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Spectrophotometric multicomponent analysis of irbesartan, hydrochlorothiazide and ramipril in pharmaceutical formulations by chemometric techniques

Abstract

Partial Least Squares (PLS) and Principal Component Regression (PCR) methods were proposed for the spectrophotometric analysis of a ternary mixture consisting of Irbesartan, Hydrochlorothiazide and Ramipril without prior separation. In these chemometric techniques the measurements of absorbance values were realized in the spectral range from 200-350 nm in the intervals of Dl=1nm at the 151 wavelengths in the zero order, first and second order spectra of the different ternary mixtures of these ingredients in 0.1 M NaOH. The prepared calibrations of both techniques using the absorbance data and concentration matrix data sets were used to predict the concentrations of the unknown concentrations of Irbesartan, Hydrochlorothiazide and Ramipril in their ternary mixture. The techniques were successfully applied to pharmaceutical formulations marked in India.

Keywords: irbesartan, hydrochlorothiazide, ramipril, chemometrics, simultaneous estimation, calibrations, absorbance, chemometric, spectrophotometric

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Abbreviations: PLS, partial least squares; PCR, principal component regression; IRB, irbesartan; HCZ, hydrochlorothiazide; RAM, ramipril; HPLC, high performance liquid chromatography; PLS, partial least squares regression; SEP, standard error prediction

Introduction

For the determination of Irbesartan (IRB), Hydrochlorothiazide (HCZ) and Ramipril (RAM), different analytical methods such as spectrophotometry,[1-6] spectrofluorimetry,[7,8] chemometrics,[9-14] high Performance Liquid Chromatography (HPLC),[15-35] LC-MS,[36-41] capillary zone electrophoresis[42] and High performance thin layer chromatography (HPTLC)[43-47] were reported individually or in combination with other drugs. But no method was reported so far for the simultaneous analysis of these drugs in ternary mixture. In comparison with conventional univariate methods, Multivariate spectrophotometric methods provide several advantages as it includes multiple variables for data analysis. These methods overcome the limitations of conventional techniques thereby applied to the simultaneous quantification of multiple analytes in mixtures.

For quantitative analysis of many pharmaceutical formulations, multivariate calibration methods like Partial Least Squares Regression (PLS) and Principal Component Regression (PCR) are predominantly used. These methods are based on the association between matricial algorithms and calibration data and the theoretical base of these methods are not fully explored by authors.

Due to the spectral overlapping simultaneous estimation of three components becomes difficult by conventional methods. Derivative spectrophotometry offers an effective quantification of multicomponent samples from their mixtures, as they resolve the common overlapping seen in the fundamental spectra. Hence derivative spectrophotometry offers greater sensitivity than the normal spectrophotometry in the simultaneous determination of two components without prior separation. An attempt has been made to overcome such spectral overlapping felt by three components in a ternary mixture by the combined use of derivative spectrophotometry and chemometric techniques.

Two multivariate procedures such as PCR and PLS algorithms are reported for the ternary mixture of drugs of interest. An effort was also made to compare the regression methods for fundamental spectra with that of first and second derivative spectra. From a calibration set comprising of 25 reference mixtures of selected analytes, the calibration models were built using a novel experimental design. In order to verify the prediction ability of the defined models, and external validation test was performed by using prediction set comprising of 10 synthetic mixtures of three analytes. The proposed methods were finally applied to the quantitative analysis of commercial samples containing one to three drugs to confirm their effectiveness in the routine quality control analysis of the real samples.

Materials and methods

Apparatus

A Perkin Elmer (Lamda 25) spectrophotometer controlled by UV winlab software and equipped with 1 cm pathlength quartz cell was used for the spectral data acquisition. The chemometric procedure was carried out using MATLAB software version 7.5 (The Mathworks) and PLS toolbox version 5.0 (Eigen Vector Technologies).

