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# Simultaneous determination of sulfamethoxazole and trimethoprim in laboratory prepared mixtures and pharmaceutical preparations using three different analytical techniques

**Ahmed Ashour[1], Maha A.Hegazy[2], Mohamed Abdel-Kawy[2], Mohamed B.El-Zeiny*[3]**

[1]Department of Analytical Chemistry, Faculty of Pharmacy, Misr International University (EGYPT)

[2]Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University (EGYPT)

[3]Department of Analytical Chemistry, Faculty of Pharmacy, Modern Sciences and Arts University (EGYPT)

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## ABSTRACT

In present paper, Sulfamethoxazole and Trimethoprim -whose spectra show complete spectral overlap in their absorbing range (200-320)- were analyzed using three analytical techniques: two multivariate techniques- Partial least squares (PLS) and principal component regression (PCR)- and univariate first derivative on ratio spectra . These techniques do not require preceding extraction steps. The models were built using thirteen calibration samples and the accuracy of the results from developed models were tested by application on independent validation samples and market samples. The developed models show mean percentages recoveries of 101.07± 0.967, 100.92± 1.174 and 100.64± 0.806 for PLS-1, PCR and Svitsky Golay derivative ratio for Sulfamethoxazole and mean percentages recoveries of 100.44± 0.993, 100.37± 1.132 and 100.20± 1.251 for PLS-1, PCR and Svitsky Golay derivative ratio for trimethoprim . Being accurate, precise, rapid and relatively inexpensive; these methods can be used for the quality control labs in the routine analytical work on this pharmaceutical mixture.



## KEYWORDS

Partial least squares;
Principal component regression;
Derivative ratio spectrophotometry;
Trimethoprim;
Sulfamethoxazole.

## INTRODUCTION

Multivariate calibration techniques which are under a branch of chemometrics had played great role in the last three decades in the analysis of multicomponent systems that were previously very difficult if not impossible to be solved using simple spectrophotometric techniques and needing expensive, time consuming techniques for their analysis like HPLC.

Classical least squares (CLS), inverse least squares (ILS), principal component regression (PCR) and partial least squares (PLS) are the most widely known and used chemometric techniques. PCR and PLS are more widely used for the analysis than CLS as CLS needs the complete knowledge of the components in the system to be analyzed while PLS and PCR techniques can work in absence of this condition. PLS and PCR were used together or at least one of them in the analysis of many pharmaceutical mixtures recently[1-8].

Also derivative spectrophotometric techniques with zero crossing points and derivative ratio spectrophotometric techniques play a great role in resolving overlap-

ping peaks and allow for simultaneous spectrophotometric analysis of drugs with overlapping spectra.

In this paper; PLS, PCR along with first derivative on ratio spectra were used for the analysis of sulfamethoxazole (4-Amino-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide) (SMZ) and trimethoprim (5-[(3,4,5-Trimethoxyphenyl)methyl]-2,4 pyrimidinediamine) (TMP) pharmaceutical mixture. This mixture is used in pharmaceutical market as antibacterial dosage form in the treatment of respiratory and urinary tract infections. This drug mixture had been analyzed in the literature using different analytical techniques including: ratio derivative spectrophotometry with simultaneous standard additions method[9], differential pulse voltammetry[10], HPLC[11-16], LC/MS/MS[17-20]. Few papers were published for the analysis of this mixture using some chemometric techniques: PLS and PCR on HPLC-DAD data at five wavelengths[21], orthogonal signal correction- Partial least squares (OSC/PLS)[22]. [PCR and PLS][23] had been used giving bad results for sulfamethoxazole of 107% with percent relative prediction errors of 10.4 and 109% with percent relative prediction errors of 10.8 for PCR and PLS, respectively.

### Principle Component Regression(PCR)

This is a chemometric technique based on the inverse expression of Beer's law. PCR is one of the most widely applicable chemometric technique as it is full spectrum technique which does not require the knowledge about interfering substances in the system to be analyzed. Its main disadvantage is that it considers the concentration matrix to be error free which is not true in the real situations.

PCR had been successfully used for the analysis of SMZ-TMP mixture as will be shown in the results section.

