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THREE MULTIVARIATE CALIBRATION METHODS FOR SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF OLMESARTAN MEDOXAMIL, AMLODIPINE BESYLATE AND HYDROCHLOROTHIAZIDE IN THEIR COMBINED DOSAGE FORM

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Olmesartan medoxamil (OLM, an angiotensin II receptor blocker), amlodipine besylate (AML, a dihydropyridine calcium channel blocker) and hydrochlorothiazide (HCT, a diuretic of the class of benzothiadiazines) are co-formulated in a single-dose combination for the treatment of hypertensive patients whose blood pressure is not adequately controlled on either component monotherapy. In this work, three multivariate calibration methods were applied for simultaneous spectrophotometric determination of OLM, AML and HCT in their combined pharmaceutical tablets The multivariate methods are classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS). The results showed the superiority of PLS over CLS and PCR for the analysis of the ternary mixture. The optimum assay conditions were established and the proposed methods were successfully applied for the assay of the three drugs in an independent validation set and combined pharmaceutical tablets with excellent recoveries. No interference was observed from common pharmaceutical additives. The results were favorably compared with those obtained by a reference spectrophotometric method.

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Keywords: Olmesartan medoxamil; Amlodipine besylate; Hydrochlorothiazide Spectrophotometry; Multivariate calibration methods; Pharmaceutical tablets.

1. Introduction

Olmesratan medoxamil (OLM, Fig. 1) is chemically known as (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)phenyl]phenyl}methylimidazol-5-carboxylate. It is a potent and selective angiotensin AT1 receptor blocker [1]. It has been approved for the treatment of hypertension in the United States, Japan and European countries. The drug contains a medoxomil ester moiety which is cleaved rapidly by an endogenous esterase to release the active olmesartan [2]. Amlodipine besylate (AML, Fig. 1) is chemically known as 3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate benzene sulphonate. It is a dihydropyridine calcium channel blocker used in the treatment of hypertension and angina pectoris [3]. AML is official in the British Pharmacopoeia (BP) which describes HPLC for its assay in the bulk powder [4]. Hydrochlorothiazide (HCT, Fig. 1) is chemically known as 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide.

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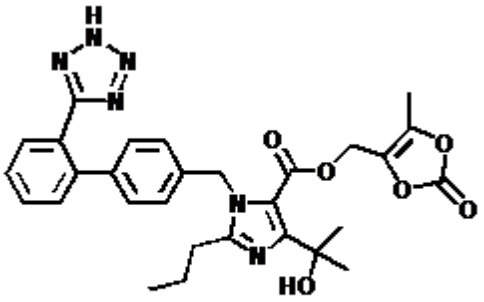
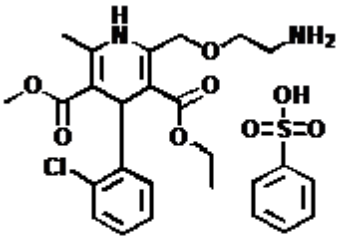
It is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which inhibits NaCl transport in distal convoluted tubule and decreases blood pressure [5].

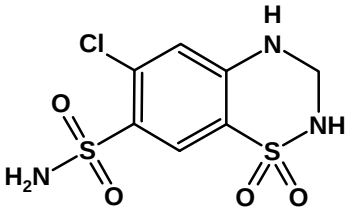
Recently, OLM has been marketed in combination with AML and HCT in tablet dosage form (TRIBENZOR® tablets). The triple combination of OLM, AML and HCT is intended for oral administration for the treatment of hypertension and are available in several different strength combinations.



Olmesartan medoxomil (OLM)



Amlodipine besylate (AML)



Hydrochlorothiazide (HCT)

Fig. 1. Chemical structures of olmesartan medoxomil (OLM), amlodipine besylate (AML) and hydrochlorothiazide (HCT).

