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SIMULTANEOUS DETERMINATION OF DOXYLAMINE SUCCINATE, PYRIDOXINE HYDROCHLORIDE AND FOLIC ACID BY CHEMOMETRIC SPECTROPHOTOMETRY

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ABSTRACT

Simultaneous estimation of three component tablets containing Doxylamine succinate (DOX), Pyridoxine hydrochloride (PYR) and Folic acid (FA) was carried out by UV spectrophotometric assisted chemometric methods. Four chemometric methods i.e. classical least square (CLS), inverse least square (ILS), principal component regression (PCR) and partial least squares (PLS) were applied to simultaneous assay of DOX, PYR and FA in tablets without any chemical separation and any graphical treatment of the overlapping spectra of three drugs. The chemometric calculations were performed by using the Chemometrics Toolbox 3.02 software (Kramer, 1995) along with MATLAB 6. The results of four chemometric methods were statistically compared with each other. These chemometric calibrations were successfully applied to the marketed tablets without any separation procedure. Mean recoveries (percent) and relative standard deviation of ILS, CLS, PCR, PLS methods were found to be 98.77/1.76, 100.59/1.53, 97.91/1.50, 97.53/1.73 for DOX; 99.79/1.22, 100.22/0.58, 100.31/1.68 and 99.33/1.10 for PYR; 99.79/1.37, 100.57/1.56 and 98.38/0.96 for FA respectively. All of the four chemometric methods in this study can be satisfactorily used for the quantitative analysis of multi-component dosage form.

KEYWORDS: **Chemometrics, Spectrophotometry, Doxylamine succinate, Pyridoxine hydrochloride, Folic acid**

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INTRODUCTION

The development of chemometric methods of multi-component analysis has allowed the resolution of the complex spectra of mixtures of analytes [1]. The chemometric quantitative analytical techniques have many applications and advantages such as the mixtures can be analyzed without any separation procedures for drug determination; the techniques are very easy to apply, very sensitive, useful and yet very inexpensive as compared to other analytical techniques for simultaneous determination of compounds in multi-component mixtures. These methods provide additional advantages where calibration can be performed by ignoring the concentration of all other components except the analyte of interest and also the speed in the determination of components in a mixture [2].

Several analytical methods for the determination of DOX or PYR or FA are available either alone or in combination with other drugs, using high-pressure liquid chromatography (HPLC) [3-6], spectrophotometry [7,8], flow injection-solid phase spectrophotometry [9], potentiometric determination [10], spectrofluorometric determination [11,12] and capillary electrophoresis methods [13,14]. Some HPLC methods [15, 16], high performance thin layer chromatography (HPTLC) [17] and micellar liquid chromatography method [18] have been reported for the determination of DOX and PYR in combined dosage forms. A number of HPLC [19, 20] and LC/UV/MS-MRM methods [21] for quantization of PYR and FA in multivitamin formulations have also been reported. Methods like first derivative spectrophotometry, ratio spectra-zero-crossing and double divisor-ratio spectra derivative have been studied for simultaneous determination of DOX, PYR, and FA [22].

In this paper, we have reported the investigation and development of rapid analytical methodology for the simultaneous determination of Doxylamine succinate (DOX), Pyridoxine hydrochloride (PYR) and Folic acid (FA). The methods are based on UV spectrophotometry, and the resulting heavily overlapping responses are processed by chemometrics. The application of chemometrics allows the interpretation of multivariate data and is vital to the success of the simultaneous determination of the organic components.

In this study, three chemometric methods have been successfully applied to the simultaneous determination of Doxylamine succinate (DOX) N,N-dimethyl-2-(1-phenyl-1-(pyridin-2-yl)ethoxy)ethanamine succinate, an antiemetic agent; Pyridoxine hydrochloride (PYR) (5-hydroxy-6-methylpyridine-3,4-diyl) dimethanol hydrochloride , an anti-inflammatory agent and Folic acid (FA) (2*S*)-2-[(4-{[(2-amino-4-hydroxypteridin-6-yl)methyl]amino}phenyl)formamido]pentanedioic acid, a dietary supplement in a commercial tablet formulation, without any prior separation procedure. Combination of these drugs is used to prevent morning sickness in pregnant women, for treatment of megaloblastic anemia and in anemias of nutritional supplements, pregnancy, infancy, or childhood.