Chemicals

IRB, HCZ and RAM reference standards were kindly supplied by Madras Pharmaceuticals, Chennai, India. The tablets IROVEL H, Sun Pharmaceuticals, XARB-H, Hetero Labs (both containing 150 mg of IRB and 12.5 mg of HCZ) and Cardace#2.5, Aventi Pharma (containing 2.5 mg of RAM and 12.5 mg of HCZ) were procured from local pharmacies. All other chemicals were of analytical reagent grade and procured from SD Fine chemicals, Mumbai, India.

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Standard solutions

Standard stock solutions (1000µg/ml) of IRB, HCZ and RAM were prepared separately in the diluent 0.1M NaOH and water in the ratio 20:80. These solutions were taken and then diluted to 10 ml with water to give a final analyte concentration desired.

Sample preparation

Ten tablets were weighed and finely powdered in a mortar. A quantity of the powder equivalent to one tablet was accurately weighed and transferred into a 100ml volumetric flask including the diluent. The flask was sonicated for 15mins and diluted to the mark with diluents. An aliquot of the solution was centrifuged at 5000 rpm for 10mins. Appropriate amount of clear supernatant was transferred into a 10 ml flask and diluted with water. Then the absorbance values were measured.

Methods

Multivariate calibration methods

In multivariate calibration methods, a calibration model is built using the absorbance values and a concentration data set. These methods are designed based on the relationship between matrices of chemical data. Initially a calibration model is built using a chemical data set i.e. absorbance values and a concentration data set. The constructed calibration model is then used to estimate the concentration of the unknown samples in the prediction set. In the PLS and PCR methods, the original variables are transformed in to smaller number of orthogonal variables called factors or principal components which are in turn linear with the original variables.

From the prepared reference samples, a relationship between spectral and concentration data, representing the variables of the system can be built using the multivariate calibration techniques. Based on the regression method, the PCs and scores can be built specific to the new matrix. The PCs corresponds to the absorptivity values of the samples at various concentration and wavelength values, whereas the scores corresponds the numerical coefficients. This allows to build the mathematical model representing the reference spectra and able to predict the component concentrations of new samples.

Determination of PC number

The prediction power of the selected methods can be improved by determining the optimal number of factors. This can be achieved by employing a full cross validation also known as leave-one-out method. This method employs leaving one sample at a time from the calibration step and performs the calibration with all other samples. The concentration of the sample removed is then predicted with the obtained model. This step is in turn repeated for each sample considered. The procedure can be repeated after fixing a different number of factors. The standard error prediction (SEP) was chosen as an optimizing criterion to select the optimal number of factors. SEP represents an estimate of the error involved in the assay of external samples by using the model. Its value depends on the number of factors used for that calibration. The number of factors giving the minimal SEP was selected as the optimal number of factors.

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}\left(\frac{\hat{c}}{c_i^2}\right)}{n}}$$

Where $\hat{c}$ denotes the added drug concentration, C_i is the predicted drug concentration and n represents the total number of synthetic mixtures.

Table 1 Concentrations of IRB, HCZ and RAM used as Calibration and Prediction sets

Calibration set (µg/ml)			Prediction set (µg/ml)		
IRB	HCZ	RAM	IRB	HCZ	RAM
0.5	0.5	5	2	0.5	1.5
1	0.5	4	2	1	2.5
1.5	0.5	3	1	1.5	2
2	0.5	2	1.5	2	2.5
0.5	1	5	0	2.5	1.5
1	1	4	0.5	3	4
1.5	1	3	1.5	3	4
2	1	2	0	0	3
0.5	0.5	5	0	1	6
1	1.5	4	1	2	1.5
1.5	1.5	3	-	-	-
2	1.5	2	-	-	-
0	2	0	-	-	-
0.5	2	5	-	-	-
1	2	4	-	-	-
1.5	2	3	-	-	-
0.5	2.5	5	-	-	-
1	2.5	4	-	-	-
1.5	2.5	3	-	-	-
2	2.5	2	-	-	-
0.5	3	1	-	-	-
1	3	1.5	-	-	-
0	0	6	-	-	-
3	0	0	-	-	-
1.5	3	2	-	-	-

