

## Simultaneous Determination of Sulphamethoxazole and Trimethoprim in Binary Mixtures and in Tablet Using Derivative Spectrophotometry

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### Abstract

Derivative spectrophotometric (first, second, third and fourth derivative) were developed for the determination of Sulphamethoxazole (SMX) and trimethoprim (TMP) antibiotic binary mixture by applying zero-crossing technique for pure synthetic mixture while simultaneous determination of (SMX) and (TMP) in tablets using  $D^4$  at wavelength 257.8 nm and 251.5 nm for (SMX) and (TMP) the correlation coefficient of calibration curves were 0.9992 and 0.9995. Linearity is maintained within a wide concentration range from 2.00 to 25.00 mg/l, with relative error (-0.11 and 0.60) the LOD (0.360 and 0.382 mg/L) and LOQ and (1.200 and 1.275 mg/L) respectively. No interference found between both determined and those of matrices. A good accuracy and precision of simultaneous determination of (SMX), and (TMP) were confirmed by statistical analysis. The percentage recovery of the individual drugs under the established conditions is ranged from 97.23% to 102.13%. This method was compared with the British pharmacopoeia method using F test. The procedures do not require any separation step.

Keywords: Sulphamethoxazole, Trimethoprim, Co-trimoxazole, Derivative spectrophotometry.

### Introduction

#### Sulphamethoxazole

Sulphamethoxazole (SMX), 4-Amino-N-(5-methylisoxazol-3-yl) benzene sulphonamide, ( $C_{10}H_{11}N_3O_3$  S), (1-a), is white, or almost white crystalline powder with molecular weight 253.3 g/mole, practically insoluble in water, freely soluble in acetone, sparingly soluble in alcohol, slightly soluble in ether. It dissolves in dilute sodium hydroxide solution.<sup>(1)</sup>

#### Trimethoprim

Trimethoprim (TMP), 5-(3,4,5-Trimethoxybenzyl) pyrimidine-2,4-diamine, ( $C_{14}H_{18}N_4O_3$ ), (1-b),

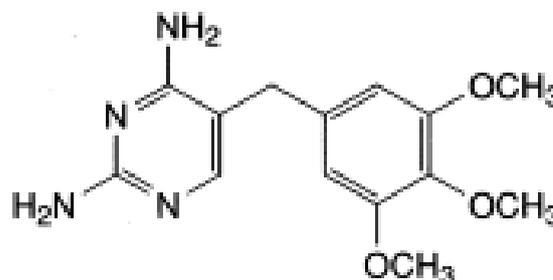
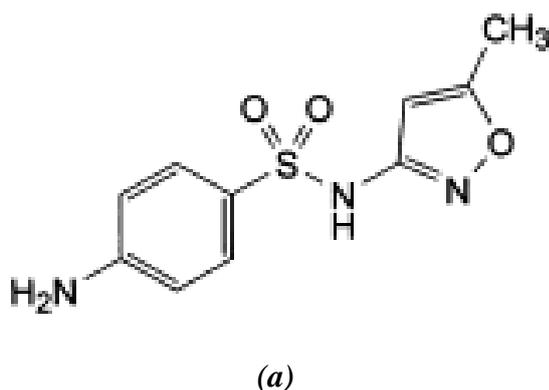


Fig.(1) Structure formula of  
(a) SMX (b) TMP.

The combination of (SMX) and (TMP) are used for the treatment of infections caused by susceptible bacterial organisms, follows the practical application of the principle that if two drugs act on sequential steps in the pathway of an obligate enzymatic reaction in bacteria, the result of their combination will be supra-additive<sup>(2)</sup>. The optimal ratio of the two agents for their synergistic activity has been found to be 5:1.<sup>(3)</sup>

Various methods have been reported for the simultaneous determination of the combination of SMX and TMP in pharmaceutical formulations and biological fluids, such as (HPLC)<sup>(4-6)</sup>, Thin layer chromatography (TLC)<sup>(7)</sup>, capillary zone electrophoresis method<sup>(8)</sup>, Voltametric methods<sup>(9-10)</sup>, Spectrophotometric methods<sup>(11-13)</sup>,

and Derivative spectrophotometric methods<sup>(14-16)</sup>.