Few methods are available for the simultaneous analysis of OLM, AML and HCT combination. These methods include HPLC [6-8] and spectrophotometry [9-11]. These methods employed intensive instrumentation (e.g. HPLC) or suffered from low robustness such as spectrophotometry because calibration procedures depend on measuring absorbances at one wavelength (univariate calibration method). So any shift in wavelength scale will lead to false results. Inclusion of many spectral wavelengths instead of using a single wavelength greatly improves the precision and predictive ability of the multivariate calibration methods [12]. The scientific novelty of the present work is that the methods used are simple, rapid, selective, less expensive and less time consuming compared with other published HPLC methods. Furthermore, these methods have high precision and accuracy as compared with the reported spectrophotometric methods because calibration procedures depend on whole spectra. So, the aim of this work was to develop simple, sensitive and validated chemometric assisted spectrophotometric methods for the simultaneous determination of OLM, AML and HCT in powdered forms, laboratory prepared mixtures and in pharmaceutical formulation. The applied chemometric methods are classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS).

2. Experimental

Apparatus

A double-beam uv–visible spectrophotometer (shimadzu, japan) model uv-1650 pc with quartz cell of 1 cm path length, connected to an IBM-compatible computer.

The spectral bandwidth was 2 nm and wavelength-scanning speed 2800 nm/min. A uv lamp with a short wavelength (254 nm).

All recorded spectra converted to ASCII format by uv-prob personal spectroscopy software version 2.21.

Software

All chemometric methods were implemented in Matlab® 7.1.0.246 (R14). PCR and PLS were carried out by using PLS-Toolbox software version 2.1. The t test, *F* test were performed using Microsoft® Excel. All calculations were performed using intel® core ™ i5-2400, 3.10 GHz, 4.00GB of RAM under Microsoft Windows 7.

Materials

OLM was obtained from AK Scientific Inc. (CA, USA). AML was obtained from Pfizer Inc. (New York, USA). HCT was kindly supplied by Al-Hekma pharmaceutical Company (Cairo, Egypt). The .purities of OLM, AML and HCT were 99.5 %, 99.5% and 99.78% respectively. Tribenzor® tablets 40/10/25 (Daiichi Sankyo inc., U.S.A) are label to contain 40 mg of OLM, 10 mg of AML base (equivalent to 13.9 mg of AML) and 25 mg of HCT (Batch number 134809). They were procured from U.S.A. market. Acetonitrile used throughout this study was of analytical grade.

Preparation of OLM, AML and HCT standard solutions

Stock solutions of OLM (250 µg mL^{-1}), AML (200 µg mL^{-1}) and HCT (250 µg mL^{-1}) were prepared by dissolving 12.5 mg of OLM, 10 mg of AML and 12.5 mg of HCT, separately in 50 mL acetonitrile. Stock solutions were stable for at least two weeks when stored refrigerated at 4 °C.

Preparation of pharmaceutical tablets sample solutions

Tribenzor® tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 40 mg of OLM , 13.9 mg of AML (equivalent to 10 mg of AML base) and 25 mg of HCT was extracted into acetonitrile with the aid of sonication and the extract was filtered. The filtrate was diluted with acetonitrile to obtain final concentrations of 200, 69.5 and 125 µg mL^{-1} for OLM, AML and HCT, respectively. 500 µL of Tribenzor ® tablet solution were transferred into a 5 mL volumetric flask and diluted to the mark with acetonitrile to get a final concentration of OLM (20 µg mL^{-1}), AML (6.95 µg mL^{-1}) and HTZ (12.5 µg mL^{-1}). Spectral acquisition and the calculations were performed in the same manner as described in **"Multivariate Calibration procedures".**

Multivariate calibration procedures

Five level, three factor calibration design [13] was used for construction of 25 samples by transferring different volumes of OLM, AML and HCT from their standard working solutions into 5 mL volumetric flasks and the solutions were diluted to the volume with acetonitrile and mixed well (Table 1). 15 samples were used to build the multivariate calibration models (training set) while 10 samples were used to test the predictive ability of the proposed models (validation set). The concentrations chosen for each compound in 25 samples were based on the calibration range of each of the two drugs, the ratio of OLM:AML:HCT in the Tribenzor tablets (4:1:2.5 respectively). The absorption spectra of the 25 samples were scanned from 200 - 400 nm against acetonitrile as a blank (Fig. 2) and transferred to Matlab for subsequent calculations. The noisy region from 200-230 nm and the zero absorbance of OLM and HCT after 340 nm accounted for the rejection of these parts from the spectra.