Results and discussion

Data Processing and model building

The UV absorption spectra of standard IRB, HCZ and RAM in 0.1 M NaOH is recorded in the range of 200-350nm in ordinary mode (Figure 1a), First derivative mode (Figure 1b) and second derivative mode (Figure 1c). Specific wavelengths for these components could not be signaled out from all the recorded spectra. Hence multivariate calibration methods appeared to be ideal to overcome the drawbacks, as the entire information from the full spectra is being utilized by these techniques.

A concentration of 25 mixtures of three compounds in the range of 0.5-3µg/ml of IRB and HCZ and 1-6 µg/ml of RAM were prepared in 0.1M NaOH (Table 1). The fundamental, first derivative and second derivative spectra of the proposed concentration set were recorded in the 200 -350 nm wavelength range. The absorption values of spectra of the concentration set were measured at the 151 wavelength points with Dl = 1 nm in the spectral region of 200 -350 nm. The concentration set and absorption data were considered as Y-block (25×3) and X-block (25×151) for the construction of PLS calibration using cross-validation procedure to reach the best calibration model. The calculations were done with PLS Toolbox 5.0 and the optimal factors were selected.

The PCR and PLS models obtained by using this training set were validated by full cross-validation and the SEP values were calculated each time that a new factor was added to the models. Table 2 & Figure 2 shows the selected numbers of factors and the corresponding SEP values. The square of the correlation coefficient (R2), which indicates the fraction of the total variance explained by the models, is also reported. The prediction values for IRB, HCZ and RAM in both models were found satisfactory (Figure 3).
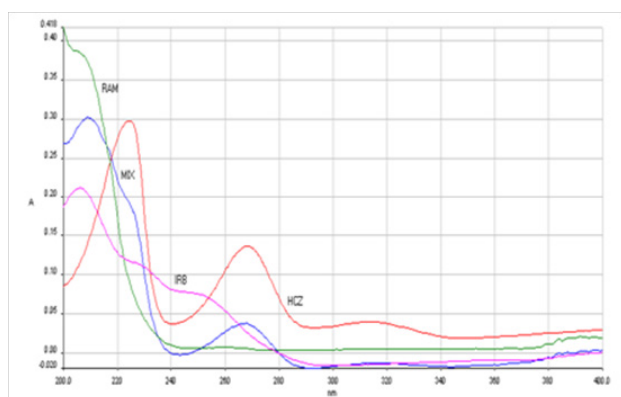
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Figure 1a Fundamental Absorption spectra of Irbesartan, Hydrochlorothiazide, Ramipril and mixture.
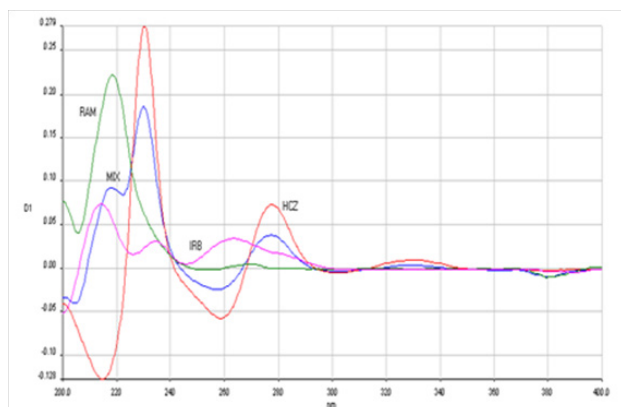