### Partial Least Squares (PLS)

It is similar to PCR being based on the inverse expression of Beer's law. PLS differs from PCR in that it considers the concentration matrix also susceptible to error and perform simultaneous decomposition of both spectral and concentration matrices.

PLS had been successfully used for the analysis of SMZ-TMP mixture as will be shown in the results section.

### Derivative Ratio Spectrophotometry

In this technique, the spectra of one component is either divided by the spectrum of the other component that gives best quantitative results or divided by the normalized spectrum of the other component and looking for the peak place at which the first component can be determined in the mixture spectra without being affected by the other component.

## EXPERIMENTAL

### Apparatus and software

A Shimadzu UV 1800 double-beam spectrophotometer connected to a computer loaded with Shimadzu software UV prob 2.32 was used (Hiroshima, Japan). UV spectra were recorded using a 1-cm quartz cell; the scan range was 200-400nm with 1nm intervals. The computations were done using the Matlab 7.1 software, and our own written codes for calculating PLS-1, PCR according to the algorithms[24] and matlab code for Savitsky Golay derivative calculation.

### Samples and reagents

### Samples

### Pure samples

### Sulfamethoxazole

Sulfamethoxazole was kindly supplied by GlaxoSmithKline pharmaceutical company certified to contain 100.3%.

### Trimethoprim

Trimethoprim was kindly supplied by GlaxoSmithKline pharmaceutical company certified to contain 99.8%.

### Market samples

A commercial pharmaceutical formulation (Septrin) tablets produced by GlaxoSmithKline pharmaceutical company Batch no.092172A labeled to contain 800 mg sulfamethoxazole and 160 mg trimethoprim per tablet was obtained from the local market.

### Reagents

Methanol was of analytical spectroscopic grade and obtained from El-Nasr Pharmaceutical Chemicals Company.

### Standard solutions

### Stock solutions

Stock solutions of sulfamethoxazole and

trimethoprim were prepared by dissolving 0.1 gm of each drug in least amount of methanol spectroscopic grade in 100 mL volumetric flask and completing to the mark with distilled water and so obtaining 1mg/mL stock solution for both components.

### Working solutions

Working solutions of sulfamethoxazole and trimethoprim were prepared by diluting 50 mL and 10 mL of stock solutions of sulfamethoxazole and trimethoprim, respectively in 250 mL volumetric flask and completing to the mark with distilled water to obtain working solutions of 200 μg/mL and 40 μg/mL of sulfamethoxazole and trimethoprim respectively.

Multilevel multifactor experimental design was used for the construction of 25 binary mixture solutions. A five-level two-factor design was used. The concentrations of sulfamethoxazole are ranging from (32 μg/mL to 48 μg/mL) and the concentrations of trimethoprim are ranging from (6.4 μg/mL to 9.6 μg/mL). From these 25 mixtures, the odd samples were chosen to be used in building the model (calibration samples), while the even samples were used to build the validation set. (TABLE 1) shows the concentrations of the two components in the 25 prepared mixtures.

### Pharmaceutical preparations

Ten tablets were accurately weighed and powdered and an amount equivalent to one eighth tablet was taken and put in contact with 30 mL of methanol in volumetric flask 100 mL and the volume completed to the mark with distilled water. The flask was subjected to mechanical shaking for 30 minutes to ensure complete dissolution of the two active ingredients, and then the solution was filtered. The receiving flask was washed with part of the filterate. 4 mL of the filterate was taken in 100 mL volumetric flask and then the volume was completed to the mark with distilled water. This solution was measured spectroscopically.

### General procedure

The absorption spectra of calibration set, validation set and pharmaceutical preparation were recorded in the range 200-400 nm. The spectra were transferred to Matlab software version 7.1 for signal processing and analysis.

