# Chemometric assisted spectrophotometric methods for the simultaneous determination of Rifampicin and Piperine in bulk and capsule

**Umang Shah*, Sharddha Patel, Manan Raval, Pankti Desai**

Department of Pharmaceutical Chemistry & Analysis, Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology, *CHARUSAT Campus, Changa – 388 421, Ta. Petlad, Dist. Anand, Gujarat, India*.

## ABSTRACT

In this work a numerical method, based on the use of spectrophotometric data coupled to Partial least squares (PLS),Principal component regression (PCR) andclassical least square (CLS) multivariate calibration, is evaluated for the simultaneous determination of rifampicin and piperine in bulk and capsule dosage form. Spectra of RIFA and PIPE were recorded at concentrations within their linear ranges 20-50 $\mu$g/ml & 1.0 - 2.5 $\mu$g/ml, respectively and were used to compute a total of 25 synthetic mixtures involving 16 calibration and 9 validation sets between wavelength range of 200 and 500 nm with the wavelengths intervals $\lambda = 5$ nm in methanol. The suitability of the models was decided on the basis of root mean square error (RMSE) values of calibration and validation data. The analytical performances of these chemometric methods were characterized by relative prediction errors and recovery studies (%) and were compared with each other. These three methods were successfully applied to pharmaceutical formulation (capsule) with no interference with excipients as indicated by the recovery study results. The proposed methods are simple, rapid and can be easily used as an alternative analysis tool in the quality control of drugs and formulation.

**Key words:** Piperine (PIPE), PCR, PLS and CLS, Rifampicin (RIFA).

## INTRODUCTION

Rifampicin (RIFA) chemically, (12Z, 14E, 24E)- (2S, 16S, 17S, 18R, 19R, 20R, 21S, 22R, 23S) - 1,2 -dihydro- 5, 6, 9, 17, 19 -pentahydroxy, 23 -methoxy- 2, 4, 12, 16, 18, 20, 22 heptamethyl -8- (4-methylpiperazin -1 yliminomethyl) -1, 11 - dioxo 2, 7 (epoxypentadeca -1, 11, 13 trienimino) naphtha [2,1-*b*] furan -21-yl acetate [Figure 1(a)] is a well-known Anti-Tuberculosis drug.[1] It is official in IP (2010), EP (2011), JP (2011), BP (2010) and USP (2013).[2-6] Piperine (PIPE) is chemically 1-[5-(1, 3-benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl] piperidine [Figure 1(b)] is a natural alkaloid use as bio - enhancer.

Literature survey reveals that many analytical methods have been reported like RP-HPLC,[7,20] HPTLC[8] and UV Spectrophotometry[9-13] and for the determination of piperine and rifampicin in individually and combination with other drugs. Multivariate calibration is a chemometric method which has been employed for determination of drugs in combined dosage.[14-17] The present work aim to develop an alternative numerical based analytical procedure on chemometric assisted spectrophotometric methods for analysis of rifampicin and piperine from capsule.

## EXPERIMENTAL

### Materials and reagents

Reference standard of rifampicin was obtained as gratis sample from Cadila Pharmaceutical Ltd. and piperine was procured from Sigma Aldrich (Purity: 97.0%). Methanol(AR grade) and Acetonitrile were procured from Loba Chemicals.

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**Correspondence Address**
***Prof. Umang Shah***
Assistant Professor, Department of Pharmaceutical Chemistry & Analysis, Ramanbhai Patel College of Pharmacy, Charotar University of Science & Technology, CHARUSAT Campus, At Changa, Ta.Petlad, Dist. Anand-388421, Gujarat, INDIA.
E-mail: umangshah.ph@charusat.ac.in