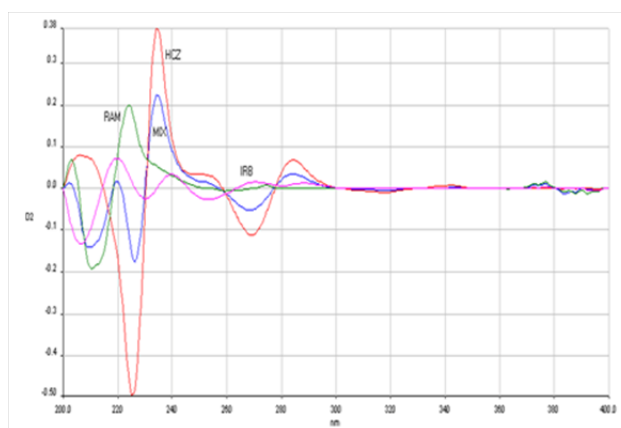**Figure 1b** First derivative spectra of Irbesartan, Hydrochlorothiazide, Ramipril and mixture.

Figure 1c Second derivative spectra of Irbesartan, Hydrochlorothiazide, Ramipril and mixture.

Application of PLS and PCR models to the Prediction set

In order to perform an external validation for the proposed PLS and PCR models, a set of 10 synthetic samples containing ternary and binary component mixtures of these selected drugs were prepared. The mixtures were prepared in the same concentration range as in the calibration set (Table 1). Such constructed prediction set was used to test the prediction ability of the models. The application of proposed PLS and PCR models to the prediction set gave the following results and are indicated in Table 3 & Figure 4.
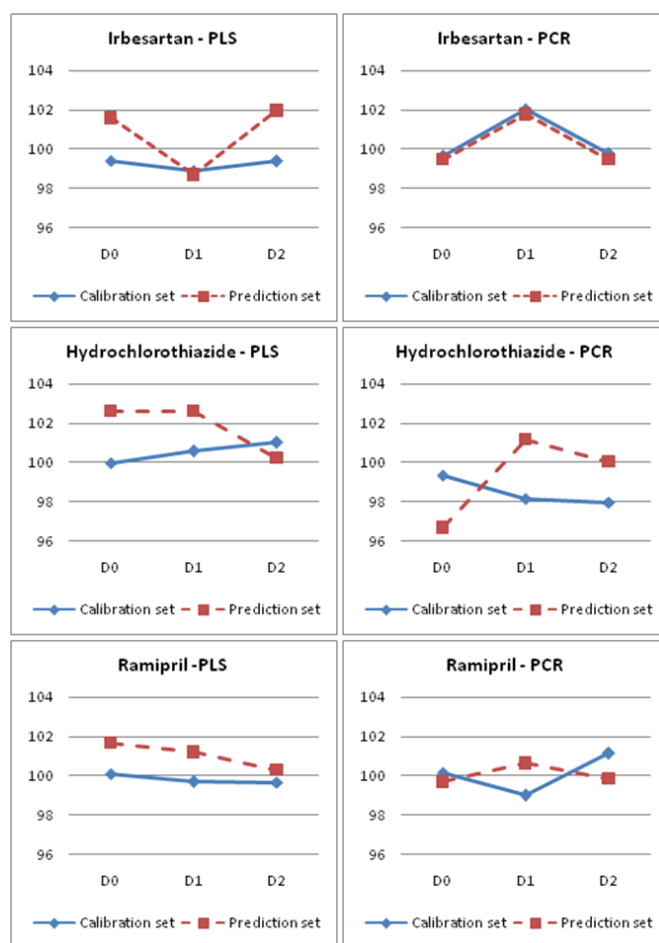

