.60	0.6
		12	99.99±0.50	0.5
		14	99.05±0.55	0.56
	GM	3	99.57±0.39	0.39
		4	99.31±0.51	0.51
		5	100.23±0.40	0.4
	CQ	12	100.35±0.56	0.56
		14	100.09±0.47	0.47
		15	99.89±0.65	0.65
Intra-day	BV	3	100.53±0.64	0.64
		4	99.75±0.48	0.48
		5	99.05±0.63	0.64
	TF	10	98.95±0.51	0.52
		12	100.43±0.61	0.61
		14	99.77±0.44	0.44
	GM	3	100.45±0.35	0.35
		4	99.84±0.39	0.39
		5	98.99±0.40	0.4
	CQ	12	100.51±0.65	0.65
		14	100.00±0.57	0.57
		16	100.05±0.49	0.49

*Average of three experiments

Table 3: Application of Intra-day and Inter-day technique to the analysis of BV, TF, GM and CQ in Mixderm® **cream by the proposed TLC–spectrodensitometric method.**

- **Selectivity**

Selectivity was confirmed by analyzing different mixtures containing drugs in different ratios within the linearity range. Satisfactory results were shown in Table 4.

	%Recovery*±SD*				
	Mix ratio,	BV	TF	GM	CQ
1	1:1:1:1	98.05±0.43	99.75±0.61	100.20±0.52	99.78±0.49
2	1:16.66:1.66:16.66**	100.21±0.55	100.31±0.591	99.81±0.52	98.95±0..51
3	1:2:3:1	98.99±0.615	99.59±0.561	99.74±0.65	99.77±0.60
4	3:1:2:1	99.49±0.505	98.76±0.53	99.74±0.35	99.99±0.45
5	2:1:3:3	99.79±0.606	99.99±0.555	99.89±0.46	100.01±0.55

*Average of three experiments

** Ratio in Mixderm® cream

Table 4: Determination of BV, TF, GM and CQ in laboratory prepared mixtures by the proposed TLC-spectrodensitometric method.

- **System Suitability**

System suitability was tested by calculating various parameters as shown in Table 5. The attained values were in the adequate ranges when compared to the reference value.

Parameter	GM	BV	CQ	TF	Reference value
R_f value	0.05±0.01	0.59± 0.01	0.74±0.01	0.91±0.02	
T_f (tailing factor)	1.1	1.13	0.85	1.05	T≤ 1.15 - 0.95 & T = 1 for symmetric peak
R_s (chromatographic resolution)	3.15	2.56		1.51	R_s> 1.5

Table 5: System suitability parameters of the proposed TLC-spectrodensitometric method of BV, TF, GM and CQ in Mixderm® cream.

Chemometric Method

Between the different regression methods current for multivariate calibration, the factor analysis based on partial least squares (PLS) and principal component regression model (PCR) regression have received considerable attention in the chemometrics literature [17]. The calibration set was built using the absorption spectra set of 15 mixtures, as listed in Table 6. The elected model was that with the smallest number of variables such that RMSECV for that model was not naturally larger than RMSECV from the model with additional variable. Four variables were found to be choicest for the mixture, as shown in Figure 3 for PLS and PCR.

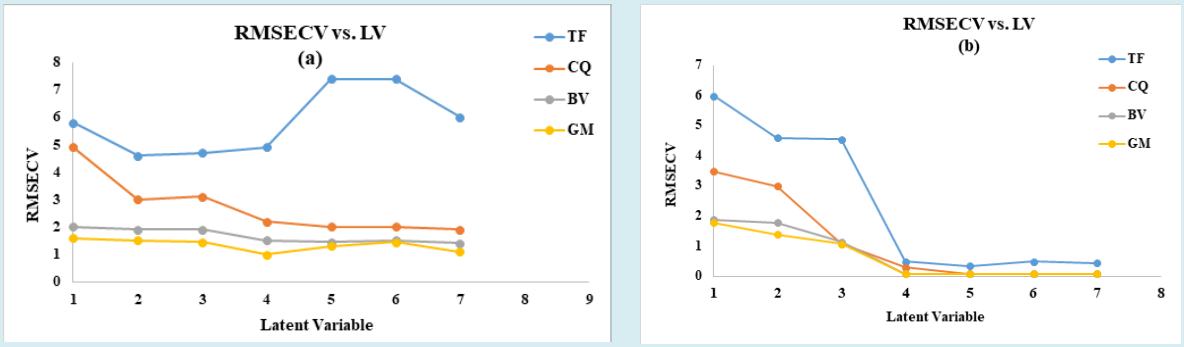


Figure 3: RMSECV of the calibration set of BV, TF, GM, and CQ as a function of latent variables of (a) PCR and (b) PLS models.