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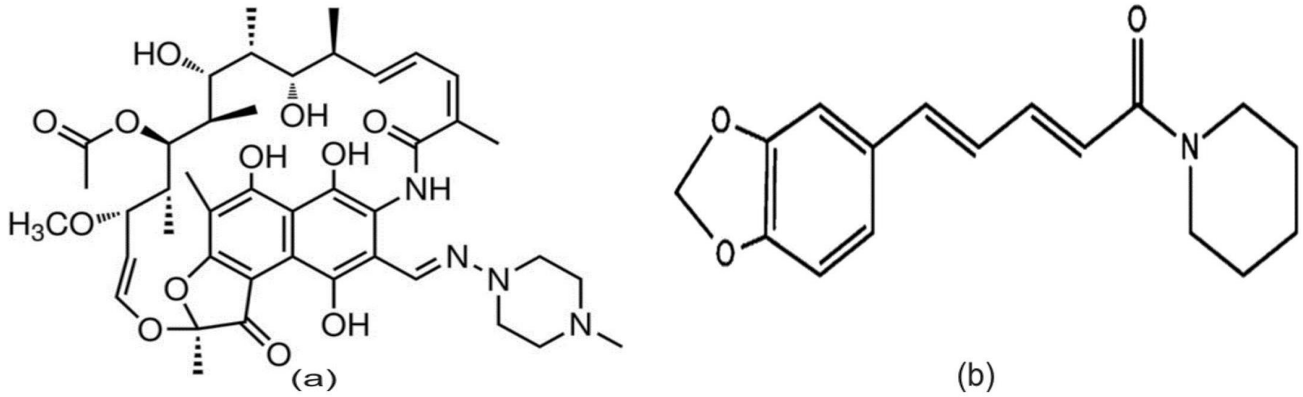


**Figure 1: (a) Chemical Structure of Rifampicin, (b) Chemical Structure of Piperine**

### Instrumentation and software

UV-Visible double beam spectrophotometer with matching pair of 1 cm quartz cell (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) was used to record UV spectra of solutions. The spectral band width is 0.5 nm. Unscrambler® and Microsoft excel were used for PCR, PLS and CLS model development and data analysis.

### Preparation of standard stock solution

Accurately weighed and transferred RIFA (10 mg) and PIPE (10 mg) into two different 100 ml and 10 ml amber color volumetric flask respectively, and volume was made up to 100 ml and 10 ml with methanol up to the mark. The final concentration of RIFA and PIPE were 100 (µg/ml) and 1000 (µg/ml) respectively.

### Preparation of working stock solution

Aliquot (1 ml) of PIPE from their stock solution was transferred into 100 ml amber color volumetric flask and volume was made up to 100 ml with methanol and it was used as a working standard solution of PIPE 10 (µg/ml). Standard stock solution of RIFA 100 (µg/ml) was use as a working solution.

### Construction of calibration and validation set

Two sets of standard solutions, a calibration set, and a validation set were prepared. Sixteen calibration standards and nine validation standard mixtures were prepared by mixing appropriate volumes of the working standard solutions of RIFA and PIPE and diluting to volume with methanol. The combination of RIFA and PIPE are illustrated in table 1. The absorption spectra of the prepared solutions were measured from 230-490 nm with 5 nm intervals. The absorbance data of the calibration set were then subjected to the Unscrambler® program for the PCR, PLS and CLS models. For validation of the PCR, PLS and CLS models, the concentrations of RIFA and PIPE in the validation set were predicted by using the proposed PCR, PLS and CLS models. The validation of all the methods was performed by ICHQ2 (R1) and IUPAC guidelines for calibration in analytical chemistry.[18-19]

### Assay of marketed formulation

Twenty capsules were accurately weighed and finely powdered. Capsule powder equivalent to RIFA (200 mg) and PIPE (10 mg) accurately weighed and transferred into 100 ml amber colored volumetric flask and 70 ml of methanol was added. The mixture was sonicated for 20 min and diluted up to the mark with methanol and filtered through a whatman filter paper no.41. From this solution 0.1 ml aliquot was withdrawn into 10 ml amber colored volumetric flask and diluted up to the mark with methanol. Solution contains RIFA 20 (µg/ml) and PIPE 1 (µg/mL). The analysis procedure was repeated six times for capsule formulation and result was shown in table 6.

## RESULT AND DISCUSSION

### Calibration matrix and selection of spectral zones for analysis by PCR, PLC and CLS

Figure 2 shows the UV spectra for RIFA and PIPE individual and the mixture of them in methanol. As shows there is clear overlapping between them. The spectral overlapping of these drugs prevents resolution of the mixtures by direct spectrophotometric measurements.

