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SIMULTANEOUS ESTIMATION OF CILOSTAZOL AND TELMISARTAN USING PCR, PLS, CLS AND ILS

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ABSTRACT

Simple, accurate, sensitive and precise four multivariate calibration approaches, comprising principal component regression (PCR), partial least square (PLS), Classical least square (CLS) and Inverse least square (ILS) have been used for the determination of cilostazol and telmisartan simultaneously. This methods are useful in spectral investigation due to the simultaneous inclusion of many spectral wavelengths instead of the single wavelength used in derivative spectrophotometry, thus a inordinate enhancement in the precision and projecting abilities of these multivariate calibrations is identified. A calibration set was assembled for the mixture and the best model was used for the calculation of the concentration of the designated drug. The projected procedures were useful efficaciously in the determination of cilostazol and telmisartan in laboratory-prepared mixtures. These chemometric calibrations for zero-order spectra were created by measuring the absorbance at full spectral points in the wavelength range 210–314 nm for a preparation set containing 5–25 µg/ml cilostazol, 5–25 µg/ml telmisartan in methanol. The chemometric calculations were accomplished by using the Unscrambler X 10.3 along with MATLAB 6. The results of four chemometric methods were statistically matched with each other. Mean recoveries (percent) and relative standard deviation of PCR, PLS, CLS, ILS methods were found to be 98.77/1.76, 100.59/1.53, 97.91/1.50, 97.53/1.73 for CLZ and 99.79/1.22, 100.22/0.58, 100.31/1.68 for TLM. All of the

four chemometric methods established and validated in this study can be satisfactorily used for the quantitative investigation of cilostazol and telmisartan in prepared synthetic mixture.

KEYWORDS: Cilostazol (CLZ), Telmisartan (TLM), Chemometrics, Spectrophotometry.

INTRODUCTION

The expansion of chemometric methods of simultaneous analysis has allowed the determination of the complex spectra of mixtures of analytes.[1] The chemometric quantitative analytical techniques have many applications and benefits such as the mixtures can be analyzed without any separation processes for drug determination.[2-7] The techniques are very easy to apply, very sensitive, useful and yet very economical as compared to other analytical techniques for simultaneous determination of compounds. These methods provide additional compensations where calibration can be achieved by discounting the concentration of all other components excluding the analytes of concern and also the speed in the determination of components in a mixture.[8] In recent years, multivariate calibration techniques, such as CLS, ILS, PCR and PLS, ongoing to be applied to the analysis of analytical data obtained in all instrumentations.[9]

Several method for approximation of Cilostazol and telmisartan either alone or in combination with other drug has been testified. Estimation of cilostazol and telmisartan individual and in combination with other drug in marketable formulation was carried by HPTLC.[10-11], Spectrophotometric determination[12-15], RP-HPLC[16-17] and HPLC.[18] Although individual and in combination with other drug method has been developed to quantify Cilostazol and Telmisartan but no method been established for simultaneous estimation of cilostazol and telmisartan in combined dosage form.

Cilostazol (CLZ) (Fig. 1) is chemically known as 6-[4-(1-cyclohexyl-1H-tetrazol-5-y1) butoxy]-3, 4-dihydro-2 (1H) – quinolinone and is a quinolinone derivative that obstructs cellular phosphodiesterase III, and is used for the inhibition of platelet aggregation and as a vasodilator. Telmisartan (TLM) (Fig. 2) is chemically known as 4' - ([4-methyl-6-(1-methyl-lH-benzimidazol-2yl) - 2-propyl-lH-benzimidazol-l-yl] methyl}-2- biphenyl carboxylic acid. Telmisartan is a new angiotensin II receptor antagonist for the treatment of essential hypertension and useful in the treatment of mild to moderate hypertension, well tolerated with a lower incidence of cough than ACE inhibitors.

In present research paper, we have reported the investigation and development of four rapid chemometrics assisted UV-spectrophotometric analytical methodology for the simultaneous determination of cilostazol (CLZ) and telmisartan (TLM).

EXPERIMENTAL

Instruments and Software

A Shimadzu model 1700 (Japan) double beam UV-Visible spectrophotometer with spectral width of 1 nm, wavelength accuracy of ± 0.1 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (Ver.2.1). The samples were weighed on electronic analytical balance (A×120, shimadzu). All the Chemometrics calculation were done using the software MATLAB Version 6.1.0.450 Release 12.1, The Mathworks, Inc. (for CLS and ILS) and The unscrambler X 10.1, CAMO software (for PCR and PLS).

Reagents and Chemicals and

Telmisartan was produced as gift sample from Alembic pharmaceutical, Vadodara and Cilostazol was purchased from Swapnroop drug Pvt. Ltd Bombay. AR grade Methanol supplied by Spectrochem pvt ltd, Mumbai.