MATERIALS AND METHODS

Commercial tablets of various brands like BOOKEY PLUS (Skymax Laboratories Pvt. Ltd., India); LAMI-6 PLUS (Srinivas Gujarat Laboratories Pvt. Ltd., India) and VOMISAFE PLUS (Kamron Laboratories Limited, India) each with composition (mg/tablet) 10 mg Doxylamine Succinate 10 mg Pyridoxine hydrochloride and 2.5 mg Folic Acid and NOMIT-OD (Speciality Meditech Pvt. Ltd., India) with composition (mg/tablet) 20 mg Doxylamine Succinate, 20 mg Pyridoxine hydrochloride and 2.5 mg Folic Acid were taken for analysis. Sodium hydroxide was purchased from Spectrochem Pvt. Ltd., (Mumbai, India). For spectrophotometric analysis, a Shimadzu UV-Vis double beam spectrophotometer equipped with 1 cm quartz cells and connected to personal computer loaded UV Probe Ver.2.10 software was used. CLS, ILS, PCR, and PLS analyses were carried out using the Chemometrics Toolbox 3.02 software (Kramer, 1995) for use with MATLAB 6. The visual BASIC program of Wahbi et al. (2005) was used for the differentiation of ratio data.

Preparation of standard solutions and calibration

For spectrophotometric measurement, stock solution (0.1 mg/ml) of DOX, PYR and FA were prepared separately by dissolving 10 mg of each drug in 100 ml 0.1M NaOH. The solutions were kept in amber colour flasks to protect them from light. The zero order and first derivative absorption spectra were recorded over the wavelength range 220-400 nm against the solvent blank. The dilutions were made in 0.1M NaOH to obtain concentrations ranging from 5-60 µg/ml for DOX, 2.5-30 µg/ml for PYR and 2.5-30 µg/ml for FA and their different synthetic mixtures by using the stock solutions and linearity was studied at respective absorbance i.e. 270, 332.8 and 309.2 nm for DOX, PYR and FA respectively.

Preparation of ternary mixtures of DOX, PYR and FA

Appropriate and accurate volume aliquots of the above stock solutions were transferred to the three sets of 10 ml calibrated flasks. The first series contained a constant concentration of PYR and FA (10 µg/ml each) and a varying concentration of DOX (5–60 µg/ml). The second contained a constant concentration of DOX and FA (10 µg/ml) and a varying concentration of PYR (2.5–30 µg/ml) and the third series contained a constant concentration of DOX and PYR (10 µg/ml) and a varying concentration of FA (2.5–30 µg/ml). The solutions were protected from light throughout the study. The absorbance data matrix was obtained by measuring the absorbance at 16 wavelength points (251 to 330 nm) with the interval of 5 nm (delta lambda 5 nm) in spectral region between 250 to 330 nm.

A calibration set of 21 mixtures was prepared in methanol, applying a multilevel multifactor design in which three levels of concentrations of DOX, PYR and FA within the stated range were introduced as shown in Table 1.

A validation set of 16 mixtures was prepared in methanol, applying a multilevel multifactor design in which three levels of concentrations of DOX, PYR and FA within the stated range were introduced as shown in Table 2.

Table 1
Composition of the concentration (calibration) set

Number of Mixtures	Concentration (µg/ml)		
	DOX	PYR	FA
1	5	10	10
2	10	10	10
3	20	10	10
4	30	10	10
5	40	10	10
6	50	10	10
7	60	10	10
8	10	2.5	10
9	10	5	10
10	10	10	10
11	10	15	10
12	10	20	10
13	10	25	10
14	10	30	10
15	10	10	2.5
16	10	10	5
17	10	10	10
18	10	10	15
19	10	10	20
20	10	10	25
21	10	10	30

Table 2
Composition of the concentration (validation) set

Number of Mixtures	Concentration (µg/ml)		
	DOX	PYR	FA
1	5	10	10
2	20	10	10
3	40	10	10
4	60	10	10
5	10	2.5	10
6	10	5	10
7	10	15	10
8	10	20	10
9	10	30	10
10	10	10	2.5
11	10	10	5
12	10	10	10
13	10	10	15
14	10	10	20
15	10	10	25
16	10	10	30

Preparation of sample solutions

In spectrophotometric methods, 20 commercial tablets of each brand (BOOKEY PLUS, LAMI-6 PLUS, VOMISAFE PLUS, NOMIT-OD) were weighed separately and powdered in mortar. An amount of the powder equivalent to one tablet was taken in a 25 ml calibrated volumetric flask and dissolved in 0.1M NaOH. After 15 min of sonication, the solution was filtered through Whatman filter paper number 41. The volume was made up to 25 ml with 0.1M NaOH. Further dilutions of the solution were made with 0.1M NaOH to reach the calibration range. All the proposed chemometric methods were applied to the solutions.