RIFA exhibit absorption maxima at 245.93, 340.65 and 477.51 nm and PIPE exhibit absorption maxima at 341 nm. The RIFA and PIPE spectra are overlapped in the absorption maxima. For this reason, two chemometric calibrations, using the zero-order spectra, were separately applied to simultaneous determination of these drugs in mixtures.

### Multivariate methods

The first step in multivariate methods involved constructing the calibration matrix. The wavelength range

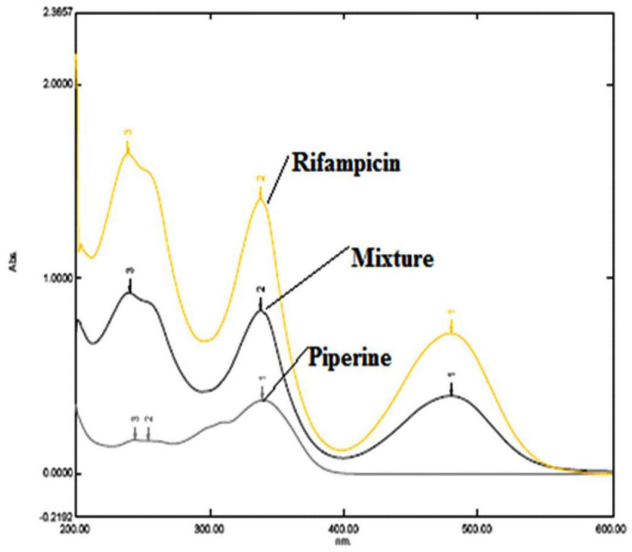


**Figure 2: Overlay spectra of RIFA, PIPE and Mixture**

used was 230-490 nm. Fifty two spectral points with 5 nm intervals were selected within this range. The compositions of the calibration mixtures were randomly designed in order to collect maximum information from the spectra of these mixtures.

The quality of muiltcomponent analysis is dependent on the wavelength range and spectral mode used. The UV absorption spectra of RIFA, PIPE and the mixture at their nominal concentrations are shown in Figure 2. The calibration set and validation set were randomly prepared with the mixture of RIFA and PIPE in methanol (Table 1). The UV spectra were observed and the absorbances were measured at 52 wavelength points in the region between 230 - 490 nm with 5 nm intervals.

The PCR, PLS and CLS models were developed by the Unscrambler® program. The predicted concentrations of the components in each sample were compared with the actual concentrations of the components in each of the validation samples, and the root mean square error of cross validation (RMSECV) was calculated for each method.

## Statistical analysis

We can define the ability of a calibration in several ways. In this subsection, we calculated the standard variation of chemo metric calibrations in the case of investigated mixtures. To validate the model, both RMSECV and RMSEP were considered; they must be as low as possible for a particular model.

RMSECV and RMSEP were calculated for each method as per equation 1 and 2.

$$RMSECV = \sqrt{\sum \frac{(C_{act} - C_{pred})^2}{I_c}} \text{----------(Equation 1)}$$

Where,

RMSECV= Root Mean Square Error of Cross Validation

$C_{act}$ = actual concentration of the calibration set samples

$C_{pred}$ = predicted concentration of the calibration set samples

$I_c$= total number of calibration set samples

$$RMSEP = \sqrt{\sum \frac{(Y_{act} - Y_{pred})^2}{I_p}} \text{---------- (Equation 2)}$$

Where,

RMSEP= Root Mean Square Error of Prediction

$Y_{act}$=actual concentration of the prediction set samples

$Y_{pred}$= predicted concentration of the prediction set samples

$I_p$= total number of prediction set samples

The RMSECV was used as a diagnostic test for examining the error in the predicted concentrations. The model is the key to achieving the correct quantization