## RESULTS AND DISCUSSIONS

**TABLE 1 : Concentrations of sulfamethoxazole and trimethoprim in the 25 laboratory prepared mixtures.**

| Sample no. | Sulfamethoxazole (μg/mL) | Trimethoprim (μg/mL) |
|---|---|---|
| 1 | 40 | 8 |
| 2 | 40 | 6.4 |
| 3 | 32 | 6.4 |
| 4 | 32 | 9.6 |
| 5 | 48 | 7.2 |
| 6 | 36 | 9.6 |
| 7 | 48 | 8 |
| 8 | 40 | 7.2 |
| 9 | 36 | 7.2 |
| 10 | 36 | 8.8 |
| 11 | 44 | 9.6 |
| 12 | 48 | 8.8 |
| 13 | 44 | 8 |
| 14 | 40 | 9.6 |
| 15 | 48 | 9.6 |
| 16 | 48 | 6.4 |
| 17 | 32 | 8.8 |
| 18 | 44 | 6.4 |
| 19 | 32 | 8 |
| 20 | 40 | 8.8 |
| 21 | 44 | 8.8 |
| 22 | 44 | 7.2 |
| 23 | 36 | 6.4 |
| 24 | 32 | 7.2 |
| 25 | 36 | 8 |

### Spectral features

The spectra in (Figure 1), show complete spectral overlap between the pure spectra of both trimethoprim and sulfamethoxazole in their spectrally active range (200-320). It is also shown that the absorbance of trimethoprim is very low in comparison to that of sulfamethoxazole in their ratio in the pharmaceutical preparation (1:5) respectively.

### Application of the models on calibration samples

For PLS-1, the model was built using the calibration samples, the number of optimum latent variables was determined by plotting the calculated predicted residual error sum of squares (PRESS) against the number of latent variables. It was found that four latent variables are the optimal number for Sulfamethoxazole built

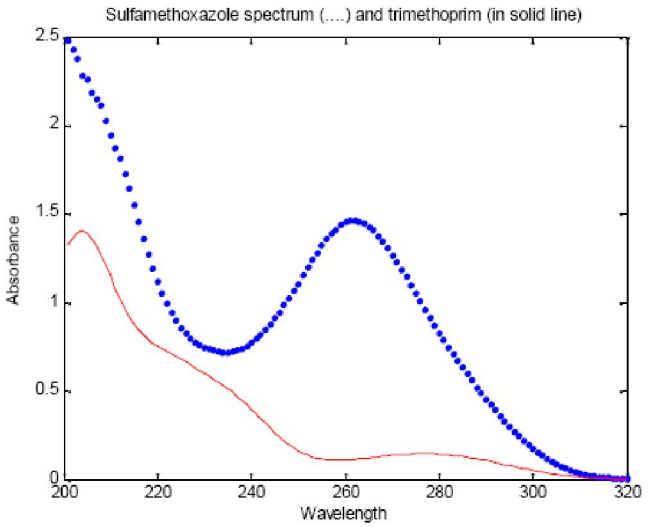


**Figure 1 : Showing the pure spectra of sulfamethoxazole (….) and trimethoprim in solid line in their ratio in the pharmaceutical preparation.**

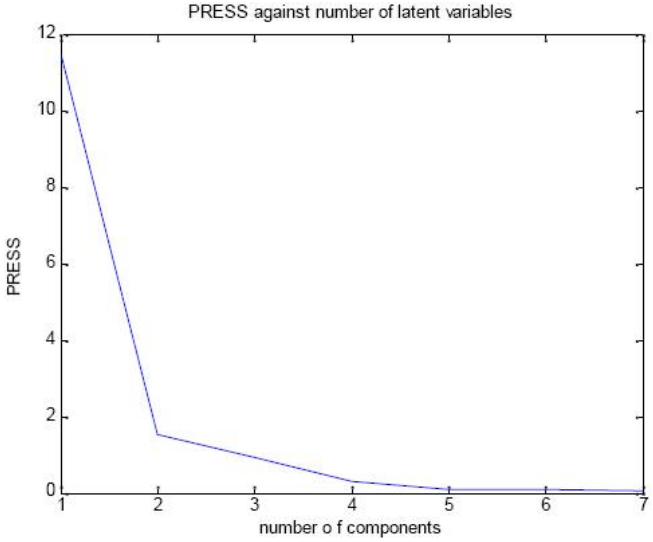


**Figure 2 : Showing the plot of PRESS against number of latent variables Showing the optimal number at four latent variables for sulfamethoxazole.**

model as shown in (Figure 2). It was found also that the optimal number of latent variables for trimethoprim is three as shown in (Figure 3).

