

**Simultaneous determination of quaternary mixture of  
sulfamethoxazole, trimethoprim, propylparaben and  
methylparaben in pharmaceutical formulations using mean  
centering of ratio spectra**

**Dr. R'afat Mahmoud Nejem \***  
**Dr. Mahmoud Mohamed Issa \***  
**Dr. Alaa Mohamed Abu Shanab \***  
**Dr. Nahed Talab Shaat \*\***

---

/ 20.0-0.8 20.0 -0.16 30.0 -2.0 64.0 -2.5

(CLS)

**Abstract**

Spectrophotometric method is proposed for the simultaneous determination of quaternary mixture of sulfamethoxazole, trimethoprim, propylparaben and methylparaben in pharmaceutical formulations without prior separation steps, using mean centering of ratio spectra. The method is based on the difference in the absorption spectra for sulfamethoxazole, trimethoprim, propylparaben and methylparaben. The method allows rapid and accurate determination of the four components. The results showed that the method was capable to simultaneous determination of 2.5-64.0, 2.0-30.0,

---

- - - - \*

- - \*\*

0.16-20.0 and 0.8-20.0  $\mu\text{g/mL}$  each of sulfamethoxazole, trimethoprim, propylparaben and methylparaben. The results obtained were statistically compared with a developed classical least squares method (CLS). No significant difference was found between the two proposed methods. The proposed method has been successfully applied to simultaneous determination of sulfamethoxazole, trimethoprim, propylparaben and methylparaben in synthetic and pharmaceutical formulations.

**Keywords:** Mean centering of ratio spectra; Classical least squares method; Sulfamethoxazole; Trimethoprim; Propylparaben; Methylparaben

### **Introduction:**

Sulphamethoxazole (SU) and trimethoprim (TR), in addition to methylparaben (MP) and propylparaben (PP), (Fig. 1), occur in a number of dosage forms. This synergism combination was used for the treatment of a variety of infections caused by Gram-positive and Gram-negative bacteria such as urinary and respiratory tract infections (Goodman, Gilman, 1980 : 1116).

The UV absorption spectrum of SU, TR, PP and MP displays considerable overlap, where the application of conventional spectrophotometry failed to resolve it. No spectrophotometric analytical methods have been reported for the simultaneous determination of SU, TR, PP and MP in a multicomponent mixture, while official methods (United State Pharmacopoeia, 2009; British Pharmacopoeia, 2009) are available for determination of SU and TR, separately or in combination. The most prominent method for simultaneous determination of SU, TR, PP and MP is the HPLC (Vander Steuijt and Sonneveld, 1987; Spreux-Varogoux *et al*, 1983; De Angelis *et al*, 1990; Laizur *et al*, 1990; Amini and Ahmadiani, 2007; Mistri *et al*, 2010). Chemometric such as H-point standard additions method (Givianrad *et al*, 2011), bivariate calibration spectrophotometric method (Lopez Martinez *et al*, 2002), multicomponent derivative analysis (Granero *et al*, 2002), ratio spectra derivative spectrophotometry (Berzas Nevado *et al*, 1992) and principal component regression analysis (Ribone *et al*, 2000) have been used for simultaneous determination of binary mixture of SU and TR. No chemometric methods were applied for simultaneous determination of quaternary mixture of SU, TR, PP and MP.

The objective of this work is to develop, a simple, sensitive and selective method for the determination of SU, TR, PP and MP in quaternary

## Simultaneous determination of ....

mixture. This is the first research paper on the determination of four component mixture using mean centering of ratio spectra method.

### Experimental:

#### Standard samples and reagent

Pharmaceutical grade of SU, TR, PP and MP with claimed purities of 99.7, 99.8, 99.9 and 99.7 %, respectively according to manufacturer certificate and were kindly donated by the Middle East pharmaceuticals and cosmetics laboratories, Palestine. Megaprime suspension, (batch number 7580) Middle East pharmaceuticals and cosmetics laboratories was used for analysis. Each 5 ml contains 200 mg SU; 40 mg TR; 1.0 mg PP and 5.0 mg MP, respectively.

#### Instrumentation:

UV-Visible double-beam spectrophotometer, Shimadzu (Kyoto, Japan) model 1650 PC, with two matched 1cm path-length quartz cells was used. The subsequent statistical manipulation was performed by transferring the spectral data to Microsoft Excel 2010 program and processing them with the standard curve fit package and matrix calculation.

#### Standard and working solutions:

Stock standard solutions of SU (32 mg/L), TR (6.4 mg/L), PP (0.16 mg/L) and MP (0.8 mg/L) were independently prepared by dissolving accurately weighed amounts of the drugs in 0.1 mol/L HCl (Merck). Working solutions were prepared by transferring appropriate volumes of the stock solutions to separate 25 ml volumetric flasks and diluting to their full marks with 0.1 mol/L HCl.

### Theoretical background

#### MCR developed method:

If a quaternary mixture (SU, TR, PP and MP) is considered and if Beer's Law is obeyed for all compounds over the whole wavelength range used, then

$$A_m = \alpha_{SU} C_{SU} + \alpha_{TR} C_{TR} + \alpha_{PP} C_{PP} + \alpha_{MP} C_{MP} \quad (1)$$

where  $A_m$  is the vector of the absorbance of the mixture  $\alpha_{SU}$ ,  $\alpha_{TR}$ ,  $\alpha_{PP}$  and  $\alpha_{MP}$  are the absorptivity vectors of SU, TR, PP and MP and  $C_{SU}$ ,  $C_{TR}$ ,  $C_{PP}$ , and  $C_{MP}$  are the concentration of SU, TR, PP and MP, respectively.