Figure 2 Comparison of Mean Recovery of PLS and PCR models for Fundamental (D0), First Derivative (D1) and Second Derivative (D2) Spectra.
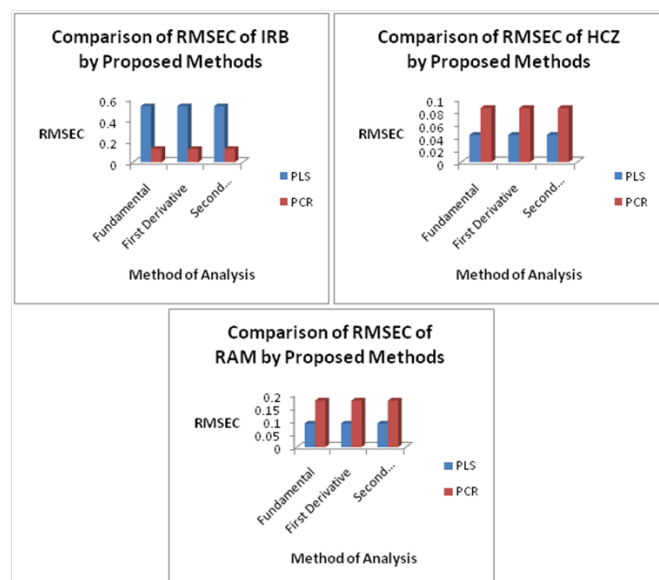

Figure 3 Comparison of Root Mean Square Error of Calibration (RMSEC) of PLS and PCR models for Fundamental (D0), First Derivative (D1) and Second Derivative (D2) Spectra.
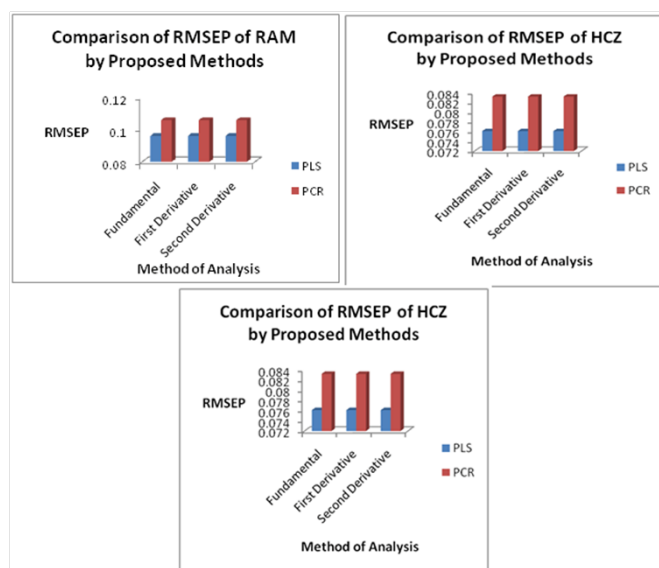
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Figure 4 Comparison of Root Mean Square Error of Prediction (RMSEP) of PLS and PCR models for Fundamental (D0), First Derivative (D1) and Second Derivative (D2) Spectra.
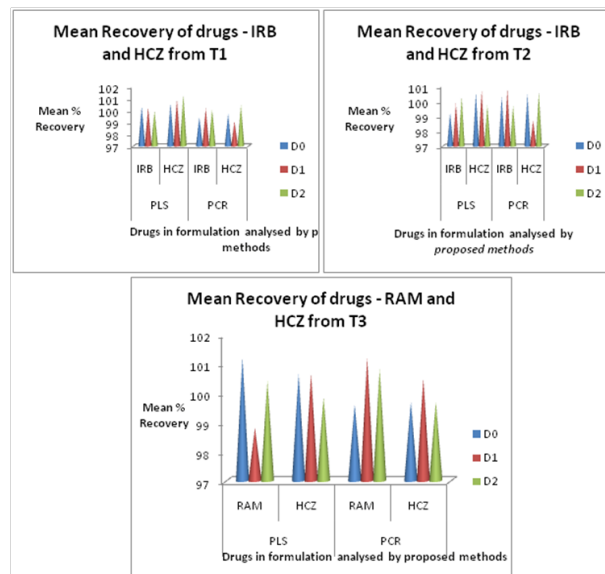

Figure 5 Comparison of % Recovery of drugs from formulations by PLS and PCR models for Fundamental (D0), First Derivative (D1) and Second Derivative (D2) Spectra.