➢ **Model Validation**

To check the prediction capability of the proposed models, an external validation set of 15 mixtures was used as shown in Table 6. The root mean squared errors of prediction (RMSEP) and the regression equations for the predicted *versus* actual concentration are shown in Table 7 as diagnostic tools for model validation.

Experimental No.	Concentration (µg mL^{-1})			
	BV	TF	GM	CQ
1	2	34	4	34
2	3	32	7	34
3	3	34	2.5	32
4	1.5	32	5.5	38
5	2.5	38	5.5	34
6	2	38	7	30
7	3	38	1	36

8	3	30	5.5	30
9	2.5	30	4	36
10	1	34	5.5	36
11	2.5	36	2.5	30
12	2.5	32	1	32
13	1	32	4	30
14	2	30	1	38
15	1	30	7	32

Table 6: Concentration of BV, TF, GM and CQ in the calibration set using PCR and PLS models.

Validation parameters	PCR				PLS			
	BV	TF	GM	CQ	BV	TF	GM	CQ
Slope	1.0541	1.0051	0.9992	0.9895	0.9935	0.9751	0.9953	1.0059
Intercept	0.1501	0.0059	0.01591	0.0342	0.1245	0.1151	0.0135	0.008
Correlation coefficient (r)	0.999	0.9999	0.9992	0.9997	0.9998	0.9996	0.9994	0.9995
RMSEP	0.1151	0.0931	0.0357	0.224	0.2765	0.1189	0.0974	0.0394

Table 7: Summary of results obtained by applying the diagnostic tools for model validation of the PCR and PLS models with BV, TF, GM and CQ.

Statistical Analysis

Table 8 showed statistical comparison of the results achieved by the suggested methods and reported methods for BV [3], TF, GM [13] and CQ for TLC-spectrodensitometric method and for chemometric method. The calculated *t* and *F* values were less than the theoretical ones indicating that there was no significant difference between the proposed and the reported methods with respect to accuracy and precision.

	BV				TF				GM				CQ			
	Reported method	TLC	PLS	PCR	Reported method	TLC	PLS	PCR	Reported method	TLC	PLS	PCR	Reported method	TLC	PLS	PCR
Mean	100.13	99.97	99.98	100.11	99.3	99.99	100.1	99.97	100.02	100.1	100.15	99.98	99.8	100.03	100.12	100.14
Standard deviation, SD	0.84	0.42	0.94	1.12	0.55	0.35	0.66	0.74	0.3	0.3	0.44	0.47	0.5	0.37	0.85	0.57
N	3	7	7	7	3	7	7	7	3	6	7	7	3	6	7	7
Variance	0.71	0.18	0.88	1.25	0.3	0.12	0.44	0.55	0.09	0.09	0.19	0.22	0.25	0.14	0.72	0.32
Student t		0.31	0.248	0.03		2.03	2	1.58		0.38	0.54	0.16		0.72	0.75	0.96
		-2.26	-2.26	-2.26		-2.26	-2.26	-2.26		-2.306	-2.26	-2.26		-2.306	-2.26	-2.26
F (19.3)		3.94	1.24	1.76		2.5	1.47	1.83		1	2.11	2.44		1.79	2.88	1.28

Table 8: Statistical comparison between the results obtained by the proposed TLC-spectrodensitometric, chemometric and the reported methods for the determination of BV, TF, GM and CQ in pure powder form.

Conclusion

This work conferred a comparative study on two analytical approaches based on UV spectrophotometry which were TLC-densitometric method and multivariate chemometric spectrophotometric method (PLS) and (PCR). Both approaches were profitably applied for the simultaneous determination of the quaternary mixture of BV, TF, GM and CQ in their pure form and pharmaceutical formulation. The TLC-densitometric method has the favor over HPLC as

it diminishes the management of reagents which backing the eco-friendly behavior of green chemistry, it diminishes the time needed for analysis, and it utilizes the benefit of handling several sample bands on TLC plate, which may be more beneficial for regulatory quality control laboratories. In addition, the method is cheap and does not require special types of stationary phases, but still, the method achieve the same validation parameters and efficiency when correlated to reported HPLC method. Meanwhile, the chemometric method has the merits of being simpler as it does not require certain chemicals or reagents, and it is considered to be cost - and time -saving, but it need special software (Matlab). It was found that PLS preceded PCR in the analysis of such complex mixtures. As a final outcome, the results earned by the two suggested methods were accurate, reliable, and precise. So, both methods can be hired for routine analysis in quality control as different methods to HPLC methods in the laboratories of quality control missing the required facilities for those costly techniques.

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