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* digitised absorbance is given by [23,24],

$$A=C\times K+E_A \quad (1)$$

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-pathlength), and E_A is the in *m* x *n* matrix of spectral errors or residuals not fit by the model.

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* digitised absorbance is given by [25, 26]:

$$C=A\times P+Ec \quad (2)$$

Where *C* and *A* are as before, *P* is the *n* × *l* matrix of unknown calibration co-efficient relating the *l* component concentrations of the spectral intensities, and *Ec* is the *m* × *l* vector of errors. 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.

Principal component regression (PCR)

In the spectral work, the following steps can explain the fundamental concept of PCR [27].

(a) The original data obtained in absorbances (*A*) and concentrations (*C*) of analytes have been reprocessed by mean-centring 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 $\mathbf{T}^T$ x A_0. Here $\mathbf{T}^T$ 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 = $\boldsymbol{C}_{mean}$ — A^T_{mean} x *b*, where A^T_{mean} represents the transpose of the matrix having the entries of the mean absorbance values and $\boldsymbol{C}_{mean}$ is the mean concentration of the calibration set.

Partial least squares (PLS)

The PLS calibration technique using the orthogonalized PLS algorithm developed by Wold[28, 29] and extensively discussed by Martens and Naes [30] 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 centred to give data matrix $\mathbf{A}_0$ and vector $\mathbf{C}_0$. The orthogonalized PLS algorithm has the following steps [31]:

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

$$W = A'_0C_0/C'_0C_0 \quad (3)$$

(b) The scores and loadings are given by:

$$t_1 = A_0W_1,$$

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

$$q1 = (C_0^T t_1)/(t_1^T t_1), \quad (4)$$

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

$$A_1 = A_0 – t_1 P_1^T,$$

$$C_1 = C_0 – t_1 q_1^T, \quad (5)$$

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

$$b = W (P^T W)^{-1} q, \quad (6)$$

$$a=C_{mean} – A^T_{mean} b, \quad (7)$$

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

RESULTS AND DISCUSSION

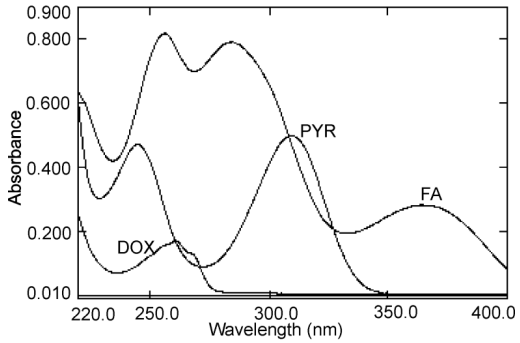

Figure 1

Overlain zero order spectra of (a) DOX (15 µg/ml), (b) PYR (15 µg/ml) and (c) FA (15 µg/ml).

Figure 1 shows the zero-order overlay spectra of DOX, PYR and FA as well as their corresponding ternary mixture in 0.1 M NaOH. As shown in the Figure 1 the spectra of DOX, PYR and FA 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.

Multivariate calibration

The calibration set of 21 standard mixture solutions which contain the concentrations with different ratio of DOX, PYR and FA was randomly prepared within the linearity range of three drugs. The UV absorbance data was obtained by measuring the absorbances in the region of 250-330 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. All the information present in the sample target should be present in the calibration data set. It has been one of the main drawbacks in development studies of multivariate method. Except ILS the remaining CLS, PCR and PLS techniques are designated as full spectrum computational procedures, thus wavelength selection is seemingly unnecessary, and so all available wavelengths are often used. Stepwise multiple linear regressions have been used for the selection of frequencies in ILS.

