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APPLICATION OF PCR AND PLS METHODS FOR THE SIMULTANEOUS DETERMINATION OF CANDESARTAN CILEXETIL AND HYDROCHLOROTHIAZIDE IN THEIR PHARMACEUTICAL PREPARATIONS

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Candesartan cilexetil (CND, an angiotensin II receptor 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, two multivariate calibration methods were applied for simultaneous spectrophotometric determination of CND and HCT in their combined pharmaceutical tablets The multivariate methods are principal component regression (PCR) and partial least squares (PLS). Both methods are useful in spectral analysis because of the simultaneous inclusion of many spectral wavelengths instead of the single wavelength used in derivative spectrophotometry. The optimum assay conditions were established and the proposed methods were successfully applied for the assay of the two 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 HPLC method.

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1. Introduction

Candesartan cilexetil (CND, Fig.1), 1-[[(cyclohexyloxy) carbonyl]oxy]ethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-benzimidazole-7-carboxylate is an angiotensin II receptor antagonist, selective for AT1 receptors, with tight binding to and slow dissociation from the receptor. It has no agonist activity. It is rapidly converted to the active substance, candesartan, by ester hydrolysis during absorption from the gastrointestinal tract [1].

Hydrochlorothiazide (HCT, Fig.1) is chemically known as 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7 sulfonamide which is a first line diuretic drug of the thiazide class. It acts by lowering blood pressure initially by increasing sodium and water excretion.This causes a decrease in extracellular volume, resulting in a decrease in cardiac output and renal blood flow. With long-term treatment, plasma volume approaches a normal value, but peripheral resistance decreases [2]

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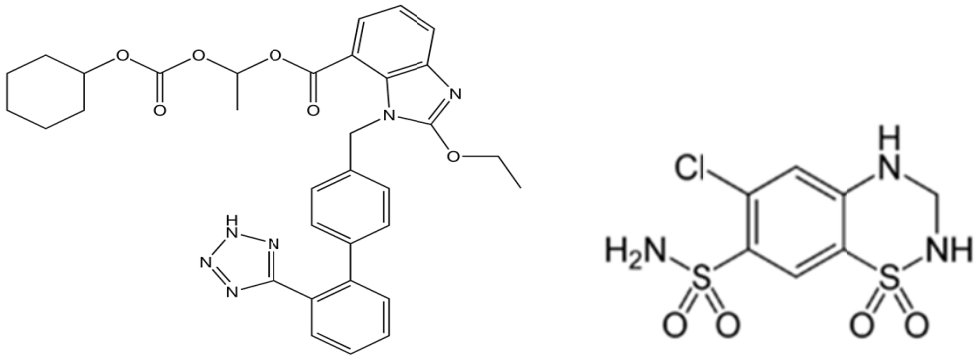

Candesartan cilexetil (CND) Hydrochlorothiazide (HCT)

Fig. 1. Chemical structures of candesartan cilexetil (CND) and hydrochlorothiazide (HCT).

The literature contains few methods for analysis of CND alone. Those reported methods include spectrophotometric [3-7], spectrflurometric[5,8], electrochemical[9] , HPLC[10-14], LC-MS [15] and capillary electrophoretic [16] methods. There were spectrophotometric [17-19], spectrflurometric [20] and HPLC [21-25] methods were proposed for the estimation of HCT.

Very few analytical methods such as spectrophotometric [26, 27], HPLC [28-32] have been developed for the determination of the two drugs in their combination.

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 [33].

In this paper we describe two simple, inexpensive, sensitive, and validated chemometric methods for the simultaneous determination of CND and HCT in pharmaceutical formulations. The applied chemometric methods are principal component regression (PCR) and partial least squares (PLS). The methods have been successfully used for quality control analysis of the drugs and for other analytical purposes.

2. Experimental

Chemicals and reagents

CND was kindly supplied from Jazeera Pharmaceutical Industries (JPI), Riyadh,Saudi Arabia. It was used as received without purification (its purity was 99.98 %). HCT was kindly provided by Chemipharm Pharmaceutical Industries (S.A.E 6th October city, Egypt). The purity was 99.80 %. It was used as received without further purification. Blopress®8 plus (Batch no156205) contains 8 mg CND and 12.5 mg HCT per tablet and Blopres®16 (batch no.156159) contains 16 mg CND and 12.5 mg HCT. These tablets were collected from the local market. The tablets manufactured by: The Arab Pharmaceutical Manufacturing Co. Ltd., Sult- Jordan. Methanol used throughout this study was of spectroscopic grade.