Selection of common solvent

After checking the solubility of drugs in different solvents methanol has been selected as Common solvent for developing spectral characteristics.

Selection of detection wavelength

Solution were scanned over the range of 200-400 nm. It was observed that the drugs showed maximum absorbance at 258 nm for Cilostazol and 296 nm for Telmisartan were selected as the wavelength for detection.

Preparation of standard stock solution

The standard stock solutions of Cilostazol and Telmisartan were prepared by dissolving 25 mg of each drug in methanol and final volume was adjusted with same solvent in 25 mL of volumetric flask to get a solution containing 1000 µg/mL of each drug. From the above solution, further dilute 10 ml of stock solution up to 100 ml in volumetric flask to get second stock of 100 µg/mL.

Construction of Calibration and Validation Set

The calibration and validation mixtures were prepared by mixing the CLZ and TLM solutions in different ratios varying within their individual linearity range viz. 4 - 24 µg/ml and 1 - 6 µg/ml respectively. The concentration of combinations was decided by design expert 8.0.4 software under two factorial design. A total of 26 sets were prepared out of which 16 were calibration sets (Table. 1) whereas, the rest 12 served as validation sets (Table. 2). All the mixtures were scanned at 200 - 400 nm range.

Preparation of synthetic mixture

Synthetic mixture was prepared using various the excipients in the pharmaceutical oral synthetic mixture (CLZ 40 mg & TLM 10 mg).Inactive ingredients of the formulation include MCC, copovidone, SSG, Mg stearate, cornstarch & Talc.

Inverse least squares (ILS)

This method treats concentration as a function of absorbance. The inverse of Beer's law model for m calibration standards with spectra of n digitized absorbance is given by.[24, 25]

$$C = A * P \quad (1)$$

Where, C and A are as before, P is the n × 1 matrix of unknown calibration co-efficient relating the 1 component concentrations of the spectral intensities. Since in ILS the number of wavelengths cannot exceed the total number of calibration mixtures, stepwise multiple linear regressions have been used for the selection of wavelengths.

Once we have matrices A and C, we can determine P by following equation, where Pseudo inverse of matrix A is calculated,

$$P = pinv(A)*C \quad (2)$$

Classical least squares (CLS)

CLS is one of the simplest methods, based on a linear relationship between the absorbance and the component concentrations at each wavelength. In matrix notation, the Beer's law models for m calibration standards containing l chemical components with spectra of n digitized absorbance is given by.[22, 23]

$$A = C * K \quad (3)$$

Where A is the m x n matrix of calibration spectra, C is the m x l matrix of component concentrations, K is the l x n matrix of absorbance-concentration proportionality constants (absorptivity-path length).

Once we have matrices A and C, we can determine P by following equation, where Pseudo inverse of matrix C is calculated,

K = *pinv* (C)*A (4)

Principal component regression (PCR)

In the spectral work, the following steps can explain the fundamental concept of PCR.[26]

(a) The original data obtained in absorbance (A) and concentrations (C) of analytes have been reprocessed by mean centering as $\mathbf{A_0}$ and $\mathbf{C_0}$, respectively.

(b) The covariance dispersion matrix of the centered matrix $\mathbf{A_0}$ was computed. The normalized eigenvalues and eigenvectors were calculated starting from square covariance matrix. The number of the optimal principal components (eigenvectors) is selected by considering only the highest values of the eigenvalues. The other eigenvalues and their corresponding eigenvectors are eliminated from our study.

Using the ordinary linear regression **C** = a + b x **A,** we calculated the coefficients a and b. To reach this objective firstly we determined the coefficient b as b = **P** x q, where P is the matrix of eigenvectors and q is the C-loadings given by q = **D** x $\mathbf{T}^{\mathbf{T}}$ x A_0. Here $\mathbf{T}^{\mathbf{T}}$ is the transpose of the score matrix **T**. **D** is a diagonal matrix having on the components the inverse of the selected eigenvalues. Knowing b we can easily find a by using the formula a = **C**mean — A^Tmean x b, where A^Tmean represents the transpose of the matrix having the entries of the mean absorbance values and **C**mean is the mean concentration of the calibration set.

Partial least squares (PLS)

The PLS calibration technique using the orthogonalized PLS algorithm developed by Wold[27, 28] and extensively discussed by Martens and Naes[29] involves simultaneously the independent and the dependent variables on the data compression and decomposition operations.