**Table 1: Composition of calibration and validation set data**

| Sr. No. | RIFA (µg/ml) | PIPE (µg/ml) |
|---|---|---|
| 1c | 20 | 1 |
| 2c | 20 | 1.5 |
| 3c | 30 | 1.5 |
| 4c | 30 | 2 |
| 5c | 40 | 1.5 |
| 6c | 30 | 1 |
| 7c | 20 | 2.5 |
| 8c | 50 | 2.5 |
| 9c | 50 | 1.5 |
| 10c | 30 | 2.5 |
| 11c | 50 | 1 |
| 12c | 20 | 2 |
| 13c | 40 | 2 |
| 14c | 40 | 2.5 |
| 15c | 50 | 2 |
| 16c | 40 | 1 |
| 17v | 28 | 1.4 |
| 18v | 14 | 2.1 |
| 19v | 42 | 2.1 |
| 20v | 42 | 1.4 |
| 21v | 28 | 2.1 |
| 22v | 42 | 0.7 |
| 23v | 14 | 0.7 |
| 24v | 14 | 1.4 |
| 25v | 28 | 0.7 |

c = solution of calibration set, v= solution of validation set

**Table 2: Recovery study of RIFA & PIPE by PCR method**

| Expected Conc. (µg/ml) | | Predicted Conc. (µg/ml) | | % Recovery | | Residual Conc. (E-P) (µg/ml) | | $(Exp - Pre)^2$ (µg/ml) | $(Exp - Pre)^2$ (µg/ml) |
|---|---|---|---|---|---|---|---|---|---|
| RIFA | PIPE | RIFA | PIPE | RIFA | PIPE | RIFA | PIPE | RIFA | PIPE |
| 28 | 1.4 | 27.731 | 1.409 | 99.03 | 100.68 | 0.268 | -0.009 | 0.0723 | 9.13E-05 |
| 14 | 2.1 | 14.091 | 2.099 | 100.65 | 99.99 | -0.091 | 7.8E-05 | 0.008 | 6.08E-09 |
| 42 | 2.1 | 41.200 | 2.11 | 98.09 | 100.76 | 0.799 | -0.016 | 0.639 | 0.00025 |
| 42 | 1.4 | 42.171 | 1.393 | 100.40 | 99.53 | -0.171 | 0.006 | 0.029 | 4.16E-05 |
| 28 | 2.1 | 27.840 | 2.118 | 99.431 | 100.876 | 0.159 | -0.018 | 0.025 | 0.0003 |
| 42 | 0.7 | 42.553 | 0.706 | 101.31 | 100.909 | -0.553 | -0.006 | 0.306 | 4.06E-05 |
| 14 | 0.7 | 14.138 | 0.697 | 100.98 | 99.627 | -0.138 | 0.002 | 0.019 | 6.81E-06 |
| 14 | 1.4 | 14.041 | 1.396 | 100.29 | 99.74 | -0.041 | 0.003 | 0.002 | 1.26E-05 |
| 28 | 0.7 | 28.369 | 0.690 | 101.31 | 98.578 | -0.369 | 0.009 | 0.136 | 9.9E-05 |
| **Mean %** | | | | | | | | 100.172 | 100.08 |
| **[a]SD** | | | | | | | | 1.103 | 0.792 |
| **[b]RSD** | | | | | | | | 1.101 | 0.792 |
| **[c]RMSEP** | | | | | | | | 0.371 | 0.00993 |

a=Standard deviation, b=Relative standard deviation c= Root meansquare error of prediction

**Table 3: Recovery study of RIFA & PIPE by PLS method**

| Expected Conc. (µg/ml) | | Predicted Conc. (µg/ml) | | % Recovery | | Residual Conc. (E-P) (µg/ml) | | $(Exp - Pre)^2$ (µg/ml) | $(Exp - Pre)^2$ (µg/ml) |
|---|---|---|---|---|---|---|---|---|---|
| RIFA | PIPE | RIFA | PIPE | RIFA | PIPE | RIFA | PIPE | RIFA | PIPE |
| 28 | 1.4 | 27.851 | 1.405 | 99.469 | 100.412 | 0.148 | -0.006 | 0.022 | 3.32698E-05 |
| 14 | 2.1 | 14.104 | 2.084 | 100.747 | 99.281 | -0.104 | 0.015 | 0.011 | 0.00022 |
| 42 | 2.1 | 41.205 | 2.111 | 98.109 | 100.548 | 0.794 | -0.011 | 0.631 | 0.00013 |
| 42 | 1.4 | 42.276 | 1.372 | 100.657 | 98.039 | -0.276 | 0.027 | 0.076 | 0.00075 |
| 28 | 2.1 | 27.827 | 2.117 | 99.385 | 100.825 | 0.172 | -0.02 | 0.029 | 0.00030 |
| 42 | 0.7 | 42.488 | 0.699 | 101.16 | 99.898 | -0.48 | 0.001 | 0.238 | 5.01547E-07 |
| 14 | 0.7 | 14.098 | 0.701 | 100.706 | 100.228 | -0.098 | -0.001 | 0.009 | 2.5568E-06 |
| 14 | 1.4 | 14.082 | 1.388 | 100.587 | 99.170 | -0.082 | 0.011 | 0.007 | 0.00013 |
| 28 | 0.7 | 28.439 | 0.708 | 101.569 | 101.217 | -0.439 | -0.008 | 0.193 | 7.25734E-05 |
| **Mean %** | | | | | | | | 100.266 | 99.958 |
| **[a]SD** | | | | | | | | 1.075 | 0.983 |
| **[b]RSD** | | | | | | | | 1.072 | 0.983 |
| **[c]RMSEP** | | | | | | | | 0.368 | 0.013 |