Table 1: The 5 level 3 factor experimental design of the training and validation set mixtures shown as concentrations of the mixture components in μg mL^{-1}.

Mix No.	OLM	AML	HCT	Mix No.	OLM	AML	HCT
1	20	5	12.5	14	20	7	15
2	20	3	10	15	25	7	10
3	15	3	15	16	25	3	13.75
4	15	7	11.25	17	15	6	10
5	25	4	15	18	22.5	3	12.5
6	17.5	7	12.5	19	15	5	13.75
7	25	5	11.25	20	20	6	13.75
8	20	4	11.25	21	22.5	6	11.25
9	17.5	4	13.75	22	22.5	4	10
10	17.5	6	15	23	17.5	3	11.25
11	22.5	7	13.75	24	15	4	12.5
12	25	6	12.5	25	17.5	5	10
13	22.5	5	15				

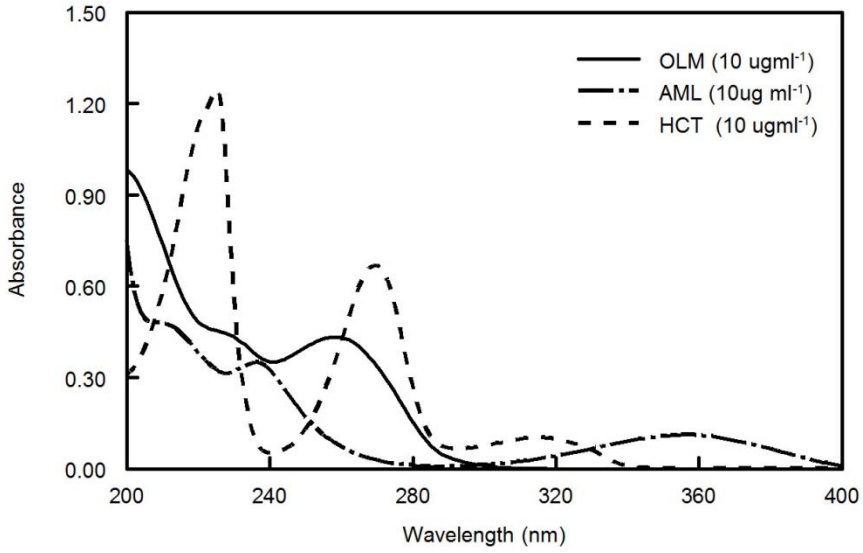


Fig. 2. Absorption spectra for OLM, AMl and HCT against acetonitrile as a blank (10 μg mL^{-1} each).

The 2D Scores plot for the first two PCs of the whole concentration matrix was obtained to confirm the well position of the mixtures in space, orthogonality, symmetry and rotatability [13] as indicated in Fig. 3. Mean centering of the data proved to be the best pre-processing method for getting the optimum results.

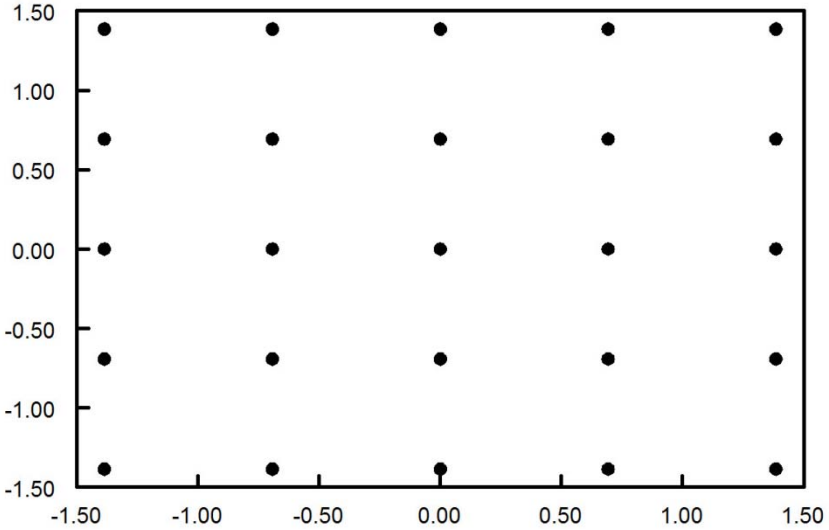


Fig. 3. Scores plot for the mean centred 25 samples concentration matrix of the five level three component experimental design.