If Equation (1) is divided by  $\alpha_{SU}$  corresponding to the spectrum of a standard solution of SU in the mixture, the first ratio spectra is obtained

$$x = \frac{A_m}{\alpha_{SU}} = C_{SU} + \frac{\alpha_{TR}}{\alpha_{SU}} C_{TR} + \frac{\alpha_{PP}}{\alpha_{SU}} C_{PP} + \frac{\alpha_{MP}}{\alpha_{SU}} C_{MP} \quad (2)$$

If the Equation (2) is mean centered, then

$$mc \ x = mc \frac{\alpha_{TR}}{\alpha_{SU}} C_{TR} + mc \frac{\alpha_{PP}}{\alpha_{SU}} C_{PP} + mc \frac{\alpha_{MP}}{\alpha_{SU}} C_{MP} \quad (3)$$

By dividing Equation (3) by  $mc \frac{\alpha_{MP}}{\alpha_{SU}}$ , the second ratio spectrum is obtained

$$y = \frac{mc \ x}{mc \frac{\alpha_{MP}}{\alpha_{SU}}} = \frac{mc \frac{\alpha_{TR}}{\alpha_{SU}}}{mc \frac{\alpha_{MP}}{\alpha_{SU}}} C_{TR} + \frac{mc \frac{\alpha_{PP}}{\alpha_{SU}}}{mc \frac{\alpha_{MP}}{\alpha_{SU}}} C_{PP} + C_{MP}$$

$$mc \ y = mc \frac{mc \ \frac{\alpha_{TR}}{\alpha_{SU}}}{mc \ \frac{\alpha_{MP}}{\alpha_{SU}}} C_{TR} + mc \ z \ C_{PP} \quad (4)$$

In the same way, the third ratio spectrum can be obtained

$$mc \ \frac{mc \ y}{mc \ z} = mc \ \frac{mc \ \frac{\alpha_{TR}}{\alpha_{SU}}}{mc \ \frac{\alpha_{MP}}{\alpha_{SU}}} C_{TR}$$

or

$$mc \ \frac{mc \ y}{mc \ z} = K \ C_{TR} \quad (K \text{ is a constant}) \quad (5)$$

Equation (5) is the mathematical foundation of multicomponent analysis which permits the determination of the concentration of each compound without interference from the other components of the mixture. In practice, the signal of the third ratio spectrum of TR is dependent only on the concentration values  $C_{TR}$  and  $\frac{A_m}{\alpha_{SU}}$ , but is independent on the concentration values  $C_{SU}$ ,  $C_{PP}$  and  $C_{MP}$  in the quaternary mixture. In the

### Simultaneous determination of ....

developed method, the concentration  $C_{TR}$  in the mixture is proportional to the amount of  $mc \frac{mc y}{mc z}$  corresponding to a maximum or minimum point. A

calibration curve could be constructed by plotting  $mc \frac{mc y}{mc z}$  against different concentrations of TR. By using the calibration curve, the concentration of TR is determined in the sample containing SU, TA, PP, and MP. The concentration of other components is determined by analogous procedures.

### Classical least squares method:

The mathematical formulation of this method is (Haaland and Thomas, 1988)

$$A = \alpha C + E$$

where the matrix A represents the absorbance matrix, C is the concentration matrix,  $\alpha$  is the absorptivity and E is a matrix of concentration prediction error. The matrix can be solved by computing

$$C = \alpha^{-1} A \quad (\alpha^{-1} \text{ is pseudo-inverse})$$
$$\alpha^{-1} = (\alpha' \alpha)^{-1} \alpha' \quad (\alpha' \text{ is transpose matrix})$$

### Analytical procedure:

Accurate aliquots of SU, TR, PP and MP were transferred from their respective working standard solutions into three series of 10 ml volumetric flasks, the volume was completed to the mark with 0.1 mol/L HCl to obtain final concentration range 2.5-64.0  $\mu\text{g/mL}$  SU, 2.0-30.0  $\mu\text{g/mL}$  TR, 0.16-20.0  $\mu\text{g/mL}$  PP, and 0.8-20.0  $\mu\text{g/mL}$  MP. The absorption spectra of the prepared solution were measured in the range of 250-285 nm.

For TR, the recorded spectra were divided by standard spectrum of 1  $\mu\text{g/mL}$  SU to obtain the first ratio spectra which then was centered. These vectors were divided by the mean center of  $\frac{\alpha_{MP}}{\alpha_{SU}}$  and, therefore the mean

centering of the second ratio spectra were obtained. In the same way, the third ratio spectra can be obtained as shown in Table 1. The mean centered values of the third ratio spectra at 260, 265, 260 and 260 for SU, TR, PP and MP, respectively were measured and plotted against the correspond concentration of each drug to construct their calibration curves.

Different synthetic mixture containing variable ratios of each SU, TR, PP and MP within their calibration ranges were prepared. The spectra of these mixtures were then recorded and the procedure that previously explained was performed to predict the concentration of each compound in the mixture.

2 ml of the Megaprime suspension was transferred to 100 ml volumetric flask and dissolved in 98.0 ml of 0.1 mol/L HCl. A 1.0 ml of this solution was transferred to 25 ml volumetric flask and the volume was completed with 0.1 mol/L HCl. The proposed method was applied to the prepared solutions.

### **Results and discussion:**

The absorption spectra of SU, TR, PP and MP, Figure 1, display considerable overlap, where the application of conventional spectrophotometry failed to resolve these overlapping. Till the time being, there are no other techniques for the simultaneous spectrophotometry determination of these drugs by chemometric method. The main objective of this work was to develop simple, sensitive and accurate analytical method for simultaneous determination of the drugs in their mixtures.

