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## ORIGINAL ARTICLE

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# Spectrometric determination of irbesartan and hydrochlorothiazide in tablets by principal component regression and partial least squares calibration approach

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**Abstract :** Two multivariate calibration-prediction techniques, principal component regression (PCR) and partial least squares (PLS) were applied to the spectrometric multicomponent analysis of the drug containing Irbesartan (IRB) and Hydrochlorothiazide (HCT) without any separation step. The selection of variables was studied. A series of synthetic solution containing different concentrations of

IRB and HCT were used to check the prediction ability of the PCR and PLS. The results obtained in this investigation strongly encourage us to apply these techniques for a routine analysis and quality control of the two drugs. © Global Scientific Inc.

**Keywords :** Irbesartan; Hydrochlorothiazide; Spectrometry; Multivariate calibration.

## INTRODUCTION

Irbesartan [IRB, 2-butyl-3-[[22-(1H-tetrazol-5-yl)[1,12-biphenyl]-4-yl]-methyl-1,3-diazaspiro[4,4]-non-1-en-4-one,] is an angiotensin II blocker. Angiotensin II receptor antagonists represent a relatively new pharmacological class<sup>[1]</sup> which acts mainly by selective blockade of AT1 receptors and reduces the effects of angiotensin II. They may be used alone or in combination with other antihypertensive or diuretic agents. Hydrochlorothiazide [HCT, 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide,] is a diuretic acting on distal convoluted tubule. Because of their synergis-

tic anti-hypertensive action, irbesartan and hydrochlorothiazide are available on the market as a combined dosage form.

The literature survey reveals that several methods were reported for the individual estimation of IRB and HCT. Several methods have been published for the determination of IRB and HCT in pharmaceutical formulations and biological samples including spectrometry<sup>[2]</sup>, spectrophotometric<sup>[3,4]</sup> voltammetry<sup>[5]</sup>, HPLC<sup>[6-10]</sup>, and LC-MS<sup>[11,12]</sup>.

During the last decade the powerful chemometric methods principal component regression (PCR) and partial least-squares (PLS) were used in spectral data analysis for the mixtures containing two or more

compounds with overlapping spectra<sup>[13-15]</sup>. These methods have wide range applications, e.g. spectrometric<sup>[16,17]</sup>, chromatographic<sup>[18]</sup> and electrochemical<sup>[19]</sup> quantitative analysis.

The multivariate calibration techniques use full spectrum, full automation, multivariate data analysis and the reduction of noise and the advantages of the selection calibration model. In addition these multivariate calibrations do not need any separation procedure, they are very cheap, very easy to apply and very sensitive. For these reasons these multivariate techniques are popular today.

In this study two chemometric methods were applied to analyse the synthetic mixtures and tablets consisting of IRB and HCT in the presence of interferences of the absorption spectra. The application of chemometrics allows the interpretation of multivariate data and is vital to the success of the simultaneous determination of the clinical drugs.

## EXPERIMENTAL

### Apparatus

A Shimadzu (Model UV-1700) UV-Visible spectrometer (Shimadzu, Kyoto, Japan), equipped with 1cm matched quartz cells was used for spectrometric measurements.

### Standard solutions

All materials used were of analytical grade. Stock solutions of 100 mg/100 mL IRB and HCT were prepared in methanol. The solutions were stable for the least two weeks if they had been stored

in a cool (< 25°C) and dark place.

### Pharmaceutical preparations

The commercial preparations; Co-Ýrda â tablet (produced by Nobel Pharm. Ind., Turkey, containing 300 mg irbesartan and 25 mg hydrochlorothiazide) per tablet were analyzed by the proposed chemometric techniques.

### Procedure for dosage forms

An accurately weighed pulverized tablets equivalent to 100 mg of the studied drugs was extracted with 10 mL of M Methanol, diluted with water, and sonicated for about 15 min. The extracts were filtered into 100 mL volumetric flasks then washed and diluted to volume with distilled water. Aliquots these solutions were transferred into a series of 10 mL volumetric flasks and the analysis were completed as spectrometric procedure. All the techniques were applied to the final solution.

## CHEMOMETRICS METHODS

### PCR and PLS

PCR and PLS are factor analysis multivariate statistical tools which have many of the full spectrum advantages and have been successfully applied to spectrophotometric analyses of multicomponent mixtures. PCR and PLS need a calibration step where the models for the spectra and the component concentrations of the unknown are estimated from the sample spectrum. Both of these methods involve spectral decomposition. The PCR decomposition is