Derivative spectrophotometry is an analytical technique of great utility for resolving some mixture of compound with overlapping spectra<sup>(17-20)</sup>. In this work, new methods were used to develop spectrophotometric methods for the simultaneous determination of the component of these binary mixtures without prior separation.

## Experimental

### Instruments and Equipment:

Double-beam UV-Visible spectrophotometer model (UV-1650 PC) SHIMADZO (Japan), interfaced with computer via a SHIMADZO UV probe data system program (Version 1.10), using 1.00 cm quartz cells, (W. Germany), Sartorius Handy 4digits Analytical Balance.

### Chemicals:

Standard drugs: (SMX) and (TMP) were purchased from the State Company of Drug Industries and Medical Appliances (IRAQ-SDI- Samara) and the mixture of sulphamethoxazole with (TRIMOL) tablets from (Julphar-UAE) were purchased from the local market, sodium hydroxide obtained from (BDH). All drugs were used as working standards without further purification.

### Preparation of Stock and working Standard Solutions

- 1- (0.1M) sodium hydroxide was prepared by dissolving an accurate amounts (4.00g) of sodium hydroxide in (1L) distilled water.
- 2- Stock solutions of (250 mg/l) standard were prepared by dissolving an accurate amounts (25.00 mg) of the studied drugs in sodium hydroxide (0.1M) in 100 ml volumetric flask, the solutions are then made up to mark with sodium hydroxide.
- 3- Two series of pure single standard (2-30mg/L) was prepared by diluting Stock solution with (0.1 M) sodium hydroxide.
- 4- Solutions for binary mixtures of standard drugs (SMX) and (TMP) solutions were prepared by two series.

First series of mixture solutions were prepared by using a fixed concentration of (20 mg/L) for (SMX) with different concentrations (2,4,6,8,10,15,20 and 25 mg/L) of (TMP), while the second series of mixture contains a fixed concentration (4 mg/L) of (TMP) with different concentration of (2,4,6,8,10,15,20 and 25mg/L) of (SMX).

- 5- (250 mg/l) stock solutions of interferences were prepared by dissolving an accurately weighed amount (0.0125 g) of each (starch, gelatin, magnesium setrate, avecil, sucrose) in 50 mL of 0.1 M NaOH, the other working solutions were prepared by the serial dilution.

### Preparation of pharmaceuticals samples

All contents of 10 tablets of the pharmaceutical Co-Trimoxazole (MITHO PRIM-400 and TRIMOL) were weighted and grinded to fine powder. Accurate weight (1/10) of one tablet (0.0519 g) and (0.0647 g) of (MITHOPRIM-400 and TRIMOL) respectively. Which was dissolved by 100ml of (0.1 M) sodium hydroxide then filtrated, the clear solution were taken and filed up to 100 ml. The resultant solution may be contained 400 mg/L (SMX) + 80mg/L (TMP) the other working solutions were prepared by the serial dilution method.

### Results and Discussion

The absorption spectra of SMX and TMP under certain experimental conditions are shown in Fig.(2-a). As can be seen, the maximum wavelengths of two compounds are close to each other and their spectra overlap, therefore, determination of SMX and TMP in the presence of each other is impractical by classical spectrophotometry, and derivative spectrophotometry can be used in this case. The zero crossing method applied on the first derivative ( $D^1$ ) to determine the concentration of SMX at valley 288 nm as shown in Fig.(2-b). On the other hand the second derivative cannot be used to determine neither SMX nor TMP as can be seen in Fig.(2-c). The Fig.(2-d) shows that both compounds can be determined in the third derivative; SMX at valley 251.4 nm, and TMP at valley 258.6 nm. And from the fourth derivative SMX can be

determined at valley 246.2 nm, and TMP at valley 251.5 nm as shown in Fig.(2-e). All these methods and there equations, slope, concentration rang, correlation coefficient and the relative errors for the mixtures containing 5:1 of each drug were listed in Table (1).