In the UV-Vis spectra, the absorbance data (A) and concentration data (C) are mean centered to give data matrix A0 and vector C0. The orthogonalized PLS algorithm has the following steps.[30]

(a) The loading weight vector W has the following expression:

$W = A'_0C_0/C'_0C_0$ (5)

(b) The scores and loadings are given by

$t_1 = A_0W_1$, (6)

$P_1 = (A_0^T t_1) / (t_1^T t_1)$, (7)

$q_1 = (C_0^T t_1) / (t_1^T t_1)$, (8)

(c) The matrix and vector of the residuals in A_0 and C_0 are

$A_1 = A_0\text{-}t_1 P_1^T$ (9)

$C_1 = C_0\text{-}t_1 q_1$, (10)

(d) From the general linear equation, the regression coefficients were calculated by

$b = W (P^T W)^{-1} q$, (11)

$a = C_{mean} – A^T_{mean} b$, (12)

As in PCR method, the builded calibration equation is used for the estimation of the compounds in the samples.

RESULTS AND DISCUSSION

The calibration set of 16 standard mixture solutions which contain the concentrations with different ratio of CLZ and TLM was randomly prepared within the linearity range of two drugs. The UV absorbance data was obtained by measuring the absorbances in the region of nm. By using the correlation between calibration concentrations and its absorbance data, the chemometric calibrations were calibrated within the CLS, ILS, PCR and PLS algorithms. The quality of multi-component analysis is dependent on the wavelength range, spectral mode used, calibration set chosen and calibration range. In this experiment range of wavelength selected for estimation is 240 to 314with the interval of 2 nm.

Fig. 3 shows the zero-order overlay spectra of CLZ and TLM as well as their corresponding binary mixture in methanol. As shown in the (Fig. 3) the spectra are overlapped in the region of their absorption maxima. Direct ultraviolet spectrophotometry cannot be used to determine the two compounds individually in their mixtures but the chemometric method seemed to offer great potential. For this reason to solve overlapped spectra, four chemometric calibrations using the zero-order spectra have been applied.

The cross validation and validation statistical parameters obtained after applying PLS, PCR, CLS and ILS to the spectrophotometric data are shown in Table. 3, showing reasonably low absolute and relative root-mean square errors of prediction. The set of 12 validation samples prepared as described above was analyzed by the proposed procedure. The results suggest that the present method is accurate in concern to the validation samples, as suggested by the low RMSEP value for this validation set. The plot of Actual Vs. predicted Concentration of both the drug CLZ (Fig. 4) and TLM (Fig. 5) indicate that the developed method is accurate and precise.

As shown in (Fig. 6) it can be observed that as the number of factors increases, explained variance in Y increases and RMSEP decreases. Factors 2 gave satisfactory results in terms of explained variance, residual variance and RMSEP. The value of explained variance in Y (Fig. 8) and Residual variance (Fig. 7) are tabulated in Table. 10**.** Thus, 2 factors were selected for prediction. The concentrations of validation set were successfully predicted using this this factor in PLS method.

From Fig. 6 it can be observed that as the number of PC increases, explained variance in Y increases and RMSEP decreases. 2 PCs gave satisfactory results in terms of explained variance, residual variance and RMSEP. From the plot of explained variance in Y (Fig. 8) and residual variance (Fig. 7) and from the value of explained variance and residual variance (Table. 9**)** the 2 PCs were selected for prediction. The concentrations of validation set were successfully predicted using PC 2 PCR method.

Statistical Parameter

The predictive applicability of a regression model is described in various ways. The most general expression is the standard error of prediction (SEP) and standard error of calibration denoted by RMSEP which is given in the following formula.

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{Added} - C_i^{Found})^2}{n}}$$

Here C_i^{Added} is the added concentration of drugs, C_i^{Fouund} is the predicted concentration of drugs and n is the total number of the synthetic mixtures. The RMSEP results obtained by applying CLS, ILS, PCR and PLS to the above mentioned validation set of the synthetic mixtures are quoted in Table 3.

To check the validity (predictive ability) of the calibration models, the simultaneous analysis of the prediction set containing 16 samples of various concentrations of CLZ and TLM were carried out. The maximum values of the mean percent errors corresponding to CLS, ILS, PCR and PLS for the same mixtures were completely acceptable because of their very smallest values. The mean recoveries and the relative standard deviations of four proposed methods were computed and indicated in Table. 4-7.

Their numerical values were completely acceptable because of their smallest values and hence found satisfactory for the validity of all calibration methods. The linearity of the proposed chemometric method for determination of CLZ and TLM was evaluated by analyzing a series of different concentrations of standard drug. The linearity was found to be ranging between 1-25 µg/ml for TLM and 1-40 µg/ml for CLZ. Each concentration was repeated four times. The results obtained were compared with expected results. The good mean recoveries and relative standard deviation mentioned in Table. 4-7 suggested good accuracy of the proposed methods and no interference from formulations excipients. The selectivity of the proposed method was also assessed by the analysis of synthetic mixtures, the result of synthetic mixture study were mentioned in Table. 8. Where satisfactory results were obtained over the stated calibration range.