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 SEC which is given in the following formula;

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

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

Table 3

Statistical parameters of chemometric methods in calibration step of Zero-order spectra

	CLS	ILS	PCR			PLS		
Component	SEC[a]	SEP[b]	SEC	PRESS[c]	RSE	SEC	PRESS	RSE
DOX	0.1928	0.1849	0.0154	0.0047	0.0669	0.0151	0.0045	0.0656
PYR	0.1617	0.1463	0.0391	0.0306	0.2882	0.0364	0.0265	0.2684
FA	0.1434	0.1124	0.0182	0.0066	0.1344	0.0183	0.0067	0.2684

[a]SEC=Standard error of calibration
[b]SEP=Standard error of prediction
[c]PRESS= Prediction residual error sum of squares

Selection of optimum number of factors for PCR and PLS

For PCR and PLS methods, 21 calibration spectra were used for the selection of the optimum number of factors by using the cross validation technique. This allows modelling of the system with the optimum amount of information and avoidance of overfitting or underfitting. The cross-validation procedure consists of systematically removing one of a group of training samples in turn and using only the remaining ones for the construction of latent factors and applied regression. The predicted concentrations were then compared with the actual ones for each of the calibration samples and mean squares error of prediction (MSEP) was calculated. The MSEP was computed in the same manner each time a new factor was added to the PCR and PLS model. The selected model was that with the fewest number of factors such that its MSEP values were not significantly greater than that for the model, which yielded the lowest MSEP. A plot of MSEP values against number of components (Figure 2 and 3 indicates that factor five and four were optimum for the estimation of principle ingredients by PLS and PCR). At the selected principal components of PLS and PCR the concentrations of each sample were then predicted and compared with known concentration and the PRESS (prediction residual error sum of squares) was calculated. PRESS value was calculated as the difference between the real and the calculated concentrations, squared and summed, over all references for each component. It was given by this equation, and values are indicated in Table 3.

$$\text{PRESS} = \sum_{i=1}^{n} \left(C_i^{Added} - C_i^{Found} \right)^2$$

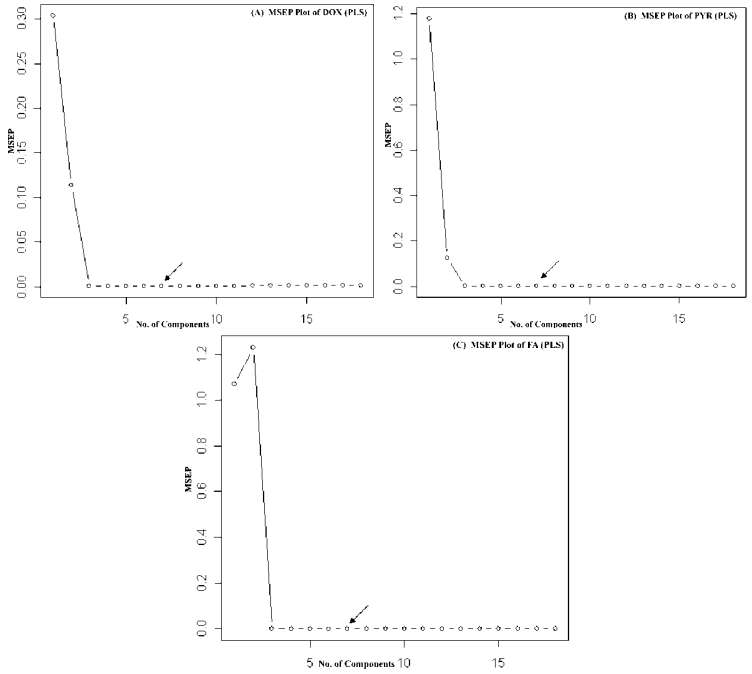

Figure 2

MSEP plots of a calibration set obtained using leave-one-out (LOO) cross validation of PLS-model for (A) DOX, (B) PYR and (C) FA in zero-order absorption data.