For PCR, It was found that three components sufficiently model the data by looking to the percentage variance and cumulative percentage variance scanned by different numbers of latent variables . It was found that three principal components scan 100% of the variance in the spectra. For derivative ratio spectroscopy, it was found that for trimethoprim the best window size is 9 points with peak at 240nm and correlation coefficients 0.993, while for sulfamethoxazole; it was found

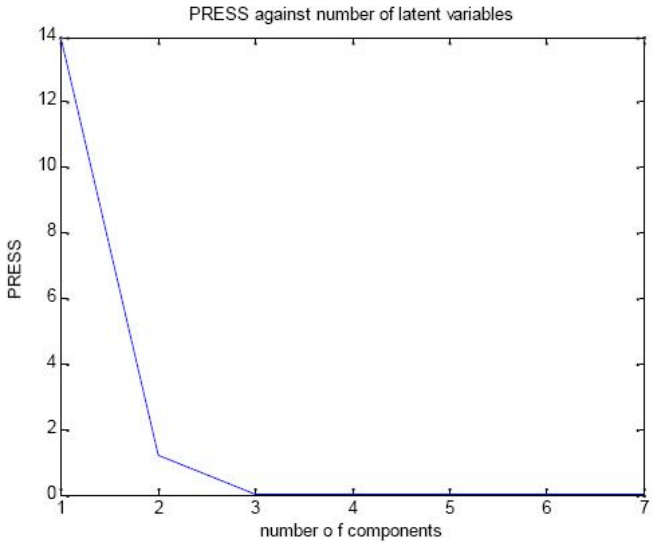


**Figure 3 : Showing the plot of PRESS against number of latent variables Showing the optimal number at three latent variables for trimethoprim.**

that the optimum window size is 7 with peak at 252nm and correlation coefficients 0.998. (Figure 4) shows the derivative ratio spectra of the calibration samples for the determination of Sulfamethoxazole, while (Figure 5) shows the derivative ratio spectra of the calibration samples for the determination of Trimethoprim.

(TABLE 2) shows the results obtained from the application of the developed models on the calibration

**Table 2 : Results from application the models on the calibration samples**

| Sample no. | Sulfamethoxazole | | | Trimethoprim | | |
|---|---|---|---|---|---|---|
| | PLS-1 | PCR | Savitsky Golay Der.rat | PLS-1 | PCR | Savitsky Golay Der.rat |
| 1 | 100.53 | 100.51 | 100.57 | 100.72 | 100.72 | 100.80 |
| 2 | 100.49 | 100.46 | 99.69 | 99.88 | 99.89 | 100.29 |
| 3 | 100.87 | 100.88 | 100.10 | 100.32 | 100.35 | 98.79 |
| 4 | 99.71 | 99.71 | 100.08 | 99.27 | 99.25 | 98.37 |
| 5 | 99.74 | 99.71 | 100.47 | 100.45 | 100.46 | 101.11 |
| 6 | 99.37 | 99.34 | 100.70 | 99.06 | 99.04 | 99.98 |
| 7 | 98.93 | 98.92 | 98.69 | 100.00 | 100.00 | 99.16 |
| 8 | 99.16 | 99.13 | 100.28 | 99.32 | 99.29 | 98.68 |
| 9 | 100.67 | 100.72 | 100.48 | 100.07 | 100.08 | 101.51 |
| 10 | 100.12 | 100.16 | 99.43 | 99.53 | 99.54 | 100.12 |
| 11 | 100.06 | 100.09 | 99.83 | 100.70 | 100.71 | 100.77 |
| 12 | 100.56 | 100.59 | 99.69 | 100.16 | 100.18 | 99.32 |
| 13 | 100.87 | 100.90 | 100.83 | 101.17 | 101.18 | 101.09 |
| mean | 100.08 | 100.09 | 100.06 | 100.05 | 100.05 | 100.00 |
| S.D. | 0.653 | 0.670 | 0.596 | 0.632 | 0.645 | 1.044 |

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samples used in building these models.

