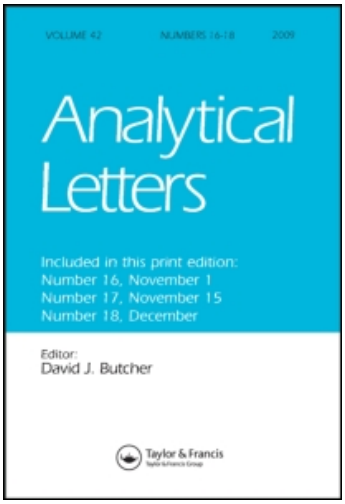

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Spectrophotometric Microdetermination of Sulfamethoxazole and Trimethoprim Using Alizarin and Quinalizarin

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SPECTROPHOTOMETRIC MICRODETERMINATION OF SULFAMETHOXAZOLE AND TRIMETHOPRIM USING ALIZARIN AND QUINALIZARIN

Key Words: Sulfamethoxazole, Trimethoprim, Alizarin, Quinalizarin, Analysis of Mixture, Spectrophotometric Determination.

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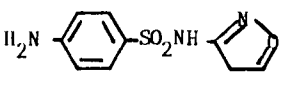
Abstract:

A spectrophotometric method has been developed for the microdetermination of sulfamethoxazole and trimethoprim drugs. The proposed method is based on the charge transfer (CT) complex formation of the drug with alizarin or quinalizarin in alkaline medium and measuring the developed absorbance at its maximum. The optimum conditions for maximum absorbance results are studied and Beer's law is obeyed in the ranges 10-130 and 10-190 μg/ml for sulfamethoxazole and trimethoprim respectively. For more accurate results, Ringbom optimum concentration ranges are 20-120 and 10-170 μg/ml respectively. The molar absorpitivity and Sandell sensitivity are also calculated. The proposed spectrophotometric method of analysis is as accurate as the USP method (USP XX. The National Formulary, NF XV, 1980 P. 925. Mack Easton, Pa., 1980) and is simpler than their official method. Applications of the suggested method to representative pharmaceutical sulfa drugs are presented and the validity of the method was assessed by applying the standard addition technique.

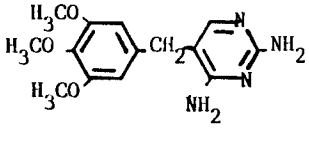
Introduction:

More than three thousand sulfonamides already synthesized warrant accurate methods for their determination either alone or in mixtures with other sulfa drugs and vitamins in pharmaceutical preparations. Among several methods of assay for sulfonamides,[1-7] the method of Bratton and Marshall[2] is the choice of biologists and pharmacologists. It involves diazotization of sulfa compounds and coupling with dihydrochloride salt of N-(1-naphthyl) ethylenediamine in acidic medium. This method was sensitive, rapid, reproducible and reliable for low concentration (i.e < 100 μg/ml). For higher concentrations this method requires multiple dilutions which are inconvenient and may introduce error. To circumvent the shortcoming, Trieff et al.[8] reported a method for the assay of P-aminobenzenesulfonamide with chloramine-T. and Srivatava et al.[9] improved Trieff's method by reducing the time required from 4 h to 30-45 min, by using N-chlorosuccinamide instead of chloramine-T.

The present investigation deals with the use of alizarin, 1,2-dihydroxyanthraquinone (I) and quinalizarin, 1,2,5,8-tetrahydroxy anthraquinone (II), as sensitive reagents for the determination of sulfamethoxazole and trimethoprim in pure form, synthetic mixtures and in commercial pharmaceutical formulations.



sulfamethoxazole



trimethoprim

Experimental:

Reagents and Materials

1. Stock solutions of sulfamethoxazole and trimethoprim (1 ml equivalent to 1 mg) were prepared by dissolving 100 mg sulfa drug in 10 ml of absolute ethanol with the aid of a few drops of 0.1 N NaOH solution, then diluted to 100-ml with bidistilled water.

2. Alizarin (1,2-dihydroxyanthraquinone) (I), and quinalizarin (1,2,5,8-tetrahydroxyanthraquinone) (II) were Aldrich products and used without any further purification. 2.5 X 10^{-3}M solution were prepared by dissolving the appropriate weight in 100-ml of absolute ethanol.