Table 2 Statistical Parameters calculated from application of PLS and PCR methods to calibration samples

Drug	Parameter	PLS			PCR		
		D^0	D^1	D^2	D^0	D^1	D^2
IRB	Mean	99.436	98.919	99.396	99.634	99.634	99.634
	RMSEC	0.5298	0.5298	0.5298	0.1262	0.1262	0.1262
	R^2	0.9943	0.9943	0.9943	0.9685	0.9685	0.9685
HCZ	Mean	99.979	100.576	101.018	99.34	99.34	99.34
	RMSEC	0.0431	0.0431	0.0431	0.0857	0.0857	0.0857
	R^2	0.9979	0.9979	0.9979	0.9919	0.9919	0.9919
RAM	Mean	100.111	99.695	99.689	100.165	100.165	100.165
	RMSEC	0.0892	0.0892	0.0892	0.1761	0.1761	0.1761
	R^2	0.9968	0.9968	0.9968	0.9877	0.9877	0.9877

Mean, Mean of 25 samples of calibration set; RMSEC, root mean square error of calibration set;
R^2, correlation coefficient obtained by plotting amount present against amount estimated.

Table 3 Accuracy (% recovery) and Precision (SD) results from application of optimized PLS and PCR models on the prediction set

Drug	Parameter	PLS			PCR		
		D^0	D^1	D^2	D^0	D^1	D^2
IRB	Mean % Recovery	101.5939	101.5939	101.5939	99.0195	99.0195	99.0195
	RMSEP	0.1481	0.1481	0.1481	0.0808	0.0808	0.0808
	SD	5.8949	5.8949	5.8949	4.4397	4.4397	4.4397
HCZ	Mean % Recovery	102.6194	102.6194	102.6194	96.6958	96.6958	96.6958
	RMSEP	0.0761	0.0761	0.0761	0.0832	0.0832	0.0832
	SD	4.8994	4.8994	4.8994	6.3584	6.3584	6.3584
RAM	Mean % Recovery	101.6947	101.6947	101.6947	99.6934	99.6934	99.6934
	RMSEP	0.0961	0.0961	0.0961	0.1059	0.1059	0.1059
	SD	2.647	2.647	2.647	3.7579	3.7579	3.7579

Mean % Recovery, mean of 10 samples of prediction set; RMSEP, root mean square error of prediction set; SD, standard deviation

The results of PLS model includes mean % recovery ranging from 98.919 to 101.018 for all the three drugs in the calibration set and from 98.704 to 102.619 in the prediction set. The SD values were found to increase with increasing spectral mode in the calibration set but seem to decrease with increasing spectral mode in the prediction set. R^2 value ranges from 0.9932-0.9979 in the calibration set and from 0.9635-0.9988 in the prediction set. RMSEC value ranges from 0.0431-0.1302 for all three drugs in the calibration set. RMSEP value ranges from 0.0469-0.1481 for all three drugs in the prediction set.

The results of PCR model includes mean % recovery ranging from 98.919 to 101.018 for all the three drugs in the calibration set and from 98.704 to 102.619 in the prediction set. The SD values were found to increase with increasing spectral mode in the calibration set but seem to decrease with increasing spectral mode in the prediction set. R^2 value ranges from 0.9932-0.9979 in the calibration set and from 0.9635-0.9988 in the prediction set. RMSEC value ranges from 0.0431-0.1302 for all three drugs in the calibration set. RMSEP value ranges from 0.0469-0.1481 for all three drugs in the prediction set.