a=Standard deviation, b=Relative standard deviation c= Root mean-square error of prediction

in PLS, CLS and PCR calibrations. The resulting models were also validated by prediction of the concentration of analyses in a separate validation set which was not used in model development. The results of prediction and the percentage recoveries are represented in Table 2 to 4. The evaluation of the predictive abilities of the models was performed by plotting the actual known concentrations against the predicted concentrations and the plot of the actual known concentrations against the predicted concentrations are mentioned in Figure 3 (a) to (f). As observed, there was good agreement between the predicted (calculated) and actual concentration of the drugs. The mean recoveries and the relative standard deviations of our proposed methods were computed and are indicated in Table 2 to 4 for RIFA and PIPE, respectively. Satisfactory correlation coefficient ($r^2$) values were obtained for each compound in the validation set by PCR, PLS and CLS optimized models indicating good predictive abilities of the models. Another diagnostic test was carried out by plotting the concentration residuals against the predicted concentrations. The

| Table 4: Recovery study of RIFA & PIPE by CLS method | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Expected Conc. (µg/ml) | | Predicted Conc. (µg/ml) | | % Recovery | | Residual Conc. (E-P) (µg/ml) | | $(Exp -Pre)^2$ (µg/ml) | $(Exp -Pre)^2$ (µg/ml) |
| RIFA | PIP | RIFA | PIP | RIFA | PIP | RIFA | PIP | RIFA | PIP |
| 28 | 1.4 | 27.748 | 1.393 | 99.101 | 99.507 | 0.251 | 0.007 | 0.063 | 4.761E-05 |
| 14 | 2.1 | 13.862 | 2.097 | 99.015 | 99.89 | 0.137 | 0.002 | 0.019 | 5.336E-06 |
| 42 | 2.1 | 41.425 | 2.127 | 98.630 | 101.321 | 0.575 | -0.027 | 0.330 | 0.00077 |
| 42 | 1.4 | 42.405 | 1.415 | 100.96 | 101.092 | -0.405 | -0.015 | 0.164 | 0.00023 |
| 28 | 2.1 | 27.897 | 2.099 | 99.634 | 99.952 | 0.102 | 0.001 | 0.010 | 1E-06 |
| 42 | 0.7 | 42.717 | 0.703 | 101.709 | 100.414 | -0.717 | -0.002 | 0.515 | 8.41E-06 |
| 14 | 0.7 | 13.912 | 0.690 | 99.37 | 98.585 | 0.088 | 0.009 | 0.008 | 9.801E-05 |
| 14 | 1.4 | 13.956 | 1.399 | 99.683 | 99.971 | 0.044 | 0.001 | 0.002 | 1.6E-07 |
| 28 | 0.7 | 28.253 | 0.698 | 100.90 | 99.686 | -0.252 | 0.002 | 0.063 | 4.84E-06 |
| Mean % | | | | | | | | 99.890 | 100.046 |
| [a]SD | | | | | | | | 1.051 | 0.825 |
| [b]RSD | | | | | | | | 1.052 | 0.824 |
| [c]RMSEP | | | | | | | | 0.361 | 0.011 |

a=Standard deviation, b=Relative standard deviation c= Root mean-square error of prediction