Instruments

The spectrophotometric measurements were made with Unicam UV/VIS Spectrophotometer no. UVA 051513 (England). The spectral bandwidth was 1.0 nm. Absorption spectra of samples were recorded using 1 cm Quarts matched cuvettes on a medium scan speed between 208-370 nm.

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.

Preparation of drugs standard solutions

Stock standard solutions of CND and HCT containing 1mg/mL were prepared separately in methanol .Working solutions were prepared (100 µg/mL) by suitably diluting the stock standard solutions with the same solvent.

Preparation of calibration (training) and validation (prediction) sets

Five level, two factor calibration design [34] was used for construction of 25 samples by transferring different volumes of CND and HCT from their standard working solutions into10 mL volumetric flasks and the solutions were diluted to the volume with methanol 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 CND: HCT in the Blopress® tablets. The absorption spectra of the 25 samples were scanned from 208 - 370 nm against methanol as a blank (Fig. 2) and transferred to Matlab for subsequent calculations. The noisy region from 200-230 nm and the near zero absorbance of CND and HCT after 320 nm accounted for the rejection of these parts from the spectra. The multivariate calibration models (PCR and PLS) were then constructed using the data obtained.

Table 1: The five level two factor experimental design of the training and validation set mixtures shown as concentrations of the mixture components in µg / mL

Mix No.	CND	HCT	Mix No.	CND	HCT
1	4	2.5	14	6	6.25
2	4	3.75	15	6	7.5
3	4	5	16	7	2.5
4	4	6.25	17	7	3.75
5	4	7.5	18	7	5
6	5	2.5	19	7	6.25
7	5	3.75	20	7	7.5
8	5	5	21	8	2.5
9	5	6.25	22	8	3.75
10	5	7.5	23	8	5
11	6	2.5	24	8	6.25
12	6	3.75	25	8	7.5
13	6	5			

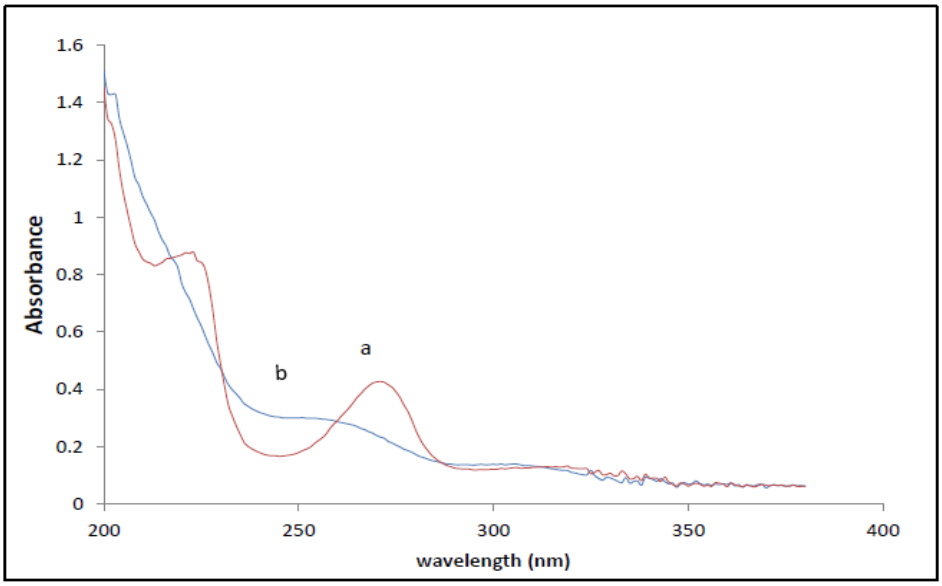

Fig. 2. UV absorption spectra of (a) 5 µg/mL of CND and (b) 6 µg/mL HCT in methanol.

Analysis of dosage forms

Fourteen tablets of Blopress®8 plus (8/12.5) or Blopress®16 (16/12.5) were powdered and mixed well; an accurately weighed amount of the powder equivalent to one tablet, was transferred into 100 mL volumetric flasks. 75 mL of methanol were added, sonicated for 30 min., filtered and then completed to volume with methanol. The solution was diluted to the same concentrations of the appropriate working solutions and proceeded according to the procedure mentioned above.