Optimisation of number of latent variables for the PCR and PLS models

Cross validation (CV) [14] was applied to predict how many are the optimum number of PLS latent variables. CV involves repeatedly dividing the data into two sets, a training set used to determine a model and a test set to determine how well the model performs so that each sample (or portion of the data) is left out of the training set once only.

Leave one out (LOO) CV is used in our study for optimizing the number of PLS components, by building the model using *I*-1 samples set (training set consisting of 14 samples) to predict the one sample left (validation sample). The root mean square error of CV (RMSECV) is calculated as

$$\mathrm{RMSECV} = \sqrt{\frac{1}{\mathrm{I}}\sum_{\mathrm{i=1}}^{\mathrm{I}}\left(c_i - \hat{c}_{i\_cv}^{A}\right)^2}$$

where I is the number of objects in the calibration set, c_i is the known concentration for sample i and $\hat{c}_{i\_cv}^{A}$ is the predicted concentration of sample i using A components. Mean centering was performed on the training set each time successive samples were left out.

3. Results and discussion

Tribenzor® tablets are combined dosage form containing the angiotensin II receptor blocker OLM, the calcium channel blocker AML and the diuretic HCT. It has been used in the treatment of hypertension. The ratio of OLM : AML : HCT in Tribenzor tablets is 4:1:2.5 respectively. This study was designed to develop simple, robust and accurate multivariate methods for the simultaneous determination of OLM, AML and HCT in Tribenzor® tablets. Because of the practical simplicity, and wide availability of spectrophotometry in quality control laboratories, it was attempted in this study. Multivariate calibration methods are very useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of using a single wavelength greatly improves the precision and predictive ability of these methods.

CLS model

The training set was used for constructing CLS model or (K) matrix (i.e. absorptivity at different wavelengths). The CLS method requires that all the components in the calibration samples must be

known. Unlike CLS, PCR and PLS methods could be used to determine the components under investigation even in the presence of unknown components (interfering substance) which gave these two methods an advantage over CLS [12]. The absorbance matrix of the calibration samples (15x111) and their corresponding concentration matrix (15x3) were used to find the absorptivity matrix (k-matrix). Then, the obtained k-matrix was further used for the calculation of the predicted concentration of the three components in both the validation and pharmaceutical formulation samples.

PCR and PLS models

In order to apply PCR and PLS to the data, the raw data of the calibration samples were mean centered [15] as a preprocessing step and the cross validation method, leaving out one sample at a time and RMSECV was calculated as mentioned above, was used to select the optimum number of factors [14]. The selection of the optimum number of latent variables was a very important pre-construction step: if the number of factors retained was more than required, more noise would be added to the data; if the number retained was too small, meaningful data that could be necessary for the calibration might be discarded. The maximum number of factors used to calculate the optimum RMSECV was selected to be eight. The method described by Haland and Thomas [16] was used for selecting the optimum number of factors. After the PCR and PLS models have been constructed, it was found that the optimum number of LVs described by the developed models was three factors for both PCR and PLS methods as shown in Fig. 4.