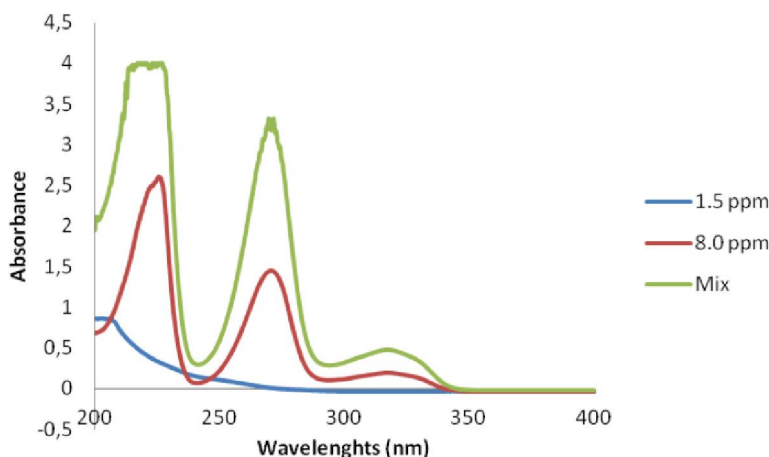


Figure 1 : Original absorption spectra of 1.5 µg/mL IRB, 8.0 µg/mL HCT and their mixture in methanol

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based entirely on spectral variations without regard for the component concentrations. In PLS, the spectral decomposition is weighted to the concentration. The major difference in the predictive abilities of these two methods is that PLS seems to predict better than PCR when there are random linear baselines or independently varying major spectral components which overlap with the spectral features of the analy-

sis. The optimal of calibration method depend on the particular experimental conditions. However, PLS seems to a reasonable choice over a wide range of conditions.

## RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra for IRB

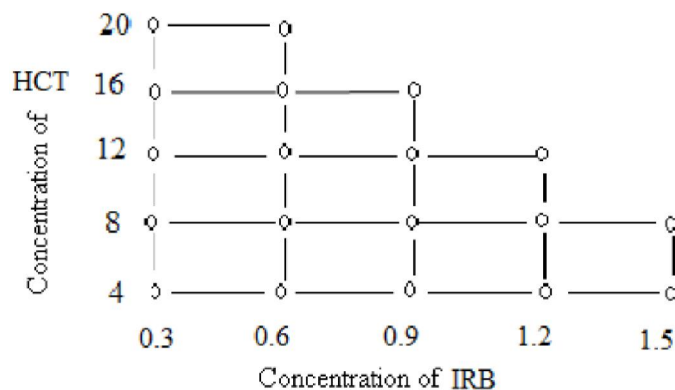


Figure 2 : Concentration set design for the preparation of PCR and PLS calibrations

TABLE 1 : Results obtained for IRB and HCT indifferent synthetic mixtures by using PCR technique

Mixture ( $\mu\text{g/mL}$ )		Recovery (%)			
IRB	HCT	IRB	HCT	IRB	HCT
0.3	4.0	0.27	4.03	90.88	100.81
0.6	4.0	0.68	4.33	114.41	100.83
0.9	4.0	0.86	4.04	95.58	101.03
1.2	4.0	1.17	3.97	97.79	99.40
1.5	4.0	1.51	3.91	101.29	97.90
0.3	8.0	0.27	7.90	91.09	98.85
0.6	8.0	0.58	8.10	96.67	101.32
0.9	8.0	0.86	8.12	96.36	101.56
1.2	8.0	1.21	7.95	101.65	99.44
1.5	8.0	1.47	7.91	98.56	98.90
0.3	12.0	0.31	11.88	102.27	99.06
0.6	12.0	0.57	11.94	96.07	99.51
0.9	12.0	0.87	12.06	97.35	100.57
1.2	12.0	1.17	12.10	97.50	100.84
0.3	16.0	0.26	15.98	87.54	99.92
0.6	16.0	0.64	15.93	108.10	99.57
0.9	16.0	0.80	16.07	89.81	100.48
0.3	20.0	0.27	19.99	90.29	99.95
0.6	20.0	0.56	20.00	94.79	100.01
			$\bar{X}$	97.26	100.00
			RSD*	6.51	0.96

RSD\*: Relative Standard Deviation.

and HCT and their mixture in methanol. A concentration set of 19 mixture solutions consisting of IRB and HCT in the concentration range of 0.3 - 1.5 and 4.0 – 20.0 µg/mL for IRB and HCT in the same solvent were symmetrically prepared from the prepared stock solutions respectively (Figure 2). Symmetric set of calibration is preferred. The reason for this is to minimize errors in calibration may occur during analysis. To check the proposed methods we used an independent validation set consisting of the synthetic mixture solutions of IRB and HCT in the above working concentration ranges. The absorbance data matrix were obtained by measuring at the 12 wavelengths with the intervals  $\Delta\lambda = 5$  nm in the 225 – 280 nm spectral region. The prepared calibrations of three techniques using the absorbance data sets were used to predict concentration of the unknown values of IRB and HCT in their mixture. Linearity range was 0.3-12.0 µg/mL for IRB and 4.0-20.0 µg/mL for HCT in the multivariate calibration proposed.

A calibration for each technique was computed

in the MAPLE 7.0 and PLS Toolbox 4.0 software by using set consisting of two drugs and their absorbance data. The multivariate calibrations of three techniques were used to predict the unknown concentrations of IRB and HCT in the samples.

Some statistical parameters were given for the validation of the constructed calibrations for the training set and synthetic binary mixtures of drug.