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

Cross validation (CV) [35] was applied to predict how many are the optimum number of 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 latent variables for PCR or PLS, 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

Blopress® tablets are combined dosage form containing the angiotensin II receptor blocker CND and the diuretic HCT. It has been used in the treatment of hypertension. This study was designed to develop simple, robust and accurate multivariate methods for the simultaneous

determination of CND and HCT in Blopress ® tablets. Because of the practical simplicity, and wide availability of spectrophotometry in quality control laboratories, it was attempted in this study. Multivariate calibration methods, such as PCR and PLS, 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. Determining of the number of factors (latent variables) to be used in the calibration is a key step in factor based techniques (PCR and PLS). Only those factors that contain analytical information must be kept. The discarded factors should contain noise only. The PLS-Toolbox software offers some indicators that could be used for determining the optimum number of latent variables. The cross validation procedure leaving out one sample at a time was applied to the mean centered data. The RMSECV was calculated as mentioned above. The root mean squared error of calibration (RMSEC) was also determined and calculated as follows:

$$\text{RMSEC} = \sqrt{\frac{\sum_1^n (C_i - \acute{C}_i)^2}{n-(q+1)}}$$

Where C_i is the measured concentration of the ith sample, $\acute{C}_i$is the predicated concentration for ith sample, using a model with q variables, and n is the number of samples in the calibration set [35]. To develop the PCR and PLS models, a rank of two latent variables was found to be the optimum rank for theses two chemometric models according to RMSECV and RMSEC values as shown in Table 2.

Table 2: Statistical parameters for the proposed multivariate methods for CND and HCT.

Parameters	Methods			
	PCR		PLS	
	CND	HCT	CND	HCT
RMSECV (µg ml-1)	0.1407	0.5616	0.1420	0.5616
RMSEC (µg ml-1)	0.1021	0.5324	0.1026	0.5324
RMSEP (µg ml-1)	0.1096	0.1371	0.0534	0.1452
Number of Latent variables	2	2	2	2

RMSECV: root mean squared error of cross validation.
RMSEC: root mean squared error of calibration.
RMSEP: root mean squared error of prediction.

The predictive ability of both models can be defined using several validation diagnostics. These include the mean squared error of prediction (RMSEP) which characterizes both the accuracy and the precision of prediction [35]. It is calculated as follow:

$$\text{RMSEP} = \sqrt{\frac{\sum_1^n (C_i - \tilde{C}_i)^2}{n}}$$

Where C_i is the true concentration. $\tilde{C}_i$is the predicted concentration and n is the total number of validation samples. The value of RMSEP is indicated in Table 2. The small value of the RMSEP indicates the negligible error of prediction and the high predictive ability of the proposed methods. The proposed methods were applied for determination of CND and HCT in the validation set (Table 3).

Table 3: Assay results for the prediction of the validation set for determination of CND and HCT by the proposed chemometric methods.

Compounds		Methods							
		PCR				PLS			
CND	HCT	CND		HCT		CND		HCT	
True		Found	R	Found	R	Found	R	Found	R
µg/ml		µg/ml	%	µg/ml	%	µg/ml	%	µg/ml	%
7	2.5	7.11	101.62	2.34	93.6	7.05	100.71	2.36	94.50
7	3.75	7.08	101.2	3.75	100	7.003	100.04	3.77	100.41
7	5	6.97	99.57	4.97	99.40	7.01	100.19	4.97	99.47
7	6.25	7.05	100.76	6.13	98.08	7.08	101.10	6.12	97.85
7	7.5	6.83	97.58	7.27	96.93	6.95	99.29	7.25	96.63
8	2.5	8.08	101	2.28	91.2	8.04	100.53	2.29	91.72
8	3.75	8.14	101.7	3.7	98.67	8.02	100.28	3.70	98.60
8	5	7.97	99.66	4.96	99.20	7.99	99.86	4.95	99.08
8	6.25	7.90	98.80	6.21	99.36	8.07	100.89	6.19	99.03
8	7.5	7.82	97.73	7.3	97.33	8.10	101.28	7.26	96.83
$\bar{X}$			99.96		97.38		100.43		97.41
S.D			1.54		2.85		0.62		2.63
%R.S.D			1.54		2.92		0.61		2.70

N.B. Each result is the average of three determinations.

The results of the proposed methods for the analysis of validation set (synthetic mixtures) were compared with those obtained by the reference method [31]. The reference method depends on the determination of CND and HCT by reversed phase HPLC method using tetra butyl ammonium hydrogen sulphate: methanol (15:85, V/V) as mobile phase at flow rate of 1mL/min and UV detection at 270 nm. Linearity was observed in the concentration range of 0.8-80 µg/mL and 0.65-62.5 µg/mL for CND and HCT respectively. Statistical analysis of the results obtained showed no significant difference in the performance of the two methods using student`s t test and F test values (Table 4).

Table 4: Assay of CND and HCT in synthetic mixtures using the proposed multivariate methods and reference methods.