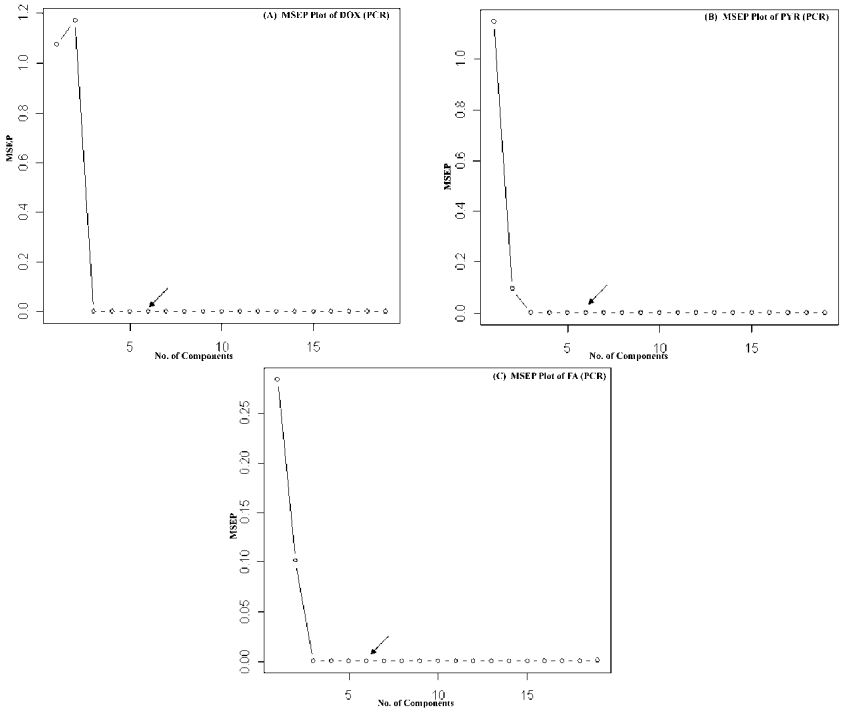

Figure 3

MSEP plots of a calibration set obtained using leave-one-out (LOO) cross validation of PCR-model for (A) DOX, (B) PYR and (C) FA in zero-order absorption data.

Validation of the developed methods

To check the validity (predictive ability) of the calibration models, the simultaneous analysis of the prediction set containing 16 samples of various concentrations (in triplicates) of DOX, PYR and FA 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 our 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 DOX, PYR and FA was evaluated by analysing a series of different concentrations of standard drug. The linearity was found to be ranging between 5-60 µg/ml for DOX and 2.5-30 µg/ml for both PYR and FA. Each concentration was repeated three times.

The accuracy study was performed by increasing standard addition of known amounts of studied drugs to an unknown concentration (constant volume) of the commercial pharmaceutical formulations. A constant volume of the unknown solution was added to each of six 10 ml volumetric flasks. Then a series of increasing volumes of working standard solutions were added. Finally, each flask was made up to the mark with 0.1M NaOH and mixed well. The resulting mixtures were analyzed and chemometric recoveries were determined. The results obtained were compared with expected results. The good mean recoveries and standard deviation 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, where satisfactory results were obtained over the stated calibration range

.

Table 4
Analysis of validation set by CLS method

Added Conc. (µg/ml)			Measured conc.* (µg/ml)			Recovery (%)		
DOX	**PYR**	**FA**	**DOX**	**PYR**	**FA**	**DOX**	**PYR**	**FA**
5	10	10	4.99	10.05	10.04	99.81	100.46	100.42
20	10	10	20.06	10.02	9.99	100.30	100.19	99.89
40	10	10	40.08	9.98	10.02	100.21	99.80	100.17
60	10	10	59.54	10.01	9.69	99.24	100.06	96.90
10	2.5	10	10.00	2.20	10.16	99.97	101.82	101.58
10	5	10	10.10	5.06	10.01	100.95	101.17	100.14
10	10	10	9.89	9.97	10.10	98.87	99.72	100.98
10	20	10	9.85	19.55	9.87	98.49	97.76	98.66
10	30	10	9.54	30.29	9.90	95.35	100.97	99.05
10	10	2.5	10.01	10.01	2.08	100.06	100.05	101.11
10	10	5	9.82	10.07	5.14	98.23	100.68	102.72
10	10	15	9.96	9.89	14.99	99.60	98.90	99.92
10	10	20	9.72	9.90	19.78	97.23	98.98	98.92
10	10	25	9.51	9.87	24.84	95.11	98.72	99.37
10	10	30	9.81	9.75	29.90	98.09	97.54	99.68
Mean						98.77	99.79	99.97
RSD (%)						1.76	1.22	1.37