## Testing the model through validation samples

The developed models were tested for their predictability using independent validation samples not used in building the model and the obtained results are presented in (TABLE 3). As shown from the results; all the methods almost perform equally well for sulfamethoxazole as shown from the mean and standard deviation of recoveries of the validation samples. On the other hand, there are two samples in the derivative ratio are not well predicted (samples 8,9). These samples correspond to the lowest concentration (6.4 µg/mL) –periphery of the space scanned by the experimental design- that is why the concentration of the market sample should fall in the middle of the space scanned by a good experimental design where the best prediction will take place.

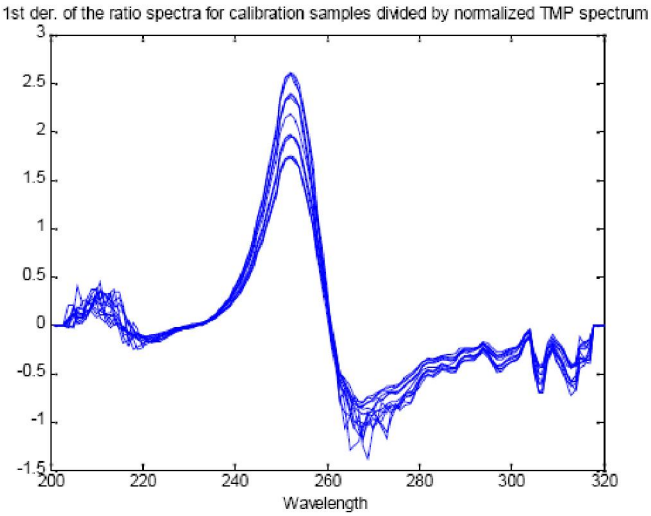


**Figure 4 : Shows the first derivative of the ratio spectra for calibration samples divided by normalized trimethoprim spectrum (peak at 252nm, window size= 7).**

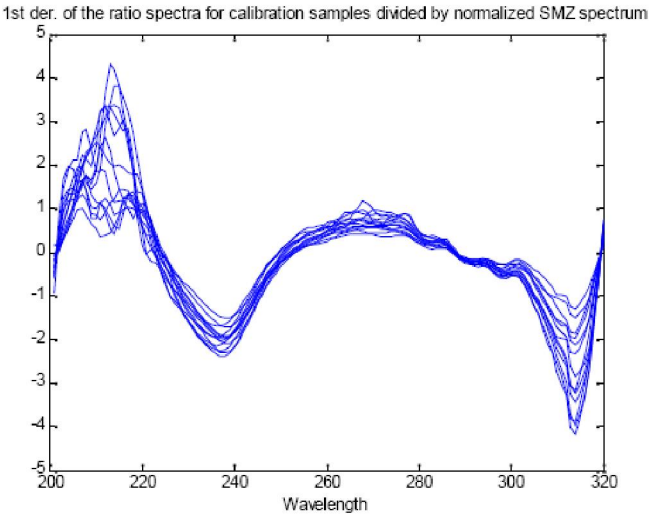


**Figure 5 : Shows the first derivative of the ratio spectra for calibration samples divided by normalized sulfamethoxazole spectrum (peak at 240nm, window size= 9).**

Being multivariate, PLS-1 and PCR had given good results for these samples as they extract information from many wavelengths (220-300), so they more robust in judging the concentrations than derivative ratio (univariate technique) as they judge through information of many wavelength not single one so if one erroneous others will reduce or cancel this error.

## Application of the model in the market samples

The developed models were applied for the analysis of the drug mixture in the pharmaceutical preparation and the obtained results were given in (TABLE 4).