TABLE LEGENDS

Table. 1: Composition of the concentration (Calibration) set

Table. 2: Composition of the concentration (Validation) set

Table. 3: Root Mean Square Error of Prediction

Table. 4: Analysis of validation set by PCR method

Table. 5: Analysis of validation set by PLS method

Table. 6: Analysis of validation set by CLS method

Table. 7: Analysis of validation set by ILS method

Table. 8: Analysis of synthetic mixture

Table. 9: Explained Y variance and Residual variance (PLS)

Table. 10: Explained Y variance and Residual variance (PCR)

FIGURE LEGENDS

Figure. 1: Chemical structure of Cilostazol

Figure. 2: Chemical structure of Telmisartan

Figure. 3: zero-order overlay spectra of CLZ, TLM and their corresponding binary mixture

Figure. 4: Plot of actual vs. Predicted concentration obtained by PCR, PLS, CLS and ILS for Cilostazol

Figure. 5: Plot of actual vs. Predicted concentration obtained by PCR, PLS, CLS and ILS for Telmisartan

Figure. 6: Root Mean Square error of prediction of Validation for PCR and PLS method

Figure. 7: Total residual Y variance for PCR and PLS

Figure. 8: Total explained Y variance for PCR and PLS

Table. 1

No of Mixture	Concentration (µg/ml)	
	Telmisartan	Cilostazol
1	2	12
2	4	12
3	2	8
4	3	16
5	4	20
6	5	16
7	2	20
8	3	8
9	2	16
10	5	8
11	4	16
12	4	8
13	3	20
14	5	20
15	3	12
16	5	12

Table. 2

No of Mixture	Concentration (µg/ml)	
	Telmisartan	Cilostazol
1	3	18
2	3	10
3	2	14
4	4	14
5	5	10
6	2	10
7	4	10
8	3	14
9	5	10
10	2	8

Table. 3

RMSEP				
Method	**PCR**	**PLS**	**CLS**	**ILS**
CLZ	0.067408	0.067408	0.111665	0.106792
TLM	0.012087	0.012087	0.071533	0.029835

Table. 4

Added Conc. (µg/ml)		Measured conc.*(µg/ml)		Recovery (%)	
CLZ	**TLM**	**CLZ**	**TLM**	**CLZ**	**TLM**
18	3	18.0094	3.028	100.0522	100.93333
10	3	10.0879	3.0179	100.879	103.93
14	2	14.004	1.9743	100.0286	98.715
14	4	13.917	3.973	99.40714	99.325
10	5	10.0958	4.9698	100.958	97.396
10	2	10.2083	2.0597	102.083	102.985
10	4	10.2236	3.9581	102.236	98.9525
14	3	14.4581	3.0651	103.2721	102.17
10	5	10.3501	5.0271	103.501	100.542
8	2	7.9316	2.0302	99.145	103.01
Mean				**101.1562**	**100.5125**
%RSD				**1.5354**	**1.421**

Table. 5

Added Conc. (µg/ml)		Measured conc.*(µg/ml)		Recovery (%)	
CLZ	**TLM**	**CLZ**	**TLM**	**CLZ**	**TLM**
18	3	18.0094	3.028	100.0522	100.93333
10	3	10.0879	3.0179	100.879	103.93
14	2	14.004	1.9743	100.0286	98.715
14	4	13.917	3.973	99.40714	99.325
10	5	10.0958	4.9698	100.958	97.396
10	2	10.2083	2.0597	102.083	102.985
10	4	10.2236	3.9581	102.236	98.9525
14	3	14.4581	3.0651	103.2721	102.17
10	5	10.3501	5.0271	103.501	100.542
8	2	7.9316	2.0302	99.145	103.01
Mean				**101.1562**	**100.5125**
%RSD				**1.5354**	**1.421**

Table. 6

Added Conc. (µg/ml)		Measured conc.*(µg/ml)		Recovery (%)	
CLZ	**TLM**	**CLZ**	**TLM**	**CLZ**	**TLM**
18	3	18.0218	3.0259	100.1211	100.8633
10	3	10.1092	3.0212	101.092	100.7067
14	2	13.9157	1.9731	99.39786	98.655
14	4	13.9119	3.9755	99.37071	99.3875
10	5	10.0956	4.9762	100.956	99.524
10	2	10.176	2.0611	101.76	103.055
10	4	10.1816	3.9629	101.816	99.0725
14	3	14.1273	3.0655	100.9093	102.1833
10	5	10.0605	5.0336	100.605	100.672
8	2	7.9332	2.0229	99.165	101.145
Mean				**100.2152**	**100.8399**
%RSD				**1.139934**	**1.485014**