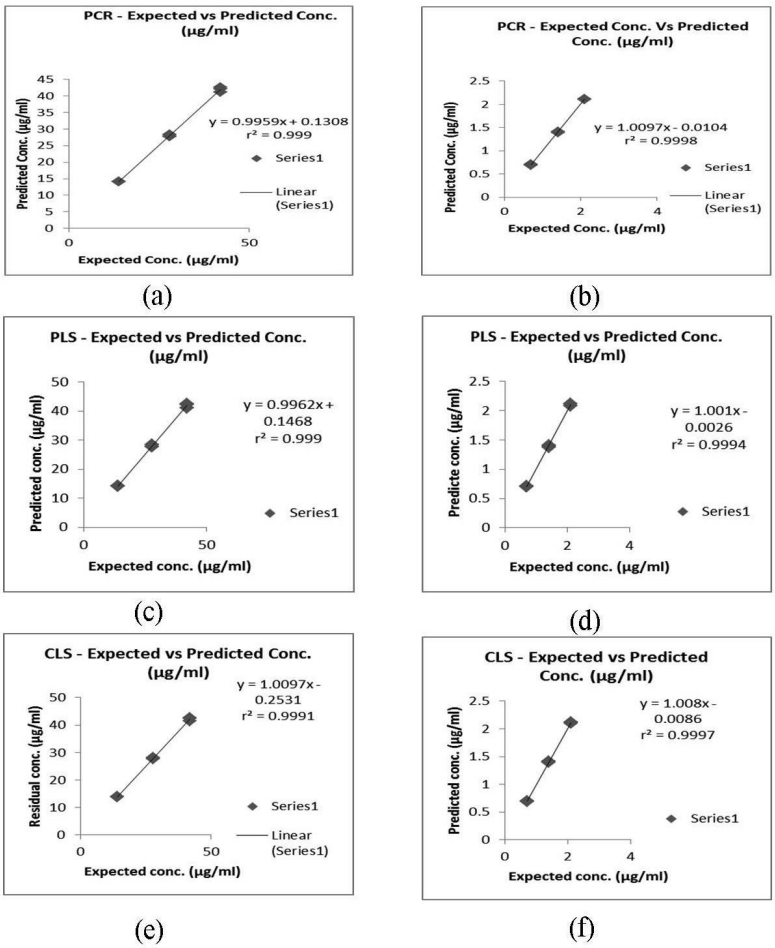


**Figure 3: (a) PCR – Expected Vs. Predicted conc. of RIFA (b) PCR – Expected Vs. Predicted conc. of PIPE (c) PLS – Expected Vs. Predicted conc. of RIFA (d) PLS – Expected Vs. Predicted conc. of PIPE (e) CLS – Expected Vs. Predicted conc. of RIFA (f) CLS – Expected Vs. Predicted conc. of PIPE**