### **Mean centering of ratio spectra method (MCR):**

The developed MCR method depends on the mean centering of ratio spectra to eliminate the derivative steps and therefore signal ratio was enhanced and it has been applied for resolving the four component mixture (Afkhani and Bahram, 2005).

In order to optimize the developed MCR method, effect of divisor on the selectivity of the method has been tested. Different concentrations of each drug was tested. Table 1 shows that the divisor has a remarkable effect on the selectivity of determination of SU, TR, PP and MP where reproducible and good results have been obtained upon using concentration of 1  $\mu\text{g/ml}$  of SU (for TR, PP and MP) and 1  $\mu\text{g/ml}$  TR (for SU) as divisors. On the other hand, changing the concentration of the divisor had a great effect on the slop, intercept and correlation of calibration equation recorded in Table 2. The amount of  $\Delta\lambda$  has no effect on the mean centering of ratio spectra, a  $\Delta\lambda$  of 5 nm was used.

The absorption spectra of the standard solutions of TR were divided by the spectrum of 1  $\mu\text{g/ml}$  SU and the first ratio spectrum was obtained. The

### Simultaneous determination of ....

third ratio spectrum according to equation (5) was obtained; while the concentration of TR was determined by measuring the amplitude at 265 nm corresponding to a minimum wavelength in the third ratio spectrum as shown in Figure 2. For the prediction of concentration of TR in synthetic mixtures and real samples, the same procedure was used.

In the same way, the first and third ratio spectra for PP, MP and SU were obtained. The concentration of PP, MP and SU was determined by measuring the amplitude at 260 nm as shown in Figures 3-5, respectively.

Beer's law was obeyed in the concentration 2.5-64.0 µg/ml for SU, 2.0-30.0 µg/ml TR, 0.16-20.0 µg/ml PP and 0.8-20.0 µg/ml MP. Table 2 shows the linear regression parameters for calibration data for simultaneous determination of SU, TR, PP and MP in their four component mixtures. Detection limits are defined as the concentration equivalent to three times the standard deviation of replicate measurements of the blank are shown in Table 2.

To check the reproducibility of the method, the relative standard deviation (R.S.D) for five replicate determinations of 5.0 µg/ml each of SU, TR, PP and MP, in quaternary mixture was obtained as 2.21, 1.97, 1.57 and 1.34 %, respectively. The mean recoveries for simultaneous determination were obtained as 99.9, 99.5, 100.5 and 100.3 % for SU, TR, PP and MP, respectively.

In order to obtain the accuracy and precision of the method, several synthetic mixtures with different concentration ratios of SU, TR, PP and MP were analyzed using the proposed method. The results are summarized in Table 3 where the prediction error of single component in the mixtures was calculated as the relative standard error (R.S.E.) of the prediction concentration (Abdollahi, 2001):

$$\text{R.S.E. (\%)} = \left( \frac{\sum_{j=1}^N (\hat{C}_j - C_j)^2}{\sum_{j=1}^N (C_j)^2} \right)^{\frac{1}{2}} \times 100$$

where N is the number of samples,  $C_j$  the concentration of component in the mixture and  $\hat{C}_j$  the estimated concentration. The total prediction error of N so is calculated as follows:

$$R.S.E._t(\%) = \left( \frac{\sum_{i=1}^M \sum_{j=1}^N (\hat{C}_{ij} - C_{ij})^2}{\sum_{i=1}^M \sum_{j=1}^N (C_{ij})^2} \right)^{\frac{1}{2}} \times 100$$

where  $C_{ij}$  is the concentration of the component in the  $j^{th}$  samples and  $\hat{C}_{ij}$  its estimation. Table 3 also shows the single and total relative errors for the ternary mixtures.

### Classical least squares (CLS) method

The absorptivity ( $\alpha$ ) were calculated for each drug using the absorbance's measured at 8.0 points (range 250-285 nm) with intervals of  $\Delta\lambda=5$  in the spectrum as shown in Table 4.

Equation (6) is a mathematical foundation of multicomponent analysis which permits the determination of the concentration of each compound without interference from the other components of the mixture, where,  $C_{SU}$ ,  $C_{TR}$ ,  $C_{PP}$ , and  $C_{MP}$  are the concentration of SU, TR, PP and MP respectively. The absorbance values at 8.0 points (250-285 nm) for samples were introduced into the above equations. The concentration of the four component mixtures were calculated.

$$\begin{bmatrix} -147.89931 & -9.8854084 & 62.846657 & 97.125981 & 34.211415 & 1.5619125 & -28.976263 & 10.752815 \\ 88.842415 & -20.084728 & -21.454948 & -82.701609 & -24.929848 & 79.307535 & 9.2153549 & 14.621975 \\ -41.272911 & -102.676179 & 246.93092 & -184.14509 & -144.841806 & 481.01261 & -291.18561 & 6.4894007 \\ 41.615834 & 93.5375387 & -206.48359 & 155.405129 & 120.465815 & -412.74923 & 242.37723 & -10.689198 \end{bmatrix} \begin{bmatrix} A_{250} \\ A_{255} \\ A_{260} \\ A_{265} \\ A_{270} \\ A_{275} \\ A_{280} \\ A_{285} \end{bmatrix} = \begin{bmatrix} C_{SU} \\ C_{TR} \\ C_{PP} \\ C_{MP} \end{bmatrix} \dots\dots\dots (6)$$

Beer's law was obeyed in the concentration 2.5-64.0  $\mu\text{g/ml}$  SU, 2.0-30.0  $\mu\text{g/ml}$  TR, 0.16-20.0  $\mu\text{g/ml}$  PP and 0.8-20.0  $\mu\text{g/ml}$  MP. Table 5 shows the linear regression parameters for calculation data for simultaneous determination of SU, TR, PP and MP in their mixtures.