3. Thiel buffer solutions of pH values 2-12 were prepared as previously recommended[10].

Equipment

An Orion Research Model 601 A/Digital Ionalyzer pH-meter was used for measuring the pH values of the buffer solutions. A Perkin-Elmer Lambda 3B spectrophotometer using a 1-cm cell was used for all absorbance measurements.

Procedures

1. Determination of sulfamethoxazole: Prepare working standard solutions by delivering suitable volumes of dilute standard sulfamethoxazole into 10-ml measuring flasks. To each flask add 1-ml of 2.5 X 10^{-3}M reagents (I or II), 1.5 ml of ethanol to 25% (v/v) ethanol, 5 ml thiel buffer solution of pH 8.5. Complete to the mark with bidistilled water, mix well and measure the absorbance of the reddish brown complex instantaneously at 510 and 555 nm using I and II respectively against a reagent blank.

2. Determination of trimethoprim: For construction of the calibration curve, proceed as described for sulfamethoxazole and allow the flasks to stand at room temperature for 20 min., or raise the temperature upto 45±2°C for 5 min. Measuring the absorbance against the blank solution containing the same ingredients, except trimethoprim, at 510 and 555nm using I and II, respectively.

   A rectilinear curve passing through the origin is obtained, indicating that Beer's law is followed over the concentration range 10-130 µg/ml for sulfamethoxazole and 10-190 µg/ml for trimethoprim.

3. Determination of mixtures of sulfamethoxazole and trimethoprim: An accurate concentration of both sulfamethoxazole and trimethop-

rim ranging from 10-130 μg/ml was transferred to a 10-ml measuring flask, 1 ml of 2.5 X 10^{-3}M reagent solution, 1.5 ml ethanol and 5 ml of pH 8.5 were added, then dilute to the mark with bidistilled water. Measure the absorbance instantaneously at room temperature against a reagent blank. The concentration of sulfamethoxazole is thus determined using a calibration curve prepared from pure sample. The flask content is allowed to stand at room temperature for 20 min. or placed in a water bath for 5 min. at 45±2°C with shaking, then the absorbance is measured at the recommended wavelength. The increase in absorbance is due to trimethoprim complex. The procedure may be followed using two identical mixtures, measuring one instantaneously for sulfamethoxazole and the other after 20 min. or with heating, for trimethoprim.

4. For tablets: Powder 20 tablets of the drug to be analyzed, then weigh accurately an amount equivalent to 100 mg of the active constituent into a 100-ml measuring flask. Dissolve the compound in 10-ml of ethanol with the aid of a few drops of 0.1 N NaOH, then adjust to volume with bidistilled water. Proceed as described above for color development.

5. For syrup: 2.5 ml of oral suspension containing a fixed weight of the active constituent is diluted into a 100 ml measuring flask, then dissolved in 10-ml of ethanol with the aid of a few drops of 0.1 N NaOH. Then adjust to volume with bidistilled water. The concentrations of sulfamethoxazole and trimethoprim per 5 ml of oral suspension were determined using the general procedure.

Results and Discussion:

A new method for the microdetermination of sulfamethoxazole and trimethoprim drugs has been developed. The proposed method is based on charge transfer complex formation of the drug with alizarin (I) or quinalizarin (II) in alkaline medium. The developed reddish brown colour was then measured against a reagent blank at the wavelength of maximum absorbance. Careful investigations were carried out to demonstrate the most favorable conditions to achieve maximum absorbance in a quantitative determination of the drug. The effect of each of the following variables on the reaction was examined.

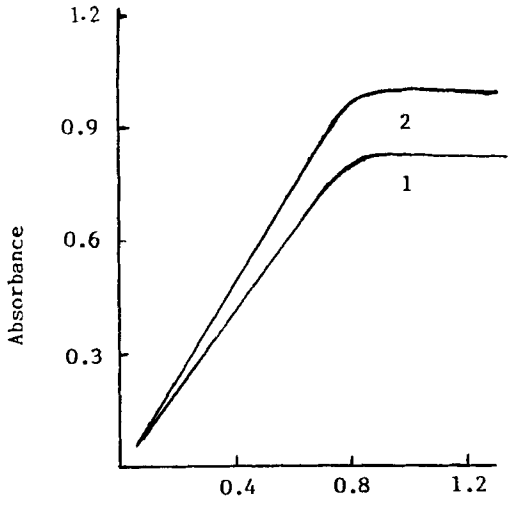


Fig. (1) Effect of pH values on the complex formation between sulfamethoxazole(I) and trimethoprim using alizarin.