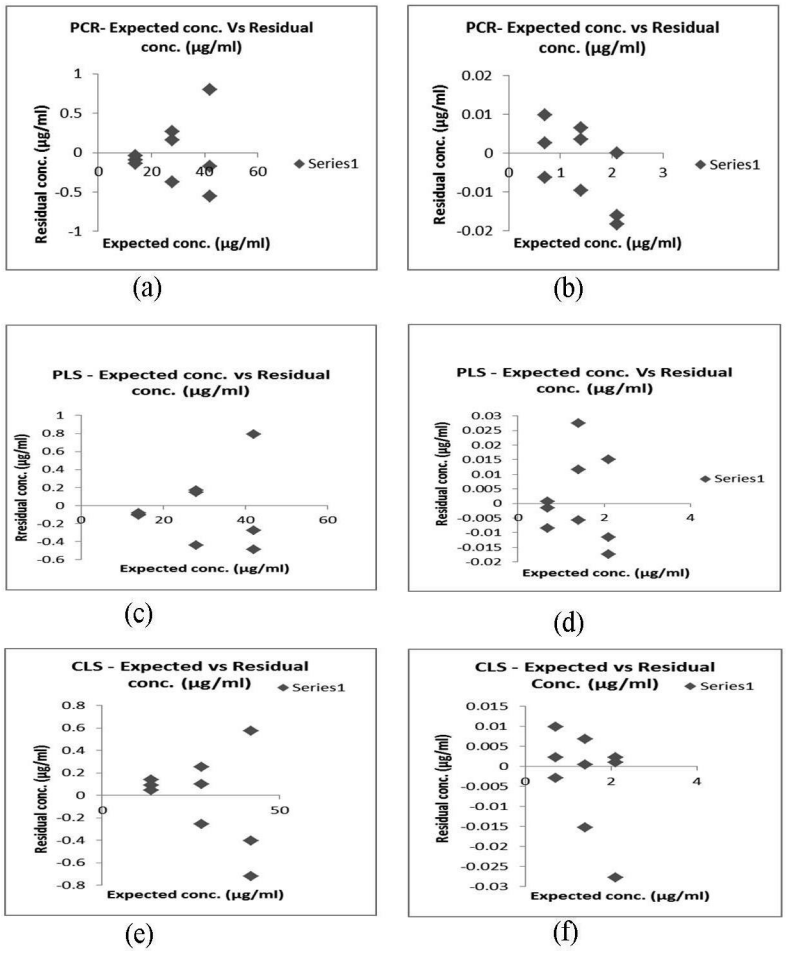


**Figure 4: (a) PCR – Expected Vs. Residual conc. of RIFA (b) PCR – Expected Vs. Residual conc. of PIPE (c) PLS – Expected Vs. Residual conc. of RIFA (d) PLS – Expected Vs. Residual conc. of PIPE (e) CLS – Expected Vs. Residual conc. of RIFA (f) CLS – Expected Vs. Residual conc. of PIPE**

**Table 5: Accuracy data of RIFA by PCR, PLS and CLS methods**

| % Level | Amount Taken (µg/ml) | Amount Found (µg/ml) | | | Mean % ± SD[a] | | | % RSD[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PCR | PLS | CLS | PCR | PLS | CLS | PCR | PLS | CLS |
| 80% | 36 | 36.54 | 36.64 | 35.99 | 101.18±0.47 | 101.07±0.65 | 100.62±0.65 | 0.46 | 0.64 | 0.65 |
| | | 36.23 | 36.34 | 36.20 | | | | | | |
| | | 36.49 | 36.17 | 36.47 | | | | | | |
| 100% | 40 | 39.29 | 39.81 | 39.84 | 99.32±0.94 | 99.61±0.86 | 100.40±0.96 | 0.95 | 0.86 | 0.96 |
| | | 40.01 | 40.20 | 40.59 | | | | | | |
| | | 39.88 | 39.51 | 40.04 | | | | | | |
| 120% | 44 | 43.40 | 43.59 | 44.74 | 99.070±1.22 | 99.659±1.42 | 101.07±0.96 | 1.24 | 1.42 | 0.95 |
| | | 43.16 | 44.56 | 44.68 | | | | | | |
| | | 44.19 | 43.38 | 43.98 | | | | | | |

a=Standard deviation, b=Relative standard deviation

residuals appear randomly distributed around zero, indicating adequate model building.

Another diagnostic test was carried out by plotting the residuals concentration against the predicted concentrations. Figure 4(a) to (f) shown the residuals appear randomly distributed around zero, indicating adequate models building. Satisfactory correlation coefficient ($r^2$) and slope values were obtained for each compound in the validation set by PLS, CLS and PCR optimized models indicating good predictive abilities of the models.

## Accuracy study

The accuracy of the method was carried out at three levels 80, 100 and 120% of the working concentration