The application competence of a calibration model can be explained in several ways. We can also examine these results numerically. One of the best ways to do this by examining the predicted residual error sum-of-squares or PRESS. To calculate PRESS we compute the errors between the expected and predicted values for all the samples, square them, and sum them together.

$$\text{PRESS} = \sum_{i=1}^n (C_i^{\text{added}} - C_i^{\text{found}})^2$$

**Strikingly speaking, this is not a correct way to normalize the PRESS values when not all of the data sets contain the same number of samples. If we want**

**TABLE 2 : Results obtained for IRB and HCT indifferent synthetic mixtures by using PLS technique**

Mixture (µg/mL)		Recovery (%)			
IRB	HCT	IRB	HCT	IRB	HCT
0.3	4.0	0.30	4.01	101.75	100.36
0.6	4.0	0.70	4.04	117.65	101.11
0.9	4.0	0.77	4.07	86.60	101.83
1.2	4.0	1.15	3.97	95.92	99.38
1.5	4.0	1.56	3.89	104.11	97.35
0.3	8.0	0.26	7.92	87.59	99.01
0.6	8.0	0.61	8.09	102.11	101.15
0.9	8.0	0.91	8.09	102.16	101.20
1.2	8.0	1.17	7.98	98.01	99.77
1.5	8.0	1.52	7.91	101.70	98.89
0.3	12.0	0.32	11.87	107.60	98.98
0.6	12.0	0.63	11.93	105.47	99.45
0.9	12.0	0.89	12.01	99.51	100.10
1.2	12.0	1.14	12.18	95.20	101.50
0.3	16.0	0.30	16.03	100.99	100.23
0.6	16.0	0.65	15.91	108.37	99.44
0.9	16.0	0.84	16.05	94.28	100.31
0.3	20.0	0.30	19.98	100.41	99.91
0.6	20.0	0.60	20.00	100.50	100.03
			$\bar{x}$	100.52	100.00
			RSD*	7.10	1.08

RSD\*: Relative Standard Deviation.

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TABLE 3 : Statistical parameters in the calibration-prediction

Parameter	Method	IRB	HCT
PRESS	PCR	0.0299	0.0982
	PLS	0.0452	0.1156
SEP	PCR	0.0397	0.0719
	PLS	0.0487	0.0780
r	PCR	0.9913	0.9998
	PLS	0.9846	0.9998
Intercept	PCR	-0.0154	0.0019
	PLS	0.0023	0.0119
Slope	PCR	0.9998	1.0008
	PLS	0.9998	0.9846

TABLE 4 : Assay results for the pharmaceutical formulation (mg/tablet)

Drug	PCR	PLS
IRB		
Mean ± SD	298.50±1.50	299.48±1.20
HCT		
Mean ± SD	24.05±2.67	24.56±2.42

Results obtained are average of six experiments for each technique; \*SD : Standard deviation

correctly compare PRESS values for data sets that contain differing numbers of samples, we should convert to standard error of prediction (SEP), which is given by following formula.

$$SEP = \sqrt{\frac{\sum_{i=1}^n (C_i^{added} - C_i^{found})^2}{n-1}}$$

Where  $C_i^{added}$  the added concentration of drug is,  $C_i^{found}$  is the found concentration of drug and n is the total number of the synthetic mixtures. The SEP can provide a good measure of how well, on average, the calibration model performs. Often, however, the performance of the calibration model varies depending on the analyte level.

In the application of two chemometric techniques to the synthetic mixtures containing two drugs in variable compositions, the mean recoveries and relative standard deviations for PCR and PLS were found to be 97.26%, 6.50 and 100.52%, 7.10 respectively for IRB and 100.00% and 0.95, 100.00% and 1.08 respectively for HCT (TABLE 1 and 2).

According to the added concentration and the concentration found in samples, the SEP and PRESS values of PCR and PLS techniques were calculated 0.0397, 0.0299 and 0.0487, 0.0452 respectively for

IRB, 0.0719, 0.0982, and 0.0780, 0.1156 respectively for HCT (TABLE 3).

The linear regression analysis of the added concentration and the concentration found in the synthetic mixtures were realized for each drug and for each calibration technique. In this regression analysis, the correlation coefficient (r), intercept, slope and relative standard deviation values were found satisfactory for the proposed chemometric techniques in TABLE 3. As can be seen, all the statistic values indicated that all techniques are convenient for the determination of two drugs in synthetic mixtures.

A summary of the assay results for the pharmaceutical formulation is given TABLE 4. The results of all methods were very to each other as well as to the label value of commercial drug formulation.

## CONCLUSION

Two chemometric technique in spectrometric analysis, PCR and PLS, were proposed for the simultaneous determination of IRB and HCT in their binary mixtures. These techniques were applied with great success to two commercial pharmaceutical tablets. The resolution of highly overlapping drug mixtures was achieved by the use of PCR and PLS tech-

niques. A selection of working wavelength having high correlation values with concentration due to interference coming from matrix sample or additional analytes outside the working range. The proposed chemometric techniques can be applied for the routine analysis of two drugs in the tablet formulation without any a priori chemical separation and without time consuming.

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