Effect of pH:

Thiel buffer solutions covering pH values from 2.0 to 12.0 were used as optimum buffer media for charge transfer (CT) complex formation. The results as shown in Fig. (1) indicated that the complexation is slow and weak in acidic media of pH 2.0 to 6.5, whereas the band of the complex develops starting from pH 7.0 to 10.0 exhibited maximum absorbance at pH 8.5. Then, the absorbance of the CT band decreases as the pH increases. Thus, pH 8.5 is the optimum medium for further study, since the results are highly reproducible at this pH value. The amount of pH 8.5 buffer added to a 10-ml solution was also studied and found to be 5 ml to produce high and constant absorbance value.

Effect of time and temperature:

The results obtained indicated that sulfamethoxazole complexes are formed instantaneously at room temperature (24±3°C) and the absorbance remains constant on raising the temperature upto 60°C, above which destruction of the complex takes place. Whereas for trimethoprim

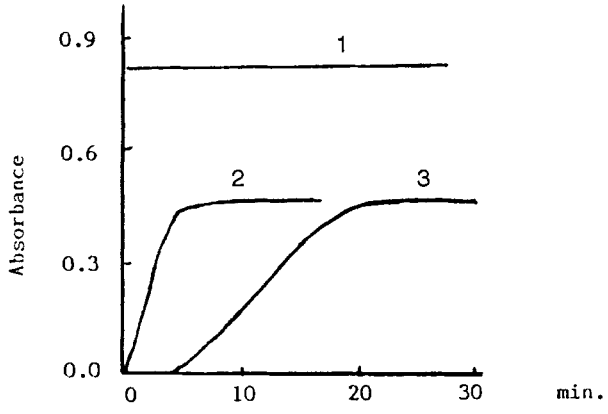
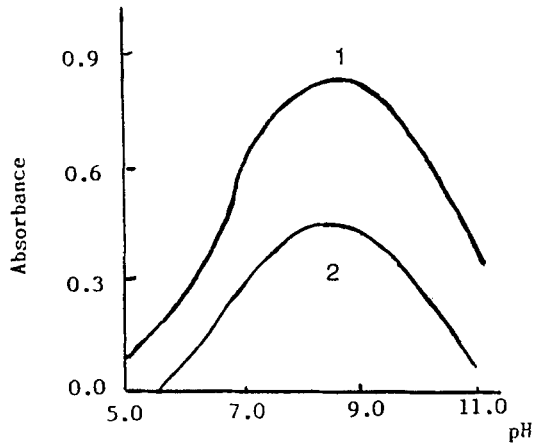


Fig. (2) Effect of time on (1) sulfamethoxazole-I complex at 24±3°C (2)trimethoprim-I at 24±3°C and (3)trimethoprim-I at 45°C.



2.5×10^{-3}M

Fig. (3) Effect of reagent concentration on sulfamethoxazole-I- complex(*1*), sulfamethoxazole-II-complex(2)

complexes, the complexation start after 5 min. of mixing and the absorbance increases gradually, achieving a maximum value after 20 min. [Fig. 2]. Raising the temperature upto 45±2°C, the time required for complex formation decreases to only 5 min.,with the same absorbance obtained after 20 min. standing at room temperature. The sulfamethoxazole complex remains stable for 24 hrs. while that of trimethoprim remains constant for 8 hrs. after which it starts to slowly fade.

Effect of reagent concentration:

The results obtained indicate that at least 0.8 ml solution of 2.5 X 10^{-3}M reagent should be present to achieve maximum color development [Fig. 3]. However, 1 ml of 2.5 X 10^{-3}M reagent was used in the present study to insure quantitative reaction at the upper limit of the calibration curves.