**Table 6: Accuracy data of PIPE by PCR, PLS and CLS methods**

| % Level | Amount Taken (µg/ml) | Amount Found (µg/ml) | | | Mean % ± SD[a] | | | % RSD[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PCR | PLS | CLS | PCR | PLS | CLS | PCR | PLS | CLS |
| 80% | 1.8 | 1.83 | 1.822 | 1.810 | 100.26 ± 0.75 | 100.47 ± 0.69 | 100.64 ± 0.72 | 0.75 | 0.69 | 0.72 |
| | | 1.79 | 1.80 | 1.82 | | | | | | |
| | | 1.81 | 1.82 | 1.79 | | | | | | |
| 100% | 2.0 | 2.003 | 2.01 | 2.02 | 99.83 ± 1.01 | 99.63 ± 1.06 | 100.01 ± 0.99 | 1.01 | 1.06 | 0.99 |
| | | 1.97 | 1.99 | 1.979 | | | | | | |
| | | 2.01 | 1.96 | 2.00 | | | | | | |
| 120% | 2.2 | 2.19 | 2.20 | 2.16 | 99.50 ± 0.90 | 99.39 ± 1.10 | 99.63 ± 0.94 | 0.90 | 1.10 | 0.95 |
| | | 2.21 | 2.19 | 2.20 | | | | | | |
| | | 2.17 | 2.15 | 2.19 | | | | | | |

a=Standard deviation, b=Relative standard deviation

**Table 7: Summary parameters of chemometric methods**

| Parameters | RIFA | | | PIPE | | |
|---|---|---|---|---|---|---|
| | PCR | PLS | CLS | PCR | PLS | CLS |
| Range (µg/ml) | 20 - 50 | | | 1 – 2.5 | | |
| | 230 - 490 | | | 230 - 490 | | |
| Δλ (nm) | 5 | | | 5 | | |
| Factor | 7 | 7 | - | 7 | 7 | - |
| % Recovery | 99.859 | 100.116 | 100.7 | 99.867 | 99.833 | 100.094 |
| SD | 1.103 | 1.075 | 1.051 | 0.792 | 0.982 | 0.825 |
| RSD | 1.101 | 1.072 | 1.052 | 0.791 | 0.983 | 0.824 |
| Correlation coefficient ($r^2$) | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 |
| Intercept | 0.130 | 0.146 | -0.253 | -0.010 | -0.002 | -0.008 |
| Slope | 0.995 | 0.996 | 1.009 | 1.009 | 1.001 | 1.008 |
| [a]RMSECV | 0.311 | 0.294 | 0.439 | 0.015 | 0.016 | 0.038 |
| [b]RMSEP | 0.371 | 0.367 | 0.361 | 0.009 | 0.013 | 0.011 |

a= Rootmeansquare error of cross-validation, b= Rootmeansquare error of prediction

**Table 8: Assay results of RIFA & PIPE by developed methods**

| Drug | % Assay by Different Methods | | |
|---|---|---|---|
| | PCR | PLS | CLS |
| RIFA | 99.636 | 100.798 | 98.804 |
| | 100.962 | 99.997 | 99.871 |
| | 100.514 | 100.012 | 100.486 |
| PIPE | 100.651 | 99.562 | 100.15 |
| | 99.783 | 100.053 | 99.926 |
| | 101.004 | 99.258 | 101.135 |

of sample. Calculated amount of standard solution of RIFA and PIPE were spiked with added sample solution to prepare level 80, 100 and 120% of the working concentration. The analysis procedure was repeated for three times. Result was shown in Table 5 and 6. The statistical parameters of validation set and calibration set were illustrated in Table 7.

## Analysis of market formulation

The validated chemometrics-assisted UV spectrophotometric methods were used in the analysis of the marketed formulation Resorine capsule with label claim of 200 mg RIFA and 10 mg PIPE per capsule. The results for drug assays show good agreement with the label claims Table 8.

## CONCLUSION

Conventional multi component UV spectroscopic methods are not suitable for combination drugs having narrow difference in $\lambda_{max}$. In such cases, chemometry serves as an alternative to other sophisticated methods like HPLC. Once the calibration matrix is built and stored in the data computation device, the samples can simply be prepared, diluted and absorbance measured and concentration of the sample read from the stored matrix. Three chemometric methods (PCR, PLS and CLS) were applied successfully to simultaneous determination of RIFA and PIPE in laboratory mixtures and pharmaceutical formulation. On the other hand, the fundamental advantages of investigated methods are the simultaneous analysis of the mixture of the subject drugs, without chemical pretreatment, speed of analysis and cost effectiveness. Model that gave lowest RMSEC values when used for predicting the unknown samples, predicted well by giving lowest RMSEP values and as per these values we can conclude that all methods canbe applied to the routine analysis and quality control of mixtures.

## CONFLICT OF INTEREST

There is no conflict of interest.

## ACKNOWLEDGEMENT

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