To check the reproducibility of the CLS method, the (R.S.D.) for five replicates determinations of 5  $\mu\text{gml}^{-1}$  each of SU, TR, PP and MP, in the mixtures were obtained as 1.91, 2.22, 1.75 and 1.89 %, respectively. The mean recoveries were obtained at 100.4, 100, 99.2 and 99.7% for SU, TR, PP and MP, respectively.

### Simultaneous determination of ....

In order to obtain the accuracy and precision of the CLS method, several synthetic mixtures were analyzed. The results are given in Table 6 where the standard error of prediction (S.E.P.) was calculated as indicated by (Dinc *et al*, 2002)

$$\text{S.E.P.} = \left( \frac{\sum_{j=1}^N (\hat{C}_j - C_j)^2}{N} \right)^{\frac{1}{2}}$$

where N is the number of samples,  $C_j$  the concentration of component in  $j^{\text{th}}$  mixture and  $\hat{C}_j$  the estimated concentration.

### Analysis of pharmaceutical suspension:

The proposed MCR and CLS methods were applied to the simultaneous determinations of SU, TR, PP and MP in commercial suspension. Five replicates determinations were carried out. Satisfactory results were obtained for each compound in similar results with label claims (Table7). No published method has been reported for simultaneous determination of the four components of this mixture. So, the results of the developed MCR method were compared with those of the proposed CLS method. Statistical comparison between the results was performed with regards to accuracy and precision using student's t-test and F-ratio at 95% confidence limit (Table 7). There is no significant difference between the results with respect to accuracy and precision.

### Conclusions:

The proposed method has the advantage of high sensitivity, extremely low detection limit, good selectivity, rapid analysis and inexpensive instrument. Furthermore, while working with this method. No need to use toxic organic solvents, In other words, they belong to green chemistry. The developed method was found to be suitable for the routine simultaneous determination of SU, TR, PP and MP in pharmaceutical suspension.

**REFERENCES:**

- [1] Goodman, Gilman. 1980: The Pharmaceutical Basis of Therapeutics, sixth ed., Macmillan, New York, 1116-1119.
- [2] United State Pharmacopoeia 32. 2009: National Formulary 27, USP convention Rockville, Md, USA.
- [3] British Pharmacopoeia. 2009: HMSO Publication Centre, vol. 3, HMSO Publication Centre, London, UK.
- [4] vander Steuijt V. and Sonneveld P. 1987: Concurrent analysis of methotrexate, trimethoprim, sulphamethoxazole and their major metabolites in plasma by high-performance liquid chromatography, J. Chromatogr. 422, 328-333.
- [5] Spreux-Varogaux O., Chapalain J.P., Cordonnier P., Adveurier C., Pays M. and Lamine L. 1983: Determination of trimethoprim, sulphamethoxazole and its N4-acetyl metabolite in biological fluids by high-performance liquid chromatography J. Chromatog. 274, 187-199.
- [6] De Angelis D.V., Woolley J.L. and Sigel C.W. 1990: High-Performance Liquid Chromatographic Assay for the Simultaneous Measurement of Trimethoprim and Sulfamethoxazole in Plasma or Urine, Ther. Drug Monit. 12, 382-392.
- [7] Laizure S.C., Holden C.L. and Stevens R.C., Ion-paired high-performance liquid chromatographic separation of trimethoprim, sulfamethoxazole and N4-acetylsulfamethoxazole with solid-phase extraction., J. Chromatog. 528(1), 235-242.
- [8] Amini H. and Ahmadiani A. 2007: Rapid and simultaneous determination of sulfamethoxazole and trimethoprim in human plasma by high-performance liquid chromatography, J. Pharm. Biomed. Anal. 43, 1146-1150.
- [9] Mistri H.N., Jangid A.G., Padage A., Alayshah A. and Shrivastar P.S. 2010: Simultaneous determination of sulfamethoxazole and trimethoprim in microgram quantities from low plasma volume by liquid chromatography–tandem mass spectrometry, Microchem. J. 94(2), 130-138.
- [10] Givianrad M.H., Saber-Tehrani M., Aberoomand-Azar P. and Mohagheghian M. 2011: H-point standard additions method for simultaneous determination of sulfamethoxazole and trimethoprim in