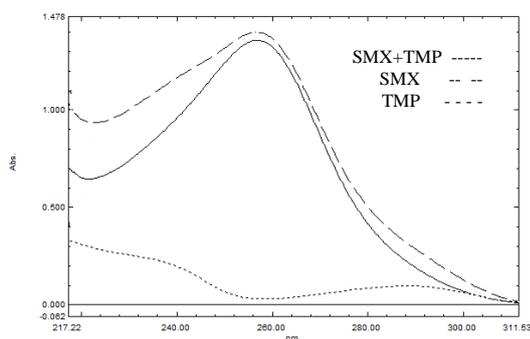


Fig. (2-a)

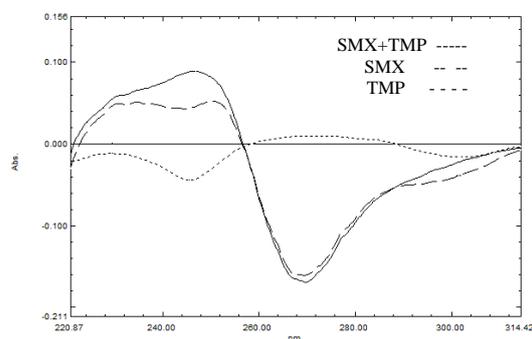


Fig. (2-b)

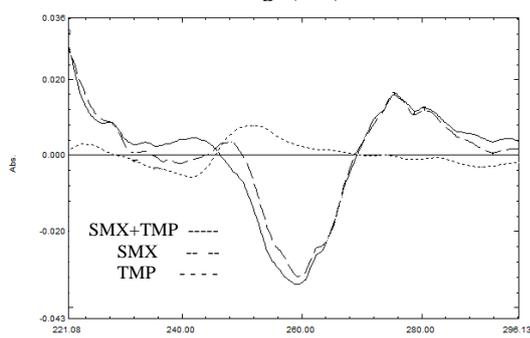


Fig. (2-c)

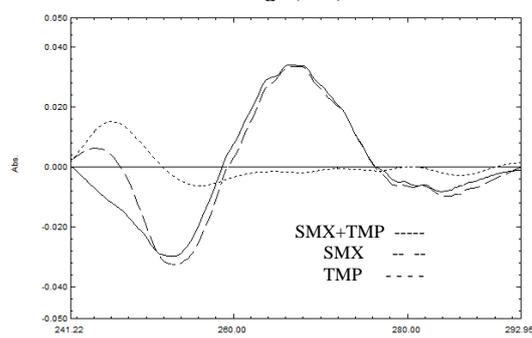


Fig. (2-d)

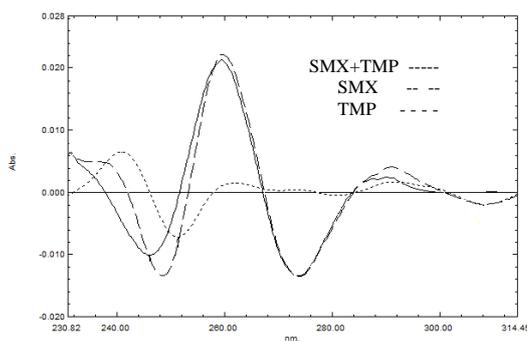


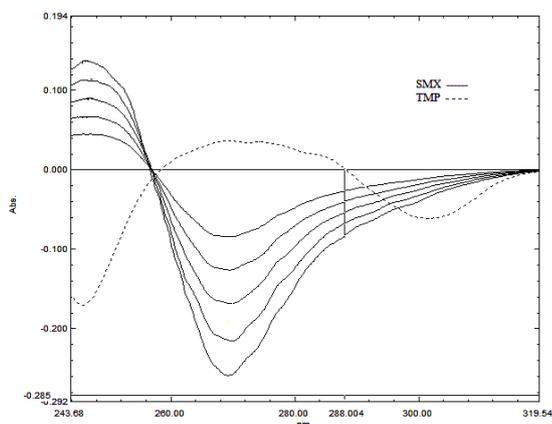
Fig. (2-e)

**Fig. (2) Spectra of 20 mg/L SMX, 4 mg/L TMP and a mixture of 20 mg/L SMX + 4 mg/L TMP.**  
*a- Normal spectrum b- First derivative c- Second derivative d- Third derivative*  
*e- Fourth derivative.*