Table. 7

Added Conc. (µg/ml)		Measured conc.*(µg/ml)		Recovery (%)	
CLZ	**TLM**	**CLZ**	**TLM**	**CLZ**	**TLM**
18	3	18.0647	3.0488	100.3594	101.6267
10	3	10.1282	3.1002	101.282	103.34
14	2	13.946	2.0515	99.61429	102.575
14	4	13.8817	3.9705	99.155	99.2625
10	5	10.1534	4.9958	101.534	99.916
10	2	10.026	2.052	100.26	102.6
10	4	10.1726	4.0034	101.726	100.085
14	3	14.0129	3.0815	100.0921	102.7167
10	5	10.1238	5.0652	101.238	101.304
8	2	7.9162	2.0504	98.9525	102.52
Mean				**100.4213**	**101.5946**
%RSD				**0.990173**	**1.382495**

Table. 8

Method	Synthetic Mixture		% Found	
	CLZ	**TLM**	**CLZ**	**TLM**
PCR	40 mg	10 mg	99.1541	102.7583
PLS	40 mg	10 mg	99.1541	102.7583
CLS	40 mg	10 mg	98.8516	103.1533
ILS	40 mg	10 mg	98.8133	102.8000

Table. 9

	Total Explained Y Variance		Total Residual Y Variance	
	Calibration	**Validation**	**Calibration**	**Validation**
PC-1	82.40049	80.12135	1.869948	2.403108
PC-2	99.81627	99.7345	0.01952121	0.0320958
PC-3	99.82397	99.71234	0.01870305	0.0347743

PC-4	99.82493	99.67924	0.01860168	0.0387753
PC-5	99.89415	99.73315	0.0112463	0.0322599
PC-6	99.90289	99.70831	0.01031731	0.0352623
PC-7	99.93699	99.77955	0.00669466	0.0266504

Table.10

	Total Explained Y Variance		Total Residual Y Variance	
	Calibration	**Validation**	**Calibration**	**Validation**
Factor-1	83.39206	81.09637	1.764594	2.285238
Factor-2	99.81628	99.7345	0.01952	0.03209522
Factor-3	99.9345	99.7322	0.006959	0.03237402
Factor-4	99.95374	99.76463	0.004916	0.02845344
Factor-5	99.97382	99.76753	0.002781	0.02810277
Factor-6	99.98047	99.77573	0.002075	0.02711107
Factor-7	99.98795	99.73072	0.00128	0.03255267