### Simultaneous determination of ....

- Pharmaceutical formulations and biological fluids with simultaneous addition of two analytes, *Spectrochimica Acta Part A* 78, 1196-1200.
- [11] Lopez Martinez L., Lopez De Alba P.L., De-leon-Rodriguez L.M. and Yepez-Murrieta M.L. 2002: Simultaneous determination of binary mixtures of trimethoprim and sulfamethoxazole or sulphamethoxy pyridazine by the bivariate calibration spectrophotometric method, *J. Pharm. Biomed. Anal.*, 30, 77-85.
- [12] Granero G., C. Granero and Longhi M. 2002: Second derivative spectrophotometric determination of sulfamethoxazole and trimethoprim in the presence of hydroxylpropyl-beta-cyclodextrin. *J. Pharm. Biomed. Anal.*, 29, 51-59.
- [13] Berzas Nevado J.J., Gallego J.L. and Penalvo G.C. 1992: Determination of sulfamethoxazole and trimethoprim by ratio spectra derivative spectrophotometry, *Fresenius J. Anal. Chem.*, 342, 723-728.
- [14] Ribone M.E., Pagani A.P., Goicoechea H.C. and Olivieri A.C. 2000: *Chem. Educ.*, 5, 236-241.
- [15] Haaland D.M. and Thomas E.V. 1988: Partial least-squares methods for spectral analysis. 1. Relation to other quantitative calibration methods the extraction of qualitative information, *Anal. Chem.*, 60, 1193-1202.
- [16] Afkhami A. and Bahram M. 2001: Mean centering of ratio spectra as a new spectrophotometric method for the analysis of binary and ternary mixtures, *Talanta* 66, 712-770.
- [17] Abdollahi H. 2001: Simultaneous spectrophotometric determination of chromium(VI) and iron(III) with chromogenic mixed reagents by H-point standard addition method and partial least squares regression, *Anal. Chim. Acta*, 442(2), 327-336.
- [18] Dinc E., Baydan E., Kanbur M. and Onur F. 2002: spectrophotometric multicomponent determination of sunset yellow, tartrazine and allura red in soft drink powder by double divisor-ratio spectra derivative, inverse least-squares and principal component regression method, *Talanta*, 58, 579-594.

**Table 1. The first, second and third ratio spectra data**

Drug	x	y	z	Divisor
TR	$\frac{A_m}{\alpha_{SU}}$	$\frac{mc x}{mc \frac{\alpha_{MP}}{\alpha_{SU}}}$	$\frac{mc \frac{\alpha_{PP}}{\alpha_{SU}}}{mc \frac{\alpha_{MP}}{\alpha_{SU}}}$	1 µg/mL SU
PP	$\frac{A_m}{\alpha_{SU}}$	$\frac{mc x}{mc \frac{\alpha_{MP}}{\alpha_{SU}}}$	$\frac{mc \frac{\alpha_{TR}}{\alpha_{SU}}}{mc \frac{\alpha_{MP}}{\alpha_{SU}}}$	1 µg/mL SU
MP	$\frac{A_m}{\alpha_{SU}}$	$\frac{mc x}{mc \frac{\alpha_{PP}}{\alpha_{SU}}}$	$\frac{mc \frac{\alpha_{TR}}{\alpha_{SU}}}{mc \frac{\alpha_{PP}}{\alpha_{SU}}}$	1 µg/mL SU
SU	$\frac{A_m}{\alpha_{TR}}$	$\frac{mc x}{mc \frac{\alpha_{MP}}{\alpha_{TR}}}$	$\frac{mc \frac{\alpha_{PP}}{\alpha_{TR}}}{mc \frac{\alpha_{MP}}{\alpha_{TR}}}$	1 µg/mL TR

**Table 2. Analytical characteristic for analysis of SU, TR, PP and MP by MCR method**

Analyte	λ (nm)	Calibration equation	R <sup>2</sup>	Linear range	LOD (µg/ml)
SU	260.0	y = 130.7 C - 6.667	0.9998	2.5-64.0	0.22
TR	265.0	y = -898.7 C + 3.321	0.9999	2.0-30.0	0.13
PP	260.0	y = -8.143 C - 1.322	0.9981	0.16-20.0	0.05
MP	260.0	y = -30.13 C - 4.233	0.9992	0.8-20.0	0.06

Simultaneous determination of ....

**Table 3. Results for several experiments for analysis of SU, TR, PP and in synthetic mixture by MCR method.**

Taken ( $\mu\text{g/mL}$ )				Found ( $\mu\text{g/mL}$ )				Recovery (%)			
SU	TR	PP	MP	SU	TR	PP	MP	SU	TR	PP	MP
32.0	6.4	0.16	0.8	31.70	6.44	0.164	0.81	99.1	100.6	102.5	101.3
2.50	2.50	2.50	2.50	2.45	2.46	2.530	2.57	98.0	98.4	101.2	102.8
40.0	8.0	0.20	1.0	39.30	8.12	0.204	1.02	98.3	101.5	102.0	102.0
5.0	5.0	5.00	5.0	5.08	4.96	5.060	4.95	101.6	99.2	101.2	99.0
48.0	9.6	0.24	1.2	48.70	9.55	0.233	1.23	101.5	99.5	97.1	102.5
10.0	10.0	10.0	10.0	10.10	9.91	10.20	9.91	101.0	99.1	102.0	99.1
64.0	12.8	0.32	1.6	63.20	12.60	0.312	1.55	98.8	98.4	97.5	97.5
20.0	20.0	20.0	20.0	20.10	19.80	20.10	19.70	100.5	99.0	100.5	98.5
Mean recovery								99.9	99.5	100.5	100.3
R.S.E. Single (%)								1.349	1.109	1.014	1.422
R.S.E. (total) (%)								1.322			

**Table 4. Experimental parameters calculated for the determination of SU, TR, PP and MP**

$\lambda$	$\alpha_{\text{SU}}$	$\alpha_{\text{TR}}$	$\alpha_{\text{PP}}$	$\alpha_{\text{MP}}$
250.0	129.84	224.53	780.0	950.00
255.0	142.74	183.96	850.0	1029.17
260.0	160.48	186.79	790.0	950.00
265.0	179.19	204.72	640.0	787.50
270.0	164.52	216.04	470.0	587.50
275.0	143.55	209.43	340.0	416.66
280.0	111.29	173.58	150.0	208.33
285.0	87.900	127.35	70.0	100.0