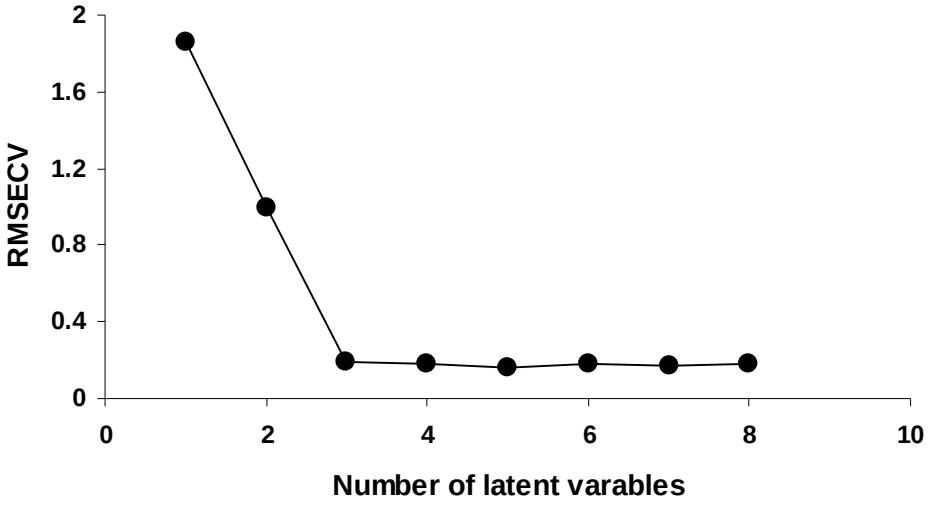


Fig. 4. RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables used to construct the PLS or PCR models.

After optimization of parameters and calibration (training) step, all models were applied successfully for analysis of OLM, AML and HCT in training set (Table 2) and in validation set (Table 3). The recoveries mean recoveries, standard deviation, root mean square of calibration (RMSEC), root mean square of prediction (RMSEP) values are summarized in Tables 2 and 3. RMSEC and RMSEP were calculated as the same manner as RMSECV for calibration and validation set, respectively. The proposed methods were then applied for the simultaneous determination of the three analytes in Tribenzor tablets (Table 4). It was clear that PCR and PLS had superiority over CLS in the analysis of the dosage form, which is expected, may be due to the excepients effect on the absorption spectra of the tablet extract. This fact was further assessed by the statistical comparison of t and F values of the proposed models and the reference spectrophotometric method [11] (Table 5) showing that there is a significant difference between CLS and the reference one regarding both accuracy and precision.

Table 2: Analysis results for the prediction of the training set by the proposed multivariate calibration methods.

Method			CLS						PCR						PLS					
OLM	AML	HCT	OLM		AML		HCT		OLM		AML		HCT		OLM		AML		HCT	
True (μg ml^{-1})			Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%
25	7	10	24.6	98.40	7.03	100.43	9.85	98.50	24.82	99.28	6.94	99.14	10.02	100.20	24.82	99.28	6.94	99.14	10.02	100.20
15	3	15	14.58	97.20	3.11	103.67	14.76	98.40	14.74	98.27	3.01	100.33	14.88	99.20	14.74	98.27	3.01	100.33	14.88	99.20
15	4	12.5	14.71	98.07	4.16	104.00	12.46	99.68	14.8	98.67	4.05	101.25	12.53	100.24	14.8	98.67	4.05	101.25	12.53	100.24
15	5	13.75	14.92	99.47	5.03	100.60	13.75	100.00	15.02	100.13	4.96	99.20	13.82	100.51	15.02	100.13	4.96	99.20	13.82	100.51
15	6	10	14.85	99.00	6.12	102.00	9.78	97.80	14.95	99.67	5.94	99.00	9.86	98.60	14.95	99.67	5.94	99.00	9.86	98.60
15	7	11.25	14.98	99.87	7	100.00	11.26	100.09	14.95	99.67	6.95	99.29	11.22	99.73	14.95	99.67	6.95	99.29	11.22	99.73
17.5	3	11.25	17.52	100.11	3.15	105.00	11.26	100.09	17.58	100.46	3.06	102.00	11.32	100.62	17.58	100.46	3.06	102.00	11.32	100.62
17.5	7	12.5	17.74	101.37	7	100.00	12.72	101.76	17.71	101.20	7.03	100.43	12.7	101.60	17.71	101.20	7.03	100.43	12.7	101.60
17.5	4	13.75	17.91	102.34	3.91	97.75	13.88	100.95	17.87	102.11	3.98	99.50	13.85	100.73	17.87	102.11	3.98	99.50	13.85	100.73
17.5	5	10	17.8	101.71	5.13	102.60	10.11	101.10	17.72	101.26	5.11	102.20	10.06	100.60	17.72	101.26	5.11	102.20	10.06	100.60
17.5	6	15	17.68	101.03	5.71	95.17	15.17	101.13	17.55	100.29	5.88	98.00	15.06	100.40	17.55	100.29	5.88	98.00	15.06	100.40
20	5	12.5	19.85	99.25	4.78	95.60	12.19	97.52	19.98	99.90	4.82	96.40	12.29	98.32	19.98	99.90	4.82	96.40	12.29	98.32
20	3	10	20	100.00	2.94	98.00	10.14	101.40	19.83	99.15	3	100.00	10.03	100.30	19.83	99.15	3	100.00	10.03	100.30
20	4	11.25	20.36	101.80	3.94	98.50	11.41	101.42	20.21	101.05	4.05	101.25	11.3	100.44	20.21	101.05	4.05	101.25	11.3	100.44
20	6	13.75	20	100.00	6.09	101.50	13.75	100.00	19.77	98.85	6.12	102.00	13.56	98.62	19.77	98.85	6.32	102.00	13.56	98.62
Mean (%)				99.97		100.32		99.99		100.00		100.00		100.01		100.00		100.00		100.01
S.D				1.478		2.934		1.373		1.084		1.611		0.927		1.084		1.611		0.927
RMSEC (μg mL^{-1})				0.2517		0.1289		0.1587		0.1842		0.1232		0.1107		0.1841		0.1230		0.1107