**Table (1)**  
**The Methods used for determination the binary mixture of SMX and TMP.**

Drug	Method	Equation	Slop	Relative error for 5:1 mixture	R	LOD $\mu\text{g/mL}$	LOQ $\mu\text{g/mL}$
SMX	D <sup>1</sup>	V=288.0 Y=-0.00283x+0.00155	-0.00283	-1.46	0.9996	0.750	2.499
	D <sup>3</sup>	V=251.4 Y=-0.00139x-0.00041	-0.00139	-1.90	0.9981	0.505	1.684
	D <sup>4</sup>	V=246.2 Y=-0.00047x-0.00118	-0.00047	-0.85	0.9989	0.395	1.317
	D <sup>4</sup>	P = 257.8 Y= 0.00095x+0.00011	0.00095	-0.11	0.9992	0.360	1.200
TMP	D <sup>3</sup>	V=258.6 Y=-0.00114x+0.00013	-0.00114	-1.45	0.9995	0.814	2.712
	D <sup>4</sup>	V=251.5 Y=-0.00179x+0.00028	-0.00179	0.60	0.9995	0.382	1.275
	D <sup>4</sup>	P = 237.6 Y= 0.00096x+0.00135	0.00096	-1.70	0.9995	0.751	2.503

First derivative spectrum of SMX and TMP which contain a suitable wavelength for measuring SMX at 288 nm when the absorbance of TMP equal zero as shown in Fig.(3), calibration curves of D<sup>1</sup> for standards SMX and TMP solutions (2-25 mg/L) gave a linear equation and the correlation coefficient and slop were listed in Table (1). The recovery and relative error of the pure SMX and the synthetic mixtures were tabulated in Table (2). Third derivative spectrum which contain a suitable wavelength for measuring SMX by applying zero crossing at 251.4 nm shown in Fig.(4), the recovery and relative error of the pure SMX and the synthetic mixtures were tabulated in Table (3). Fourth derivative zero crossing spectrums of SMX at valley 246.2 nm and peak 257.8 nm is shown in Fig.(5), the recovery and relative error are listed in Table (4) and (5) respectively.

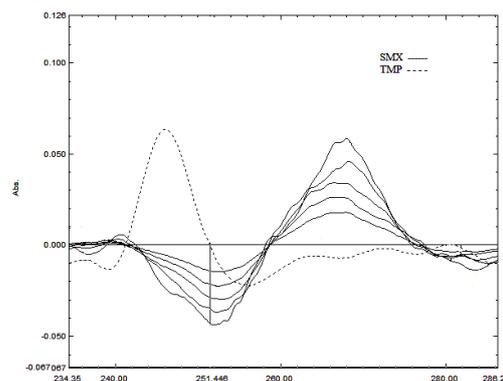


**Fig.(3) First derivative zero crossing of (4-25) mg/L SMX with(4)mg/L TMP.**

**Table (2)**  
**The relative error and recovery of SMX in the presence of TMP using D<sup>1</sup> spectrum zero crossing at valley 288 nm.**

SMX and TMP mixtures	SMX found* mg/L	Relative error%	Recovery %
30 SMX+0 TMP	30.216	0.72	100.72
20 SMX+0 TMP	19.401	-3.00	97.01
10 SMX+0 TMP	10.008	0.08	100.08
4 SMX + 0 TMP	3.894	-2.65	97.35
15 SMX+4 TMP	14.688	-2.08	97.92
20 SMX+4 TMP	19.709	-1.46	98.55
25 SMX+4 TMP	25.448	1.79	101.79
20SMX+2 TMP	20.068	0.34	100.34
20SMX+4 TMP	19.709	-1.46	98.55
20SMX+6 TMP	19.709	-1.46	98.55

\* Each concentration represents an average of at least three measurements.



**Fig.(4) Third derivative zero crossing of (4-25) mg/L SMX with(4)mg/L TMP.**

Table (3)

The relative error and recovery of SMX in the presence of TMP using  $D^3$  Spectrum zero crossing at valley 251.4 nm.

SMX and TMP mixtures	SMX found* mg/L	Relative error%	Recovery %
30 SMX+0 TMP	29.937	-0.21	99.79
20 SMX+0 TMP	19.581	-2.10	97.91
10 SMX+0 TMP	9.786	-2.14	97.86
4 SMX + 0 TMP	4.112	2.80	102.80
15 SMX+4 TMP	14.731	-1.79	98.21
20 SMX+4 TMP	19.621	-1.90	98.11
25 SMX+4 TMP	24.511	-1.96	98.04
20SMX+2TMP	20.319	1.60	101.60
20SMX+4TMP	19.621	-1.90	98.11
20SMX+6TMP	19.621	-1.90	98.11

\*Each concentration represents an average of at least three measurements.