Effect of solvent ratio:

Turbidity was observed when using 15% (v/v) ethanolic solution. Also low color formation was observed at < 35% (v/v) ethanol. Because of these reasons, 25% (v/v) ethanolic solutions were used.

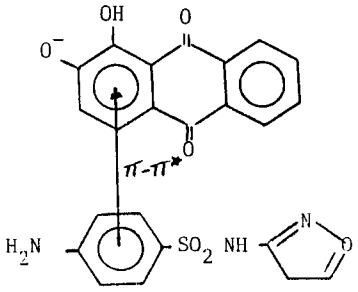
Composition of the complex:

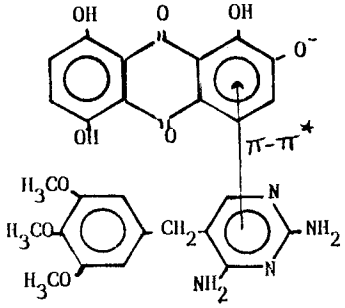
The stoichiometry of the complexes formed between sulfamethoxazole or trimethoprim with reagents I or II was investigated at pH 8.5 applying the molar ratio[11] and continuous variation[12] methods. The results indicate the existance of 1 : 1 complexes. The reaction occurs through the formation of a charge transfer complex. The log stability constant was found to be 8.6 and 8.3 for sulfamethoxazole and trimethoprim using alizarin as a reagent, whereas for quinalizarin complex it was found to be 8.8 and 8.4 for sulfamethoxazole and trimethoprim respectively. The $\pi - \pi^*$ interaction may be supported by the bathochromic shift observed from 452 nm for alizarin to 510 nm and from 487 nm for quinalizarin to 555 nm for the complexes formed. The colored reaction product can be represented, taken sulfamethoxazole-alizarin and trimethoprim-quinalizarin complexes as example, by the following structures.

TABLE 1

Quantitative parameters for the reaction of sulfamethoxazole and trimethoprim using reagents I and II.

Parameters	Values			
	Sulfamethoxazole		Trimethoprim	
	I	II	I	II
Beer's law limits, $\mu g\text{-}ml^{-1}$	10-110	10-130	10-160	10-190
Molar absorptivity, $l\text{-}mol^{-1}\ cm^{-1}$	$6.67X10^3$	$8.13X10^3$	$1.08X10^3$	$1.22X10^3$
Sandell sensitivity, $\mu g\ cm^{-2}$	0.0209	0.0173	0.0158	0.0111
Regression equation:				
Slope (b)	0.33	0.41	0.49	0.55
Intercept (a)	0.024	0.02	0.017	0.015
Correlation coefficient (r)	0.9985	0.9966	0.9938	0.9973
Standard Deviation, %	1.1	0.9	1.3	0.7
Range of error, %	±0.67	±0.5	±0.83	±0.33
Ringbom optimum concentration range, $\mu g\ ml^{-1}$	20-100	20-120	10-150	10-170





Analytical data:

Beer's law limits, molar absorptivity, the regression equation, the correlation coefficient, and the standard deviation obtained by linear least square treatment of the results are given in Table 1. The Ringbom method[13] was applied to obtain more accurate results

TABLE 2

Evaluation of accuracy and precision of the proposed method.

Reagent	Sulfamethoxazole							Trimethoprim						
	Added*	Found[a] R	Found[a] P	S.D	R.S.D%	Standard error	Confidence limits	Added*	Found[a] R	Found[a] P	S.D	R.S.D%	Standard error	Confidence limits
I	30	30.5	30.2	0.03	0.39	0.012	30.2±0.035	20	20.4	20.1	0.04	0.59	0.016	20.1±0.050
	50	49.5	50.5	0.05	0.34	0.020	50.5±0.060	50	49.5	50.3	0.03	0.42	0.012	50.3±0.035
	70	71.1	69.4	0.06	0.55	0.024	69.4±0.070	80	80.7	79.5	0.05	0.31	0.020	79.5±0.060
	90	89.2	90.8	0.08	0.63	0.033	90.8±0.095	100	99.4	100.6	0.07	0.75	0.029	100.6±0.080
	110	111.5	108.8	0.07	0.41	0.029	108.8±0.080	130	131.1	129.3	0.09	0.81	0.037	129.3±0.110
	mean				0.46	0.024						0.58	0.023	
II	40	39.7	40.2	0.04	0.56	0.016	40.2±0.050	40	39.7	40.3	0.05	0.33	0.020	40.3±0.060
	70	71.1	69.5	0.07	0.71	0.029	69.5±0.080	80	80.7	79.6	0.08	0.67	0.033	79.6±0.095
	100	99.4	100.6	0.05	0.35	0.020	100.6±0.060	110	111.5	109.1	0.12	0.89	0.049	109.1±0.140
	130	131.1	130.8	0.11	0.83	0.045	130.8±0.130	150	149.1	150.9	0.10	0.78	0.041	150.9±0.120
	160	161.5	159.0	0.09	0.78	0.037	159.0±0.110	190	191.4	188.6	0.14	0.94	0.057	188.6±0.165
	mean				0.65	0.030						0.66	0.40	