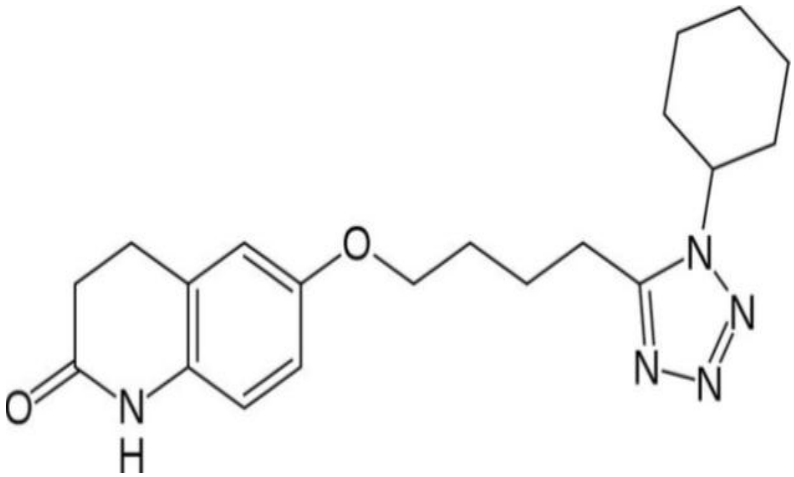


Figure. 1

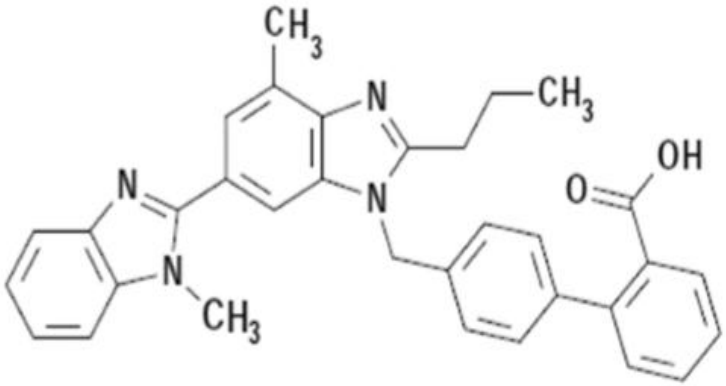


Figure. 2

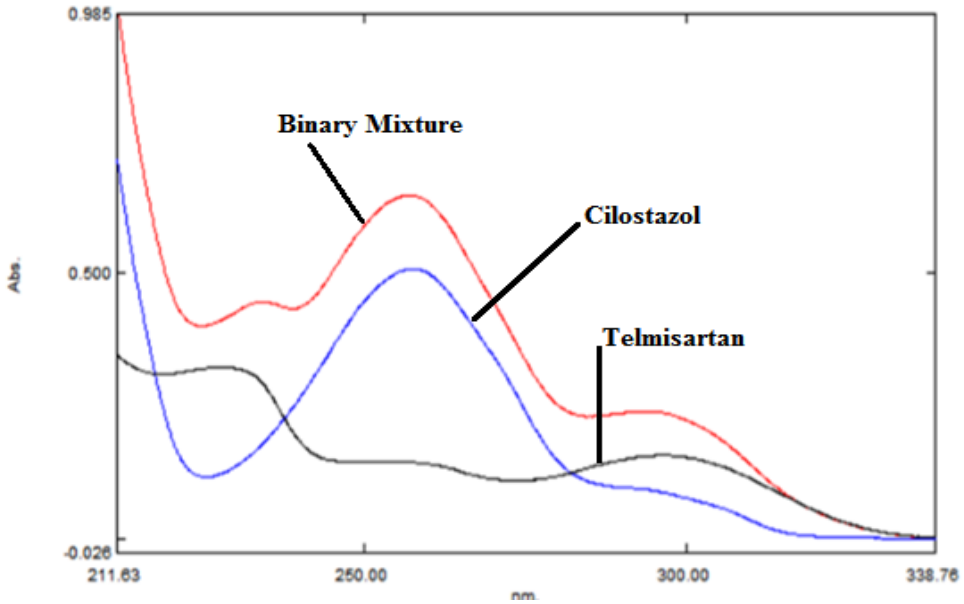