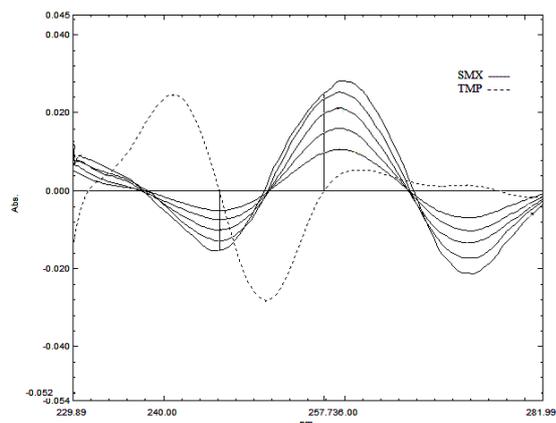


Fig. (5) Fourth derivative zero crossing of (4-25) mg/L SMX with (4)mg/L TMP.

Table (4)

The relative error and recovery of SMX in the presence of TMP using  $D^4$  spectrum zero crossing at valley 246.2 nm.

SMX and TMP mixtures	SMX found* mg/L	Relative error%	Recovery %
30 SMX+0 TMP	29.762	-0.79	99.21
20 SMX+0 TMP	19.817	-0.91	99.09
10 SMX+0 TMP	9.756	-2.44	97.56
4 SMX + 0 TMP	3.92	-2.00	98.00
15 SMX+4 TMP	14.793	-1.38	98.62
20 SMX+4 TMP	19.829	-0.85	99.15
25 SMX+4 TMP	25.454	1.82	101.82
20SMX+2TMP	19.829	-0.85	99.15
20SMX+4TMP	19.829	-0.85	99.15
20SMX+6TMP	19.829	-0.85	99.15

\*Each concentration represents an average of at least three measurements.

Table (5)

The relative error and recovery of SMX in the presence of TMP using  $D^4$  spectrum zero crossing at peak 257.8 nm.

SMX and TMP mixtures	SMX found* mg/L	Relative error%	Recovery %
25 SMX+0 TMP	25.145	0.58	100.58
20 SMX+0 TMP	19.869	-0.66	99.35
10 SMX+0 TMP	9.899	-1.01	98.99
4 SMX + 0 TMP	4.085	2.13	102.13
15 SMX+4 TMP	14.688	-2.08	97.92
20 SMX+4 TMP	19.977	-0.11	99.89
25 SMX+4 TMP	25.266	1.06	101.06
20SMX+2TMP	19.977	-0.11	99.89
20SMX+4TMP	19.977	-0.11	99.89
20SMX+6TMP	19.977	-0.11	99.89

\*Each concentration represents an average of at least three measurements.

Third derivative spectrum of trimethoprim TMP and sulphamethoxazole SMX which contains a suitable wavelength for measuring TMP at 258.6 nm when the absorbance of SMX equal zero as shown in Fig.(6),

calibration curves of  $D^3$  for standards TMP and SMX solutions (2-30 mg/L) gave a linear equation and the correlation coefficient and slope were listed in Table (1). The recovery and relative error of the pure TMP and the synthetic mixtures were tabulated in Table (6). Fourth derivative zero crossing spectrums of TMP at valley 251.5 nm and peak 237.6 nm is shown in Fig.(7), the recovery and relative error are listed in Table (7) and (8) respectively.

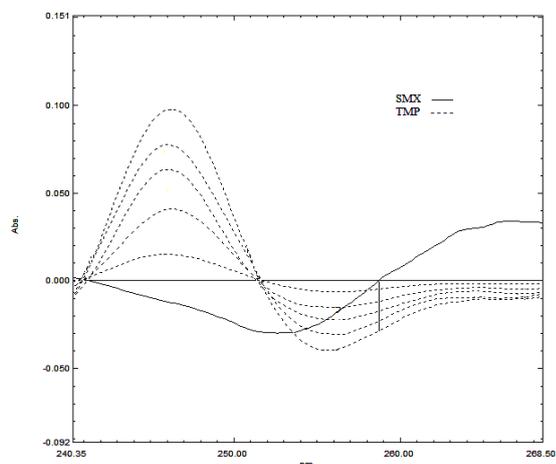


Fig.(6) Third derivative zero crossing of (4-25) mg/LTMP with(20)mg/L SMX.