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Table 4 Assay Results of optimized PLS and PCR models on Pharmaceutical preparations

Method	Tablet	Mode	IRB		HCZ	
			Amount Found* (mg/tab) ± SD	% Recovery	Amount Found* (mg/tab) ± SD	% Recovery
PLS	T1	D0	150.45 ± 0.21	100.3	12.57 ± 0.11	100.56
		D1	150.32 ± 0.11	100.21	12.61 ± 0.13	100.88
		D2	149.88 ± 0.08	99.92	12.66 ± 0.13	101.28
	T2	D0	148.82 ± 0.05	99.21	12.57 ± 0.05	100.56
		D1	149.76 ± 0.12	99.84	12.59 ± 0.11	100.72
		D2	150.43 ± 0.04	100.28	12.47 ± 0.03	99.76
PCR	T1	D0	149.11 ± 0.11	99.4	12.46 ± 0.04	99.68
		D1	150.21 ± 0.23	100.14	12.38 ± 0.13	99.04
		D2	150.08 ± 0.06	100.05	12.55 ± 0.12	100.4
	T2	D0	150.42 ± 0.03	100.28	12.56 ± 0.06	100.48
		D1	151.22 ± 0.07	100.81	12.34 ± 0.23	98.72
		D2	149.53 ± 0.03	99.68	12.57 ± 0.04	100.56

Method	Tablet	Mode	RAM		HCZ	
			Amount Found* (mg/tab) ± SD	% recovery	Amount Found* (mg/tab) ± SD	% Recovery
PLS	T3	D0	2.53 ± 0.04	101.2	12.58 ± 0.11	100.64
		D1	2.47 ± 0.03	98.8	12.58 ± 0.03	100.64
		D2	2.51 ± 0.03	100.4	12.48 ± 0.04	99.84
PCR	T3	D0	2.49 ± 0.04	99.6	12.46 ± 0.03	99.68
		D1	2.53 ± 0.03	101.2	12.56 ± 0.02	100.48
		D2	2.52 ± 0.02	100.8	12.46 ± 0.04	99.68

*Average of six estimations

T1 - IROVEL H - Sun Pharmaceuticals, J&K, Irbesartan-150 mg and Hydrochlorothiazide-12.5 mg

T2 - XARB H - Hetero Labs, HP, Irbesartan-150 mg and Hydrochlorothiazide-12.5 mg

T3 - Cardace # 2.5 - Aventis Pharma, Goa, Ramipril-2.5 mg and Hydrochlorothiazide-12.5 mg

Table Abbreviations: PLS, partial least square; PCR, principal component regression; IRB, irbesartan; HCZ, hydrochlorothiazide; RAM, ramipril; D^0, fundamental/zero order; D^1, first order; D^2, second order; RMSEC, root mean square error of calibration; RMSEP, root mean square error of prediction; SD, standard deviation; R^2, correlation coefficient

Analysis of commercial formulations

The proposed PLS and PCR models were applied to the assay of binary pharmaceutical formulations. The assay results are summarized in Table 4 & Figure 5. A good coincidence was observed between experimental results and label claim of the pharmaceutical formulations. Application of models from fundamental to second derivative spectra gave recovery between 98.72 to 101.20% and SD of not more than 1.0. No interference from the excipients was observed.

Conclusion

The spectrophotometric data of complex mixtures results in severe spectral overlapping thereby imposing difficulty in simultaneous analyses. Multivariate methods such as PCR and PLS overcomes the difficulty and proves to be valid analytical tools for quantitative estimation of analytes from complex mixtures. The prediction ability of the multivariate methods can be further magnified by acquisition of derivative spectra. The application of PLS and PCR methods on zero to second order derivative spectra of ternary mixtures of IRB, HCZ and RAM has been accomplished. Very satisfactory results were obtained when the optimized models were applied to the synthetic mixtures and commercial formulations. According to these studies, multivariate calibration methods (PLS and PCR) coupled with derivative spectral data can be recommended as a very suitable choice to resolve severe overlapped absorption spectra of drug mixtures. This approach is simple in application, inexpensive, requires an easy treatment of the samples and provides reliable analytical results.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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