Parameter	Proposed methods				Reference method [31]	
	PCR		PLS			
	CND	HCT	CND	HCT	CND	HCT
	101.62	93.60	100.71	94.50	99.60	100.74
	101.2	100.00	100.04	100.41	102.50	98.07
	99.57	99.40	100.19	99.47	100.80	101.30
	100.76	98.08	101.10	97.85	99.10	98.83
% Recovery	97.58	96.93	99.29	96.63		
	101	91.20	100.53	91.72		
	101.7	98.67	100.28	98.60		
	99.66	99.20	99.86	99.08		
	98.80	99.36	100.89	99.03		
	97.73	97.33	101.38	96.83		
$\bar{X}$	99.96	97.38	100.43	97.41	100.50	99.74
SD	1.54	2.85	0.62	2.63	1.51	1.53
Variance	2.37	8.12	0.38	6.92	2.28	2.34
Students t-test	0.60	2.00	0.10	2.06		
F-test	1.04	3.47	6.16	2.96		

Tabulated t- and F-values at p= 0.05 are 2.18 and 8.812 respectively.

The proposed method was successfully applied to the simultaneous assay of CND and HCT in commercial tablets (Blopress®8 plus (8/12.5) and Blopress®16 (16/12.5). The average percent recoveries of a certain defined concentration were based on the average of four replicate determinations (Table 5). The results shown in Table 6 are in good agreement with those obtained with the reference method [31].

Table5. Assay of CND and HCT in tablets using the proposed multiderivate methods.

Parameter	**Compound**		**Methods**							
			PCR				**PLS**			
	CND	HCT	CND		HCT		CND		HCT	
Blopress®8 plus	True µg/ml		Found µg/ml	R %	Found µg/ml	R %	Found µg/ml	R %	Found µg/ml	R %
	4	6.25	4.21	105.25	6.48	103.68	3.98	99.50	6.50	104
	4	6.25	4.10	102.50	6.43	102.88	3.85	96.25	6.45	103.2
	4	6.25	4.18	104.50	6.49	103.84	3.89	97.25	6.51	104.16
	4	6.25	4.15	103.75	6.55	104.80	3.93	98.25	6.57	105.12
$\bar{X}$				104		103.8		97.81		104.12
S.D.				1.17		0.79		1.39		0.79
%R.S.D				1.13		0.76		1.42		0.76
Blopress® 16 plus	8	6.25	7.99	99.88	6.21	99.36	7.99	99.86	6.18	98.88
	8	6.25	8.20	102.50	6.06	96.96	8.20	102.50	6.04	96.64
	8	6.25	8.28	103.50	6.09	97.44	8.29	103.63	6.07	97.12
	8	6.25	8.37	104.63	6.15	98.40	8.37	104.63	6.12	97.92
$\bar{X}$				102.63		98.04		102.33		97.64
S.D.				2.03		1.06		2.39		0.98
%R.S.D				1.98		1.08		2.34		1.00

N.B. Each result is the average of three determinations.

Table 6: Statistical comparison between the proposed multivariate methods and reference methods for the determination of CND and HCT in tablets.

Parameter	Proposed methods				Reference method [31]	
	PCR		PLS			
	CND	HCT	CND	HCT	CND	HCT
% Rec.	105.25	103.68	99.50	104	103.55	103.34
Blopress®	102.50	102.88	96.25	103.2	101.43	100.86
8 plus	104.50	103.84	97.25	104.16	100.70	99.06
	103.75	104.80	98.25	105.12	97.97	102.98
$\bar{X}$	104	103.8	97.81	104.12	100.91	101.56
SD	1.17	0.79	1.39	0.79	2.30	1.99
Variance	1.37	0.62	1.93	0.62	5.29	3.97
Students t-test	1.69	1.48	1.63	1.69		
F-test	3.86	6.40	2.74	6.40		
% Rec.	99.88	99.36	99.86	98.88	102.33	97.98
Blopress®	102.50	96.96	102.48	96.64	100.98	99.17
8 plus	103.50	97.44	103.57	97.12	101.45	99.96
	104.63	98.40	104.62	97.92	100.21	100.00
$\bar{X}$	102.63	98.04	102.33	97.64	101.25	99.28
SD	2.03	1.06	2.04	0.98	0.89	0.95
Variance	4.12	1.12	4.16	0.96	0.79	0.90
Students t-test	1.25	1.74	1.25	2.4		
F-test	5.22	1.24	5.27	1.06		

Tabulated t- and F-values at p=0.05 are 2.45 and 9.28 respectively.

4. Conclusion

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

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