Mean of three individual determinations

Table 5
Analysis of validation set by ILS method

Added Conc. (µg/ml)			Measured conc.* (µg/ml)			Recovery (%)		
DOX	**PYR**	**FA**	**DOX**	**PYR**	**FA**	**DOX**	**PYR**	**FA**
5	10	10	5.00	10.09	10.13	99.95	100.89	101.33
20	10	10	20.12	10.05	10.03	100.61	100.49	100.33
40	10	10	40.20	9.94	10.14	100.51	99.42	101.39
60	10	10	59.79	10.02	9.76	99.65	100.16	97.61
10	2.5	10	9.92	2.55	10.18	99.15	100.18	101.79
10	5	10	10.36	5.03	10.09	103.58	100.67	100.87
10	10	10	10.22	10.00	10.17	102.17	100.00	101.66
10	20	10	9.85	19.84	9.90	98.49	99.19	99.02
10	30	10	10.04	30.37	9.92	100.36	101.24	99.16
10	10	2.5	10.12	10.00	2.63	101.23	100.00	103.36
10	10	5	10.13	10.09	5.16	101.28	100.93	103.12
10	10	15	10.25	10.02	15.00	102.51	100.22	99.97
10	10	20	10.17	10.05	19.91	101.75	100.46	99.54
10	10	25	9.79	9.98	24.87	97.94	99.77	99.49
10	10	30	9.97	9.97	29.98	99.67	99.66	99.92
Mean						100.59	100.22	100.57
RSD (%)						1.53	0.58	1.56

Mean of three individual determinations

Table 6
Analysis of validation set by PCR method

Added Conc. (µg/ml)			Measured conc.* (µg/ml)			Recovery (%)		
DOX	**PYR**	**FA**	**DOX**	**PYR**	**FA**	**DOX**	**PYR**	**FA**
5	10	10	4.84	10.04	9.83	96.80	100.39	98.32
20	10	10	19.97	10.03	9.86	99.83	100.27	98.59
40	10	10	40.04	10.03	9.96	100.11	100.26	99.61
60	10	10	59.55	10.11	9.67	99.25	101.06	96.73
10	2.5	10	9.81	2.64	9.91	98.13	105.55	99.15
10	5	10	9.92	5.02	9.78	99.22	100.47	97.79
10	10	10	9.76	9.97	9.91	97.61	99.71	99.13
10	20	10	9.85	19.63	9.73	98.47	98.15	97.33
10	30	10	9.56	30.45	9.85	95.59	101.51	98.54
10	10	2.5	9.81	9.97	2.50	98.08	99.66	100.09
10	10	5	9.63	10.05	4.87	96.34	100.52	97.36
10	10	15	9.87	9.92	14.84	98.72	99.22	98.93
10	10	20	9.68	9.96	19.69	96.81	99.57	98.44
10	10	25	9.54	9.94	24.83	95.36	99.39	99.33
10	10	30	9.84	9.89	29.90	98.39	98.90	99.65
Mean						97.91	100.31	98.60
RSD (%)						1.50	1.68	0.98

Mean of three individual determinations

Table 7
Analysis of validation set by PLS method

Added Conc. (µg/ml)			Measured conc.* (µg/ml)			Recovery (%)		
DOX	**PYR**	**FA**	**DOX**	**PYR**	**FA**	**DOX**	**PYR**	**FA**
5	10	10	4.79	9.93	9.80	95.71	99.32	97.99
20	10	10	19.92	9.94	9.84	99.61	99.35	98.36
40	10	10	40.01	9.95	9.94	100.02	99.48	99.40
60	10	10	59.55	10.12	9.66	99.25	101.22	96.64
10	2.5	10	9.75	2.50	9.88	97.48	99.99	98.83
10	5	10	9.86	4.88	9.75	98.56	97.69	97.45
10	10	10	9.73	9.92	9.89	97.33	99.23	98.90
10	20	10	9.91	19.81	9.74	99.06	99.04	97.40
10	30	10	9.46	30.26	9.79	94.57	100.86	97.86
10	10	2.5	9.82	9.99	2.50	98.18	99.90	100.08
10	10	5	9.65	10.08	4.87	96.47	100.82	97.34
10	10	15	9.80	9.80	14.79	98.02	97.98	98.60
10	10	20	9.62	9.87	19.64	96.24	98.68	98.21
10	10	25	9.48	9.87	24.78	94.81	98.66	99.13
10	10	30	9.76	9.78	29.83	97.65	97.77	99.44
					Mean	97.53	99.33	98.38
RSD (%)						1.73	1.10	0.96

Mean of three individual determinations

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 DOX, PYR and FA 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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