Figure. 3

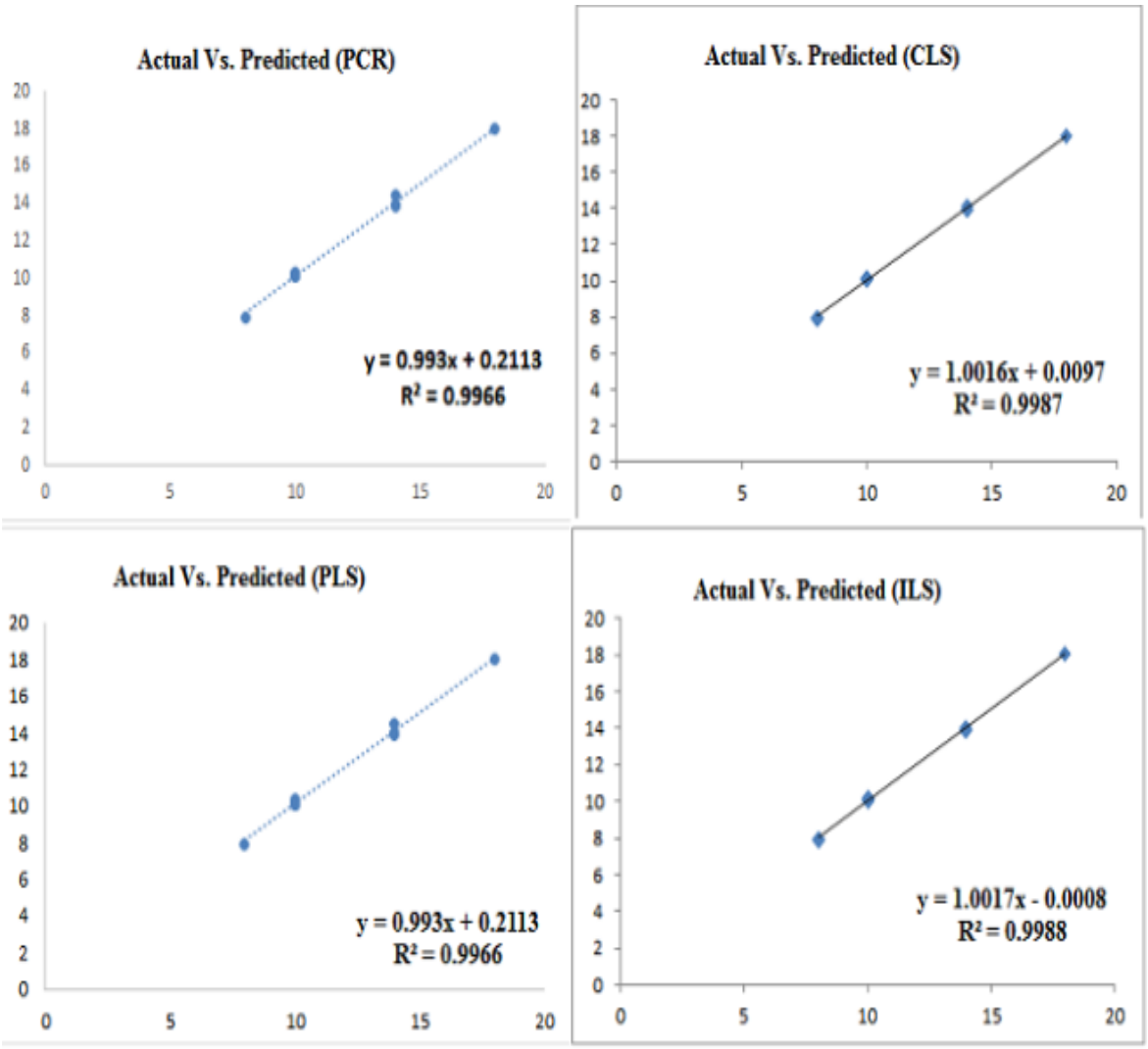
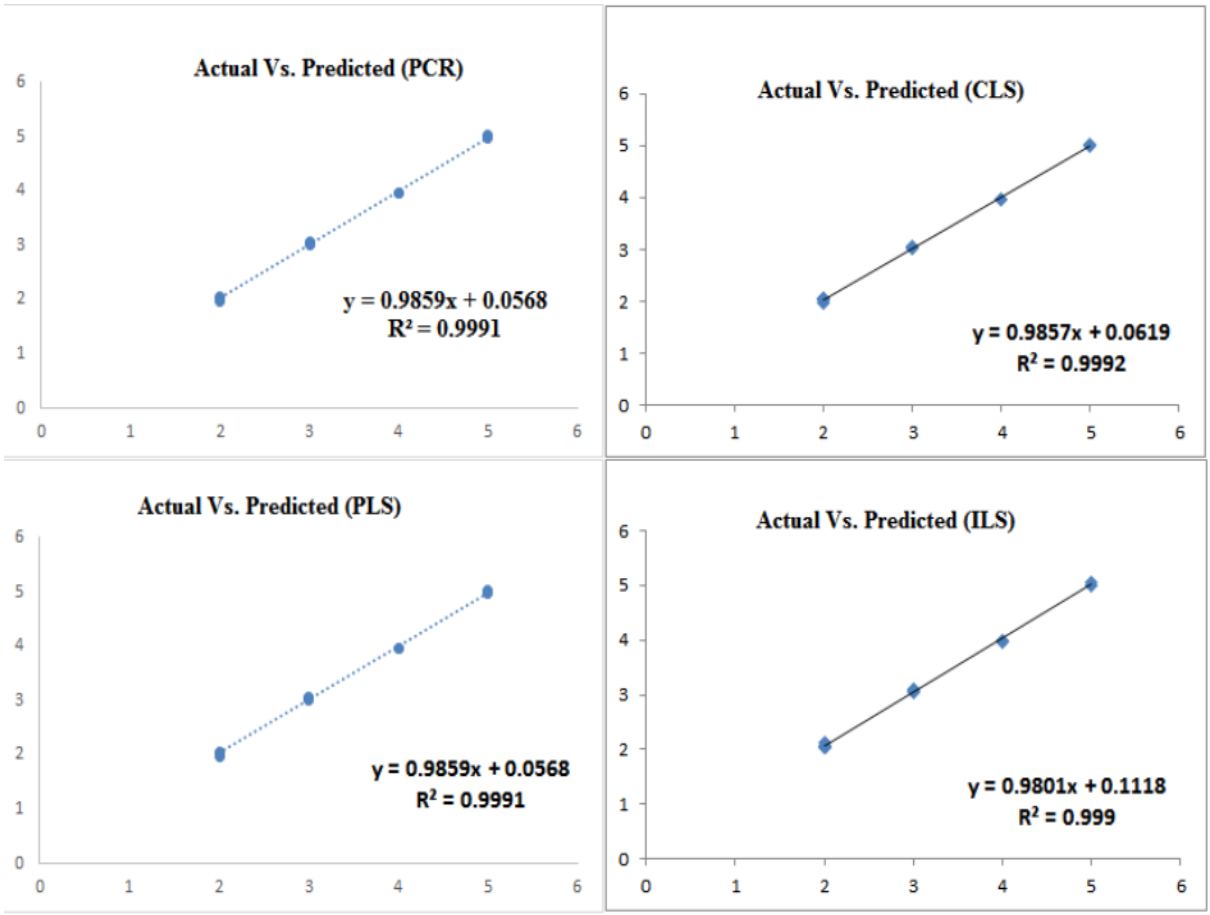
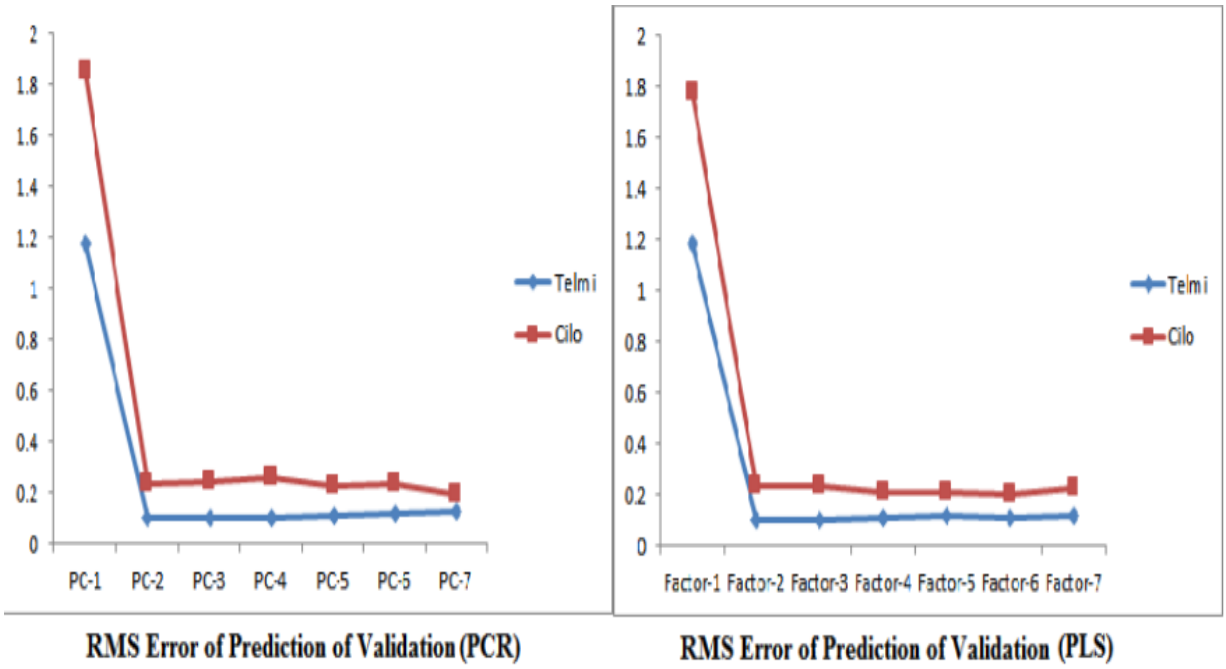