Table (6)

The relative error and recovery of TMP in the presence of SMX using  $D^3$  spectrum zero crossing at valley 258.6 nm.

TMP and SMX mixtures	TMP found* mg/L	Relative error%	Recovery %
30 TMP+0 SMX	29.537	-1.54	98.46
20 TMP+0 SMX	20.012	0.06	100.06
10 TMP+0 SMX	9.736	-2.64	97.36
4 TMP + 0 SMX	3.937	-1.58	98.43
4 TMP +15SMX	3.892	-2.70	97.30
4 TMP +20SMX	3.942	-1.45	98.55
4 TMP +25SMX	4.031	0.77	100.78
2 TMP+20 SMX	1.974	-1.30	98.70
4 TMP+20 SMX	3.942	-1.45	98.55
6 TMP+20 SMX	6.108	1.80	101.80

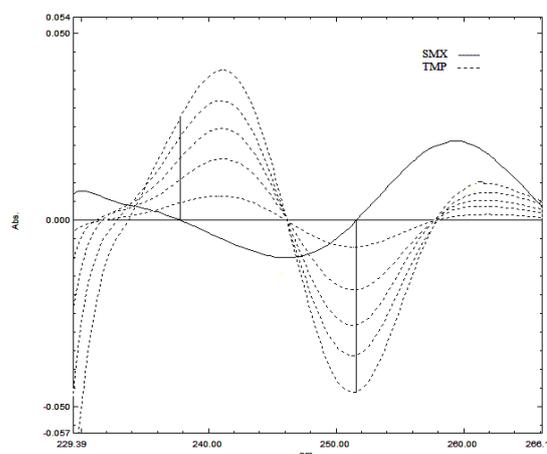


Fig.(7) Fourth derivative zero crossing of (4-25) mg/L TMP with(20)mg/L SMX.

Table (7)

The relative error and recovery of TMP in the presence of SMX using  $D^4$  spectrum zero crossing at valley 251.5 nm.

TMP and SMX mixtures	TMP found* mg/L	Relative error%	Recovery %
30 TMP+0 SMX	29.676	-1.08	98.92
20 TMP+0 SMX	19.47	-2.65	97.35
10 TMP+0 SMX	9.723	-2.77	97.23
4 TMP + 0 SMX	3.967	-0.82	99.18
4 TMP+15 SMX	3.996	-0.10	99.90
4 TMP+20 SMX	4.024	0.60	100.60
4 TMP+25 SMX	4.024	0.60	100.60
2 TMP+20 SMX	1.946	-2.70	97.30
4 TMP+20 SMX	4.024	0.60	100.60
6 TMP+20 SMX	5.933	-1.12	98.88

\*Each concentration represents an average of at least three measurements.

Table (8)

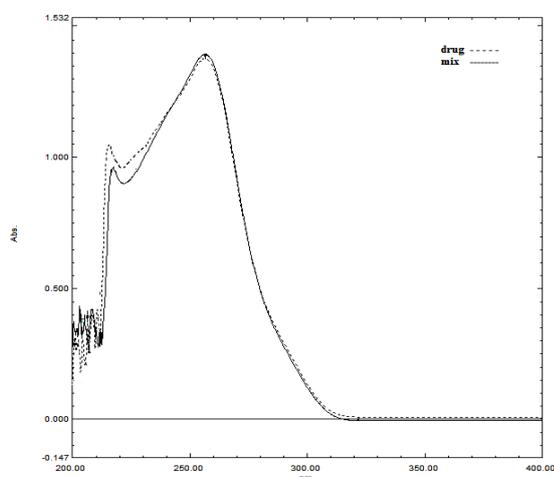
*The relative error and recovery of TMP in the presence of SMX using D<sup>4</sup> spectrum zero crossing at peak 237.6 nm.*

<i>TMP and SMX mixtures</i>	<i>TMP found mg/L</i>	<i>Relative error%</i>	<i>Recovery %</i>
30 TMP+0 SMX	29.652	-1.16	98.84
20 TMP+0 SMX	20.254	1.27	101.27
10 TMP+0 SMX	9.886	-1.14	98.86
4 TMP + 0 SMX	3.887	-2.83	97.18
4 TMP+15 SMX	3.932	-1.70	98.30
4 TMP+20 SMX	3.932	-1.70	98.30
4 TMP+25 SMX	3.932	-1.70	98.30
4 TMP+20 SMX	3.932	-1.70	98.30
6 TMP+20 SMX	5.974	-0.43	99.57

*\*Each concentration represents an average of at least three measurements.*

#### *Interferences study*

To find an effect of matrix constituents on the results of determination, comparative analysis was carried out for standard solution containing active components at concentrations (20SMX+4TMP) mg/L comparable to those of the analyzed drug contain the same concentration, they show the same normal spectra Fig.(8).