Table 3: Analysis results for the prediction of the independent validation test set by the proposed multivariate calibration methods.

Method			CLS						PCR						PLS					
OLM	AML	HCT	OLM		AML		HCT		OLM		AML		HCT		OLM		AML		HCT	
True (μg ml^{-1})			Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%
20	7	15	20.43	102.15	6.8	97.14	15.06	100.40	20.36	101.80	6.98	99.71	14.99	102.72	20.36	101.78	6.98	99.75	14.99	99.96
22.5	3	12.5	22.79	101.29	2.89	96.33	12.92	103.36	22.68	100.80	3.05	101.67	12.84	100.50	22.68	100.80	3.05	101.58	12.84	102.72
22.5	4	10	22.72	100.98	4.12	103.00	10.02	100.20	22.75	101.11	4.11	102.75	10.05	99.80	22.75	101.13	4.11	102.72	10.05	100.52
22.5	5	15	22.8	101.33	4.84	96.80	14.93	99.53	22.86	101.60	4.96	99.20	14.97	97.96	22.86	101.59	4.96	99.26	14.97	99.82
22.5	6	11.25	22.41	99.60	5.95	99.17	10.96	97.42	22.48	99.91	5.94	99.00	11.02	95.93	22.48	99.92	5.94	99.01	11.02	97.94
22.5	7	13.75	22.73	101.02	6.96	99.43	13.06	94.98	22.9	101.78	6.97	99.57	13.19	100.07	22.9	101.76	6.97	99.54	13.19	95.92
25	3	13.75	25.09	100.36	2.82	94.00	13.65	99.27	25.22	100.88	2.89	96.33	13.76	97.47	25.22	100.86	2.89	96.39	13.76	100.05
25	4	15	25	100.00	3.82	95.50	14.46	96.40	25.2	100.8	3.87	96.75	14.62	97.78	25.2	100.81	3.87	96.74	14.62	97.47
25	5	11.25	24.79	99.16	4.91	98.20	10.88	96.71	24.94	99.76	4.89	97.80	11	102.32	24.94	99.75	4.89	97.8	11	97.81
25	6	12.5	24.77	99.08	5.81	96.83	12.64	101.12	24.95	99.80	5.82	97.00	12.79	102.72	24.95	99.81	5.82	97.04	12.79	102.31
Mean (%)				100.50		97.64		98.94		100.82		98.98		99.73		100.82		98.98		99.45
S.D				1.023		2.489		2.536		0.787		2.100		2.399		0.780		2.071		2.163
RMSEP (μg mL^{-1})				0.2395		0.1289		0.1587		0.2468		0.0973		0.2787		0.2453		0.0963		0.1107

Table 4: Determination of OLM, AML and HCT in Tribenzor tablets (Batch No. *134809*) *by the proposed multivariate calibration methods.*