Figure. 4



Figur. 5



RMS Error of Prediction of Validation (PCR)

RMS Error of Prediction of Validation (PLS)

Figure. 6

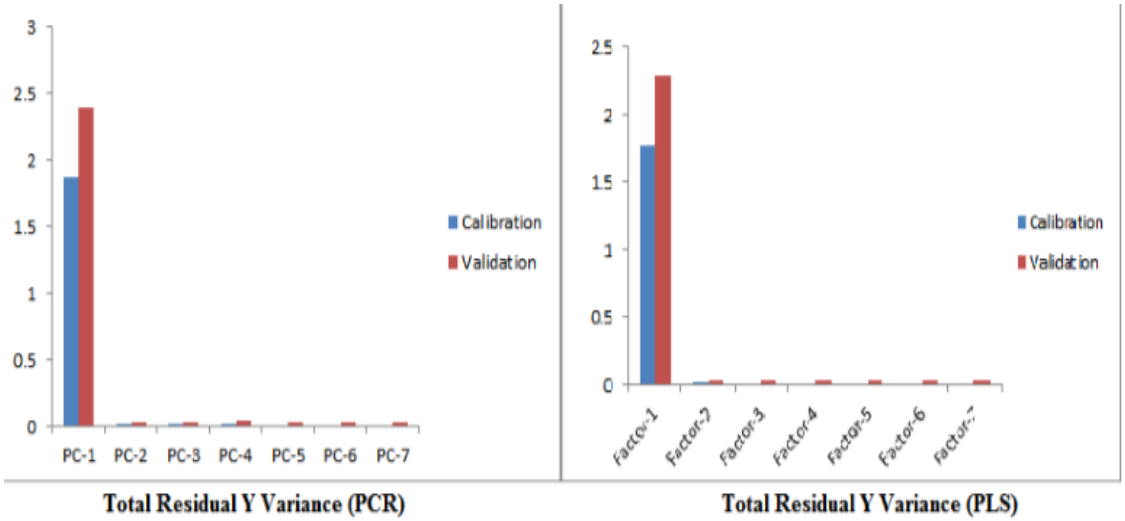


Figure. 8

CONCLUSION

Many drugs have come up in combinations in order to improvise the therapy of various ailments. These combinations have forged a challenge to use a simple method to estimate the individual drugs in combination with respect of time and complexity. Simultaneous determination of CLZ and TLM in tablet is not reported in the literature as yet. We attempted to develop four chemometric methods i.e. CLS, ILS, PCR and PLS. We found them to be simple, precise, accurate, rapid and economical methods for their simultaneous determination. The methods were successfully validated and found suitable for quality control laboratories.

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