As stated previously, although there were some bad results in the trimethoprim samples using derivative ratio spectroscopy, the prediction of the market sample is good and comparable to the results obtained by the PLS-1. This is due to the fact that the concentration chosen to be analyzed is in the center of the experimental design space where best predictions take place away from periphery which is more prone to some variability especially in presence of minor component as in our

**Table 3 : Results from application the models on the validation samples**

| Sample no. | Sulfamethoxazole | | | Trimethoprim | | |
|---|---|---|---|---|---|---|
| | PLS-1 | PCR | Savitsky Golay Der.rat | PLS-1 | PCR | Savitsky Golay Der.rat |
| 1 | 101.21 | 101.20 | 100.10 | 100.69 | 100.70 | 99.93 |
| 2 | 101.98 | 102.66 | 102.41 | 100.75 | 100.78 | 102.75 |
| 3 | 100.79 | 101.43 | 102.25 | 101.28 | 101.31 | 101.31 |
| 4 | 99.87 | 99.81 | 99.27 | 99.042 | 99.04 | 98.49 |
| 5 | 99.67 | 100.15 | 100.97 | 100.21 | 100.24 | 101.19 |
| 6 | 99.60 | 99.27 | 99.63 | 99.27 | 99.25 | 98.84 |
| 7 | 99.51 | 99.69 | 99.97 | 100.60 | 100.60 | 102.07 |
| 8 | 98.75 | 98.36 | 99.55 | 97.63 | 97.60 | 94.61 |
| 9 | 99.65 | 99.78 | 98.69 | 98.37 | 98.39 | 95.50 |
| 10 | 100.05 | 100.29 | 100.12 | 100.40 | 100.42 | 101.51 |
| 11 | 99.97 | 99.99 | 98.50 | 100.32 | 100.32 | 98.73 |
| 12 | 100.62 | 101.07 | 100.28 | 101.51 | 101.53 | 102.08 |
| mean | 100.14 | 100.31 | 100.15 | 100.01 | 100.01 | 99.93 |
| S.D. | 0.871 | 1.132 | 1.227 | 1.181 | 1.195 | 2.618 |
| RMSEV | 0.320 | 0.421 | 0.439 | 0.083 | 0.083 | 0.185 |

case.

Both F-test and T-test were applied for the results to show whether there are any significant differences between the developed PCR or derivative ratio in comparison to PLS-1 which is the considered the best quantitative chemometric technique. All the calculated values for both T-test and F-test are below the tabulated values indicating that there are no significant differences between the developed models and that they give equivalent good analytical results for the analysis of this pharmaceutical drug mixture.

**Table 4 : Results from application the models on the market samples**

| Sample no. | Sulfamethoxazole | | | Trimethoprim | | |
|---|---|---|---|---|---|---|
| | PLS-1 | PCR | Savitsky Golay Der.rat | PLS-1 | PCR | Savitsky Golay Der.rat |
| 1 | 102.44 | 100.44 | 99.94 | 100.46 | 101.28 | 101.33 |
| 2 | 100.48 | 100.99 | 101.26 | 101.28 | 100.99 | 99.47 |
| 3 | 101.04 | 102.51 | 100.11 | 100.98 | 100.46 | 101.19 |
| 4 | 100.31 | 99.75 | 99.33 | 99.04 | 98.75 | 98.82 |
| mean | 101.07 | 100.92 | 100.64 | 100.44 | 100.37 | 100.20 |
| S.D. | 0.967 | 1.174 | 0.806 | 0.993 | 1.132 | 1.251 |
| t-test 6(2.447)* | | 0.191 | 1.442 | | 0.093 | 0.297 |
| F-test (9.266)* | | 1.474 | 1.438 | | 1.3 | 1.587 |
| RMSEP | 0.543 | 0.549 | 0.287 | 0.077 | 0.084 | 0.088 |

*** Theoretical values at 95% confidence limit**

## CONCLUSION

In this paper, two multivariate models (PCR and PLS-1) along with one univariate method (first derivative ratio spectroscopy) were developed for the simultaneous spectroscopic analysis of sulfamethoxazole and trimethoprim without need for previous separation. Although the spectra of the two components are overlapping over the whole spectral range and that trimethoprim spectral contribution is very low (minor component) in comparison to sulfamethoxazole in their preparation ratio (1:5), the developed models results were accurate (mean recoveries around 100%) and precise (standard deviations are lower than 1.5). Being fast and relatively inexpensive, the developed methods can be used in quality control laboratories for the simultaneous analysis of this pharmaceutical drug mixture.

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