**Table 5. Analytical characteristics for analysis of SU, TR, PP and MP by CLS method**

Drug	Calibration equations		$R^2$	Range ( $\mu\text{gml}^{-1}$ )	LOD
SU	$-147.9A_1 - 9.9A_2 + 62.8A_3 + 97.1A_4 + 34.2A_5 - 1.56A_6 - 28.9A_7 + 10.7A_8$	$=C_{\text{SU}}$	0.9998	2.5-64.0	0.18
TR	$88.8A_1 - 20.1A_2 - 21.4A_3 - 82.7A_4 - 24.9A_5 + 79.3A_6 + 9.2A_7 + 14.6A_8$	$=C_{\text{TR}}$	0.9991	2.0-30.0	0.11
PP	$-41.2A_1 - 102.6A_2 + 246.9A_3 - 184.1A_4 - 144.8A_5 + 481.0A_6 - 291.1A_7 + 6.4A_8$	$=C_{\text{PP}}$	0.9987	0.16-20.0	0.04
MP	$41.6A_1 + 93.5A_2 - 206.4A_3 + 155.4A_4 + 120.4A_5 - 412.7A_6 + 242.37A_7 - 10.6A_8$	$=C_{\text{MP}}$	0.9990	0.8-20.0	0.06

**Table 6. Results for several experiments for analysis of SU, TR, PP and MP by CLS method**

Taken (µg/mL)				Found ( µg/mL)				Recovery (%)			
SU	TR	PP	MP	SU	TR	PP	MP	SU	TR	PP	MP
32.0	6.40	0.16	0.8	32.20	6.42	0.161	0.79	100.9	100.3	100.6	98.8
2.5	2.50	2.50	2.5	2.55	2.50	2.540	2.40	102.0	100.0	101.6	96.0
40.0	8.00	0.20	1.0	40.70	7.90	0.230	1.01	101.8	98.8	95.8	101.0
5.0	5.00	5.00	5.0	4.92	5.06	4.950	5.0	98.4	101.2	99.0	100.0
48.0	9.60	0.24	1.2	47.93	9.63	0.236	1.19	99.9	100.1	98.3	99.3
10.0	10.00	10.0	10.0	9.90	10.10	10.20	9.94	99.0	101.0	102.2	99.4
64.0	12.80	0.32	1.6	63.60	12.77	0.310	1.63	99.4	99.8	96.9	101.9
20.0	20.00	20.0	20.0	20.40	19.91	19.89	20.3	102.0	99.6	99.5	101.5
Mean recovery								100.4	100.1	99.2	99.7
S.E.P.								1.413	0.723	2.188	1.773

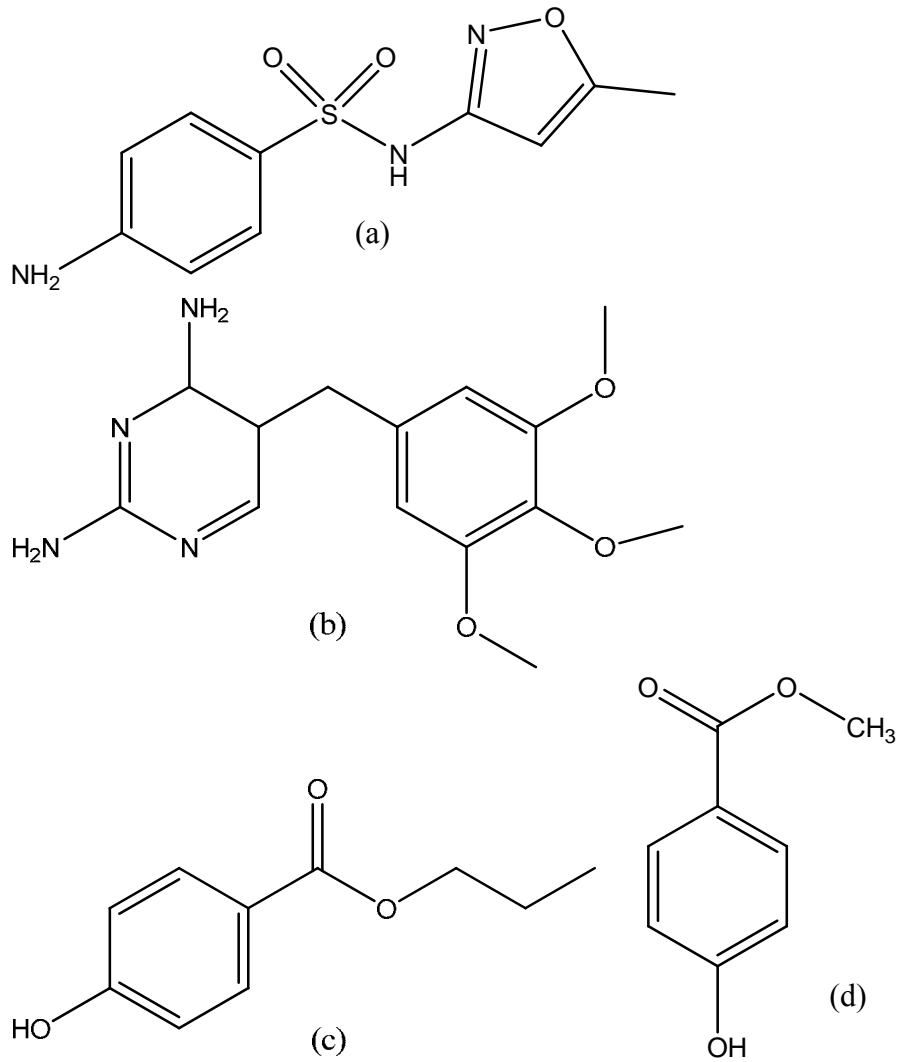
**Table 7. Determination of SU, TR, PP and MP in commercial suspension using the proposed methods**

