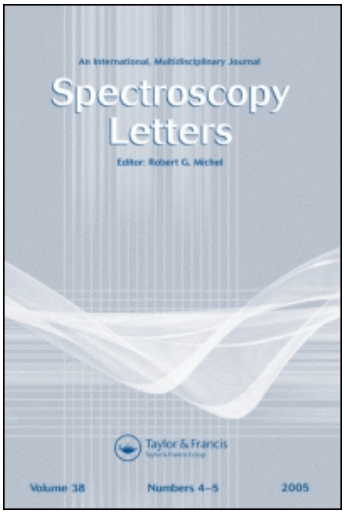

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Simultaneous Analysis of a Ternary Mixture of Pharmaceuticals Containing Trimethoprim, Sulphamethoxazole, and Phenazopyridine Hydrochloride Using Third-Derivative and Zero-Order Photodiode Array Spectrophotometry

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Simultaneous Analysis of a Ternary Mixture of Pharmaceuticals Containing Trimethoprim, Sulphamethoxazole, and Phenazopyridine Hydrochloride Using Third-Derivative and Zero-Order Photodiode Array Spectrophotometry

Keywords Trimethoprim; sulphamethoxazole; phenazopyridine hydrochloride; ternary mixture; third-derivative spectrophotometry.

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Abstract:

A simple and rapid method for the simultaneous analysis of a ternary mixture containing trimethoprim, sulphamethoxazole and phenazopyridine hydrochloride is reported. The procedure consists of

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extraction in 0.1 M hydrochloric acid, filteration and measurement of the amplitudes of the third-derivative spectra at 242 and 276 nm and the absorbance at 450 nm for the determination of trimethoprim, sulphamethoxazole and phenazopyridine hydrochloride, respectively. Good linearity, accuracy, precision and selectivity were found, and the method is proposed for routine quality control purposes, even for the uniformity of contents test.

Introduction:

Combinations of trimethoprim (TMP) and sulphamethoxazole (SMX) are used in human and veterinary medicine for the treatment of specific bacterial infections. Phenazopyridine hydrochloride (PZP) is used as urinary analgesic. This drug in combination with TMP ans SMX is used in the treatment of urinary tract infections.[1].

TMP in combination with SMX, have been determined by non-aqueous titrimetry[2], densitometry[3], potentiometry[4], colorimetry[5,6], TLC[7], and HPLC[8-10]. In addition, various spectrophotometric techniques have been reported for assay of TMP and SMX in dosage forms. These include: first-derivative technique[11], pH-induced differential spectrophotometry[12], triple-wavelength spectrophotometry[13], least squares area-fitting method[14], dual wavelength K-ratio[15], and Kalman filter spectophotometry[16]. PZP has been determined by colorimetry[17] and amperometry[18] in single preparations. In combination with nitrofurantoin, this drug has been determined using polarography[19], HPLC[20],and spectrophotometry[21]. Capsules containing a ternary mixture of PZP, tetracycline hydrochloride and sulphamethizole have been assayed by HPLC[22].

The analysis of mixtures of TMP and SMX has been presented but no work has been reported on the determination of ternary mixture of TMP, SMX and PZP. However, their simultaneous determination would

require the use of HPLC or some other separation technique. This is particularly disadvantageous in content uniformity testing and dissolution studies, in which larger number of samples are to be determined. In addition, chromatography is frequently not possible due to the widely different physicochemical and chromatographic properties of the individual components. Therefore, the present work was prompted by the need for developing a rapid and reliable method for the routine analysis of such a combination. Multiple component analysis compilcated by spectral overlap can be aided by derivative spectroscopy[23,24]. The diode array spectrophotometer performs these functions and offers distinct advantages over chromatographic methods in terms of speed of analysis.

Thus, in the present study, a method based on third-derivative UV spectrophotometry for the determination of TMP, SMX and PZP in a ternary mixture is described.

Experimental:

Materials:

Authentic samples of TMP and SMX (Pharco-Pharmaceuticals Co, Alexandria, Egypt) and PZP (Pfizer Corporation, Brussel, Belgium) wer kindly donated by their manufacturers and were used as received. Tablets were prepared in the laboratory to contain 80 mg TMP, 400 mg SMX and 100 mg of PZP per tablet together with common additives and excipients, e.g., lactose, starch, talc and magnesium stearate. All other chemicals and solvents were analytical reagent grade.

Apparatus:

Spectrophotometric measurements were performed using a Hewlett-Packard 8451 diode-array spectrophotometer which operates at high speed with built-in functional keyboard and built-in printer/plotter

and it can calculate of up to seventh-derivative with Savitsky-Golay smoothing. All third-derivative spectra were obtained using the following parameters: five smoothing points; spectral bandwidth, 2nm; and ordinate maximum and minimum settings, ±0.0015; in 10-mm quartz cells.

Procedure:

Standard drug solutions:

Accurately weighed amounts of 40 mg TMP, 100 mg SMX or 25 mg PZP were separately dissolved in 100 ml 0.1 M hydrochloric acid (heat in a water-bath if necessary).

Calibration curves:

Accurate volumes (1-5 ml) of standard durg solutions (0.4 mg ml^{-1} of TMP, 1 mg ml^{-1} of SMX and 0.25 mg ml^{-1} of PZP) were separately transferred into 100-ml calibrated flasks and diluted to volume with 0.1 hydrochloric acid solution. The third-derivative spectra of the TMP and SMX solutions were recorded against 0.1 M hydrochloric acid solutions and the third-derivative amplitudes (^3D) were graphically measured at 242 and 276 nm, respectively, and plotted against the corresponding concentrations to obtain the calibration curves. The absorbances of the PZP solutions were measured at 450 nm and plotted versus the corresponding concentrations to obtain the calibration curve.