Method			CLS						PCR						PLS					
OLM	AML	HCT	OLM		AML		HCT		OLM		AML		HCT		OLM		AML		HCT	
True ($\mu g\ ml^{-1}$)			Found ($\mu g\ ml^{-1}$)	R%	Found ($\mu g\ ml^{-1}$)	R%	Found ($\mu g\ ml^{-1}$)	R%	Found ($\mu g\ ml^{-1}$)	R%	Found ($\mu g\ ml^{-1}$)	R%	Found ($\mu g\ ml^{-1}$)	R%	Found ($\mu g\ ml^{-1}$)	R%	Found ($\mu g\ ml^{-1}$)	R%	Found ($\mu g\ ml^{-1}$)	R%
20	6.95	12.5	19.14	95.71	7.12	102.50	11.9	95.18	19.67	98.33	6.87	98.79	12.3	98.42	19.67	98.32	6.87	98.32	12.3	98.32
20	6.95	12.5	19.12	95.62	7.13	102.56	11.91	95.29	19.57	97.87	6.84	98.46	12.26	98.07	19.57	97.87	6.84	97.87	12.26	97.87
20	6.95	12.5	19.12	95.60	7.2	103.62	11.87	94.99	19.56	97.79	6.92	99.63	12.21	97.69	19.56	97.79	6.92	97.79	12.21	97.79
20	6.95	12.5	19.03	95.13	7.09	101.97	11.85	94.80	19.43	97.13	6.83	98.28	12.16	97.27	19.43	97.13	6.83	97.13	12.16	97.13
20	6.95	12.5	19.09	95.47	7.06	101.55	11.89	95.14	19.43	97.17	6.84	98.44	12.15	97.24	19.43	97.17	6.84	97.17	12.15	97.17
20	6.95	12.5	19.01	95.04	6.98	100.37	11.83	94.63	19.28	96.42	6.8	97.78	12.04	96.33	19.28	96.42	6.8	96.42	12.04	96.42
Mean (%)				95.43		102.09		95.01		97.45		98.56		97.51		97.45		98.56		97.50
S.D				0.276		1.097		0.251		0.677		0.616		0.736		0.678		0.615		0.736

Table 5: Statistical comparison of the results obtained by CLS, PCR, PLS and the reference spectrophotometric method for the analysis of Tribenzor® tablets (Batch No. 134809).

Parameters	CLS			PCR			PLS			Reference Method *		
	OLM	AML	HCT	OLM	AML	HCT	OLM	AML	HCT	OLM	AML	HCT
R%	95.71	102.50	95.18	98.33	98.79	98.42	98.32	98.79	98.42	98.69	98.50	99.54
	95.62	102.56	95.29	97.87	98.46	98.07	97.87	98.46	98.07	98.43	98.50	98.87
	95.60	103.62	94.99	97.79	99.63	97.69	97.79	99.62	97.69	98.56	98.50	98.11
	95.13	101.97	94.80	97.13	98.28	97.27	97.13	98.28	97.27	97.59	98.50	98.21
	95.47	101.55	95.14	97.17	98.44	97.24	97.17	98.44	97.24	97.90	98.50	97.80
	95.04	100.37	94.63	96.42	97.78	96.33	96.42	97.78	96.33	97.24	97.14	96.96
Mean (%)	95.43	102.09	95.01	97.45	98.56	97.51	97.45	98.56	97.50	98.07	98.28	98.25
S.D	0.276	1.097	0.251	0.677	0.616	0.736	0.678	0.615	0.736	0.581	0.552	0.886
Variance	0.076	1.203	0.063	0.459	0.379	0.542	0.459	0.380	0.541	0.337	0.305	0.785
number of samples	6	6	6	6	6	6	6	6	6	6	6	6
Student's *t* statistic	10.065**	7.616**	8.633**	1.693	0.850	1.586	1.691	0.842	1.587			
F ratio	4.421	3.943	12.469**	1.347	1.245	1.450	1.347	1.241	1.500	------		------

Note for p=0.05 and 10 degrees of freedom the critical values of T and F are 2.228 and 5.050 respectively.

* Reference spectrophotometric method is that published in the literature [11].

** Statistically different.

4. Conclusion

The proposed multivariate calibration methods were simple, rapid, sensitive and precise and could be easily applied in quality-control laboratories for the simultaneous determination of OLM, AML and HCT in pure bulk powders. Moreover, PCR and PLS could be applied for dosage form analysis as well as in pure powder form without any preliminary separation step.

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