*Fig. (8) Normal spectra for analyzed preparation.*

#### *Analysis of Pharmaceutical Sample:*

METHOPRIM and TRIMOL samples with constant concentration (20 mg/L) of SMX and (4 mg/L) of TMP was measured using the methods listed in Table (9) for determination each of Sulphamethoxazole in the presence of Trimethoprim using; D<sup>4</sup> at peak 257.8 nm, and for Trimethoprim in the presence of Sulphamethoxazole using; D<sup>4</sup> spectrum at valley 251.5 nm .

Results for the analysis of standard drugs were compared with British pharmacopoeia method <sup>(1, 11)</sup> using F test. As it is shown in Table (10), a good agreement was observed between the proposed method and standard method.

The analytical method was accepted according to the tabulated value of (F) is greater than the calculated value.

Table (9)

The pharmaceutical sample relative error, recovery, Standard deviation, statistical analysis and RSD for each method used to determine SMX and TMP in the drug.

Drug	Method	Conc. mg/L	Found* mg/L	Er%	Re%	$\delta_{n-1}$	RSD	$\mu=x \pm (t\delta)/\sqrt{n}$		
METHOPRIM	SMX	D <sup>4</sup>	Peak = 257.8	20	17.880	-10.596	89.404	0.029	0.165	17.880±0.036
	TMP	D <sup>4</sup>	Valley = 251.5	4	3.209	-19.77	80.23	0.039	1.241	3.209±0.049
TRIMOL	SMX	D <sup>4</sup>	Peak = 257.8	20	20.972	4.864	104.864	0.139	0.663	20.972±0.172
	TMP	D <sup>4</sup>	Valley = 251.5	4	3.875	-3.120	96.880	0.241	6.243	3.875±0.300

\* Each concentration is the average at least for five measurements.

Table (10)

Comparison with British pharmacopoeia method using F test, Standard deviation for SMX and TMP is (0.543 and 0.428) respectively.

Standard	Method	$\delta_{n-1}$	$F_{cal.}$	$\mu=x \pm (t\delta)/\sqrt{n}$	
20SMX+4TMP	D <sup>4</sup>	Peak = 257.8	0.202	2.688	19.977±0.251
20SMX+4TMP	D <sup>4</sup>	Valley = 251.5	0.247	1.732	4.024±0.307

$F_{tab}$  for  $n_1=6$ ,  $n_2=5$  is equal to (6.26).

## Conclusions

A fast and accurate method for determination of sulphamethoxazole and trimethoprim was developed by using derivative spectrophotometry. The advantage of this method is that both constituents can be determined directly in a single sample without the need to be separated. It was also found that D<sup>4</sup> is used for determining the drugs in tablets.

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### الخلاصة

في هذا البحث تم تقدير هذه المركبات أنيا باستخدام المشتقات الطيفية للأدوية (الأولى والثانية والثالثة والرابعة). على شكل أمزجة ثنائية للأدوية بواسطة تطبيق النقاط الصفرية.

تم تعيين السلفاميثا كسازول والترايميثوبريم انيا في الادوية باستخدام المشتقة الرابعة في (257.8nm) و (251.5nm) للسلفاميثا كسازول و الترايميثوبريم على التوالي. وكان منحنى المعايرة بمعامل ارتباط r لا يقل عن (0.9992 و 0.9995) ومدى واسع للتراكيز الخطية (2-25 mg/L) وخطا نسبي (-0.11 و 0.60) وحد الكشف (0.360 mg/L و 0.382) وحد التعيين (1.200 mg/L و 1.275) على التوالي بدون تداخل بين مكونات الادوية. النتائج الاحصائية تساند دقة وصحة الطرق المستعملة حيث بلغت نسبة الاسترجاع بين 97.23%-102.13%. هذه الطريقة سهلة لا تحتاج الى فصل أو أي معاملة.