Sample No.	Concentration (µg/mL)				Recovery (%)							
					MCR				CLS			
	SU	TR	PP	MP	SU	TR	PP	MP	SU	TR	PP	MP
1	32.0	6.4	0.16	0.8	99.1	100.6	102.5	161.3	99.2	98.3	97.9	99.4
2	40.0	8.0	0.20	1.0	98.3	101.5	102.0	167.8	100.7	99.8	99.6	97.1
3	44.0	8.8	0.22	1.1	101.1	101.4	100.5	98.2	100.3	99.7	101.0	100.9
4	48.0	9.6	0.24	1.2	101.5	99.5	97.1	102.5	98.7	101.9	98.3	100.1
5	64.0	12.8	0.32	1.6	99.8	98.4	97.5	97.5	101.5	101.4	99.9	100.8
Mean recovery					100.0	100.3	99.9	100.5	100.1	100.3	99.3	98.9
S.D <sup>a</sup> .					1.34	1.32	2.51	2.46	1.13	1.44	1.13	1.77
t <sup>b</sup>					1.291	0.97	1.13	1.43				
F <sup>b</sup>					1.41	0.84	4.93	1.93				

<sup>a</sup> Standard deviation

<sup>b</sup> Theoretical values for t and F at P=0.05 are 2.776 and 6.39, respectively.

**Simultaneous determination of ....**



Chemical structure of : a) Sulfamethoxazole, b) Trimethoprim, c) Propylparaben and d) Methylparaben.

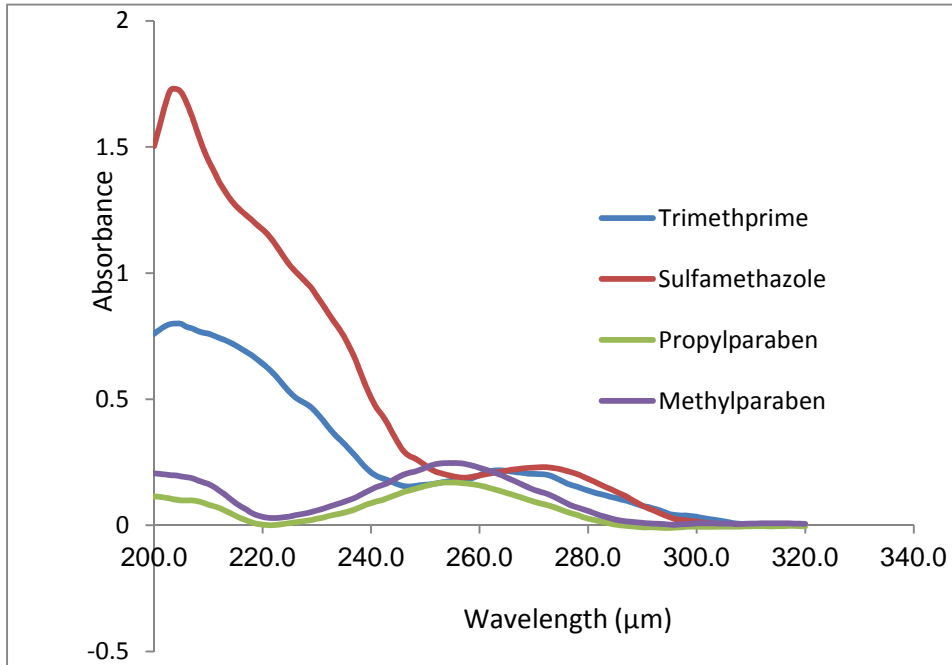


Fig. 1. The zero order spectra of sulfamethoxazole, trimethoprim, propylparaben and methylparaben.

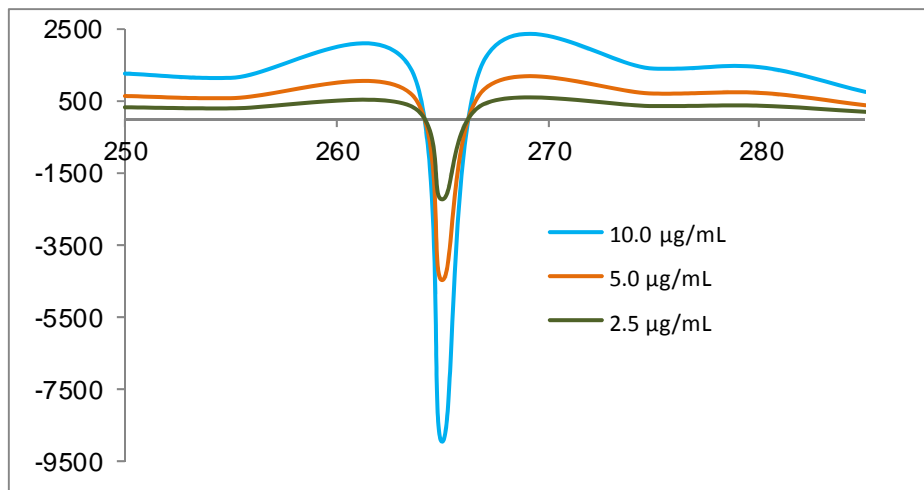


Fig. 2. The third ratio spectrum of different concentrations of trimethoprim (2.5, 5.0 and 10.0 µg/mL)