a : Average of six determinations.

R : Recommended method.

P : Proposed method.

* : μg/ml

TABLE 3

Spectrophotometric determination of sulfamethoxazole and trimethoprim in different pharmaceutical preparation using reagents I and II.

Drug	Contents		company	Sulfamethoxazole				Trimethoprim			
	Sulfamethoxazol	Trimethoprim		Found[a]		error %		Found[a]		error %	
				I	II	I	II	I	II	I	II
Tablets											
Septazole	400	80	1	395	397	-1.25	-0.75	79.4	80.3	-0.75	+0.38
Triprim	-	100	2	-	-	-	-	100.8	99.4	+0.80	-0.60
Sutrim	400	80	2	394	402	-1.50	+0.50	80.5	79.8	+0.63	-0.25
Chemotrim	400	80	3	403	406	+0.75	+0.50	79.5	79.6	-0.63	-0.50
Urotrim	400	80	3	396	398	-1.00	-0.50	80.4	80.6	+0.50	+0.75
Cotril	400	80	4	405	397	+1.25	-0.75	80.1	79.8	+0.13	-0.25
Syrup[b]											
Septazol	200	40	1	203	198	+1.50	-1.00	39.8	39.9	-0.50	-0.25
Sutrium	200	40	2	202	197	+1.00	-1.50	40.1	39.7	+0.25	-0.75
Chemotrim	200	40	3	199	202	-0.50	+1.00	40.2	40.3	+0.50	+0.75
Cotril	200	40	4	201	203	+0.50	+1.50	39.6	40.1	-1.00	+0.25

a : Average of six determinations.
b : Each 5 ml of oral suspension.
1 : The Alexandria Co. for Pharm. and Chem. Ind., Alex., Egypt.
2 : The Memphis Chemical Co., Cairo, Egypt.
3 : Kahira Pharm. & Chem. Ind. Co., Cairo, Egypt.
4 : ADWIC Pharm. Division, El-Nasr Pharm. Chem. Co., Abu-Zaabal, Egypt.

(recorded in Table 1). The performance of the present method was assessed by calculation of the t-and F- values. Mean values were obtained in a student t- and F- test at the 95% confidence limit for five degrees of freedom[14] and the results showed that the calculated t- and F- values did not exceed the theoretical values.

Sensitivity, accuracy and precision:

The mean Sandell sensitivity as calculated from Beer's law is presented in Table 1 . In order to determine the accuracy and precision of the method, solutions containing five different concentrations of sulfamethoxazole and trimethoprim were prepared and analyzed in quintuplicate. The measured standard deviation (S.D), relative standard deviation (R.S.D), the standard analytical error and confidence limits [Table 2] can be considered satisfactory, at least for the level of concentrations examined.

Comparison of the obtained percentage recovery by the proposed method with the purity of the studied compounds as determined by the titration using 0.1 M sodium nitrite in acidic medium (HCl) [USP XX and NF XV, 1980[15]] showed similar accuracy of the two methods [Table 2]. The proposed spectrophotometric procedure is simpler than the official one.

Analytical Applications:

Application of the proposed method to some pharmaceutical preparations containing 5 times as much sulfamethoxazole as trimethoprim mixtures was performed. The results, recorded in Table 3 indicate the high accuracy of the present method in this respect.

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