**Simultaneous determination of ....**

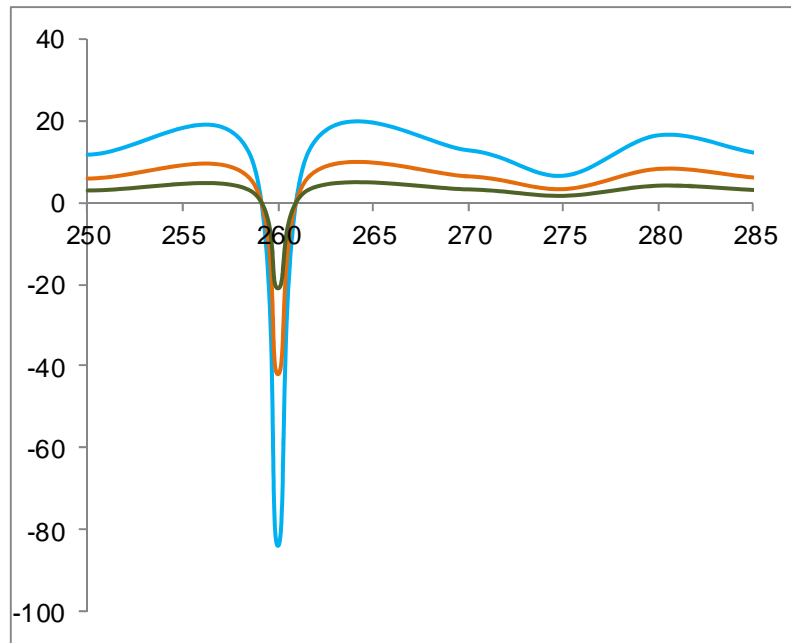


Fig. 3. The third ratio spectrum of different concentrations of propylparaben (2.5, 5.0 and 10.0 µg/mL)

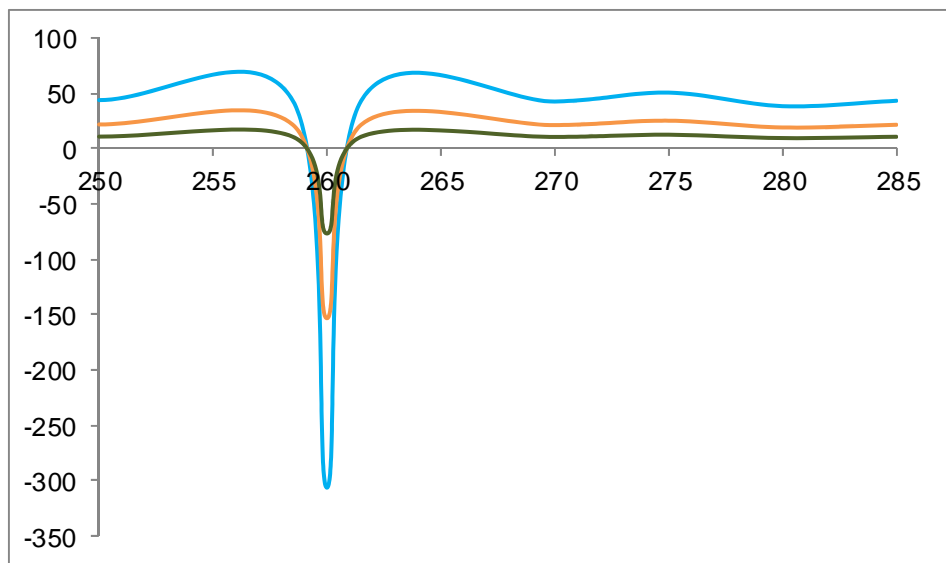


Fig. 4. The third ratio spectrum of different concentrations of methylparaben (2.5, 5.0 and 10.0 µg/mL)

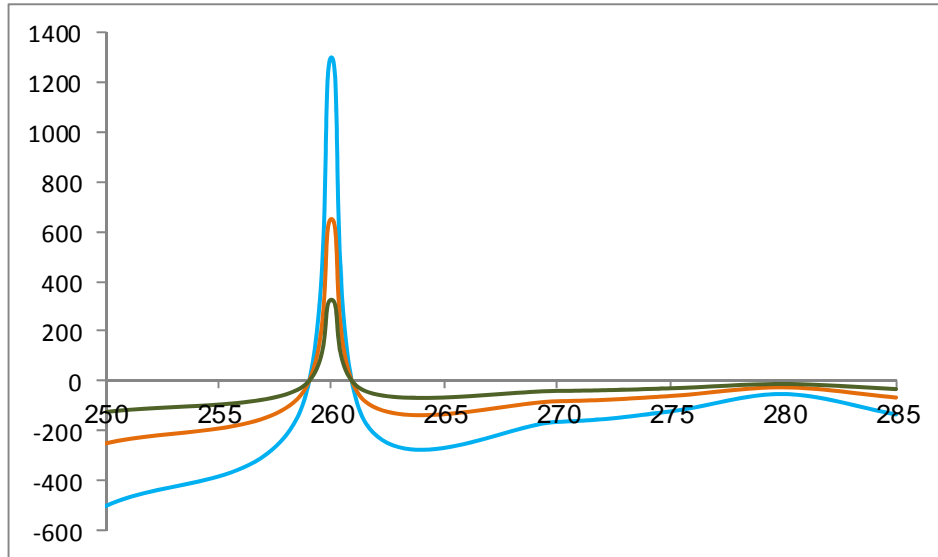


Fig. 5. The third ratio spectrum of different concentrations of sulfamethoxazole (2.5, 5.0 and 10.0  $\mu\text{g/mL}$ )