Analysis of tablets:

An accurately weighed quantity of the powdered-tablets, equivalent to about 80 mg of TMP (400 mg of SMX and 100 mg of PZP), was placed in a 100-ml calibrated flask and 50 ml of 0.1 M hydrochloric acid was added. The contents were heated in a water-bath (70 ± 1 °C)

for 10 min and shaken mechanically for 15 min, then diluted to volume with 0.1 M hydrochloric acid solution. The resulting suspension was filtered and the first few milliliters of the filtrate were discarded. A 1.0 ml of the filtrate was diluted to 100 ml with 0.1 M hydrochloric acid solution and the final solution was subjected directly to spectrophotometric analysis.

Result and Discussion :

Selection of wavelengths for analysis:

The UV absorption (zero-order) spectra of TMP, SMX, and PZP in 0.1 M hydrochloric acid solution obtained by diode-array spectrophotometer are shown in Fig. 1 (a & b). It can be seen that the zero-order spectrum of PZP exhibits significantly different spectral features in the visible region, particularly between 350-600 nm (Fig.1-a). In this region (350 - 600 nm), PZP has an absorption maximum at 450 nm whereas the absorbance of both TMP and SMX is zero at any wavelength higher than 350 nm. Hence, in this range, specifically at wavelength=450 nm, it is possible to take the absorbance of the mixture of TMP, SMX and PZP proportional to PZP concentration only i.e., at the 450 nm wavelength chosen for PZP, other components (TMP & SMX) do not absorb (Fig. 1-a). In the UV region (230-290 nm) (Fig.1-b) absorption maxima appear at 240 nm for PZP, at 265 nm for SMX, and at 274 nm for TMP. It can be observed that the absorption spectra of the three components are very similar and that non of the spectra shows prominent peaks that can be used for reliable direct absorbance measurements. This extensive overlap of the spectral bands of the three components between 230-290 nm has led to unacceptable results when the traditional spectrophotometric analysis which involves the use of three simultaneous equations[25] was tried. Such results confirms Gleen's limitations, whereby poor resutls are obtained when absorption spectra of the components are not sufficiently separated.

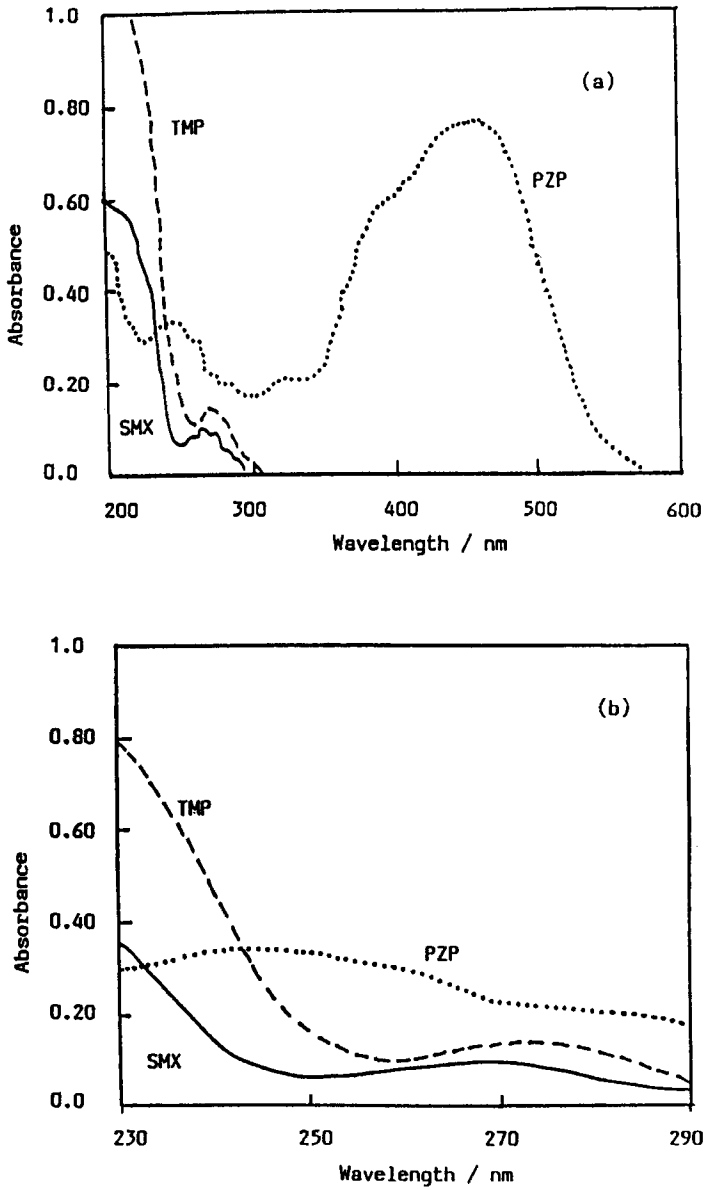

Fig.1. Zero-order absorption spectra over the wavelength range (a) 200-600 nm, and (b) 230-290 nm of 10 ug ml^{-1} of each of TMP (dashed line), SMX (solid line) and PZP (dotted line) in 0.1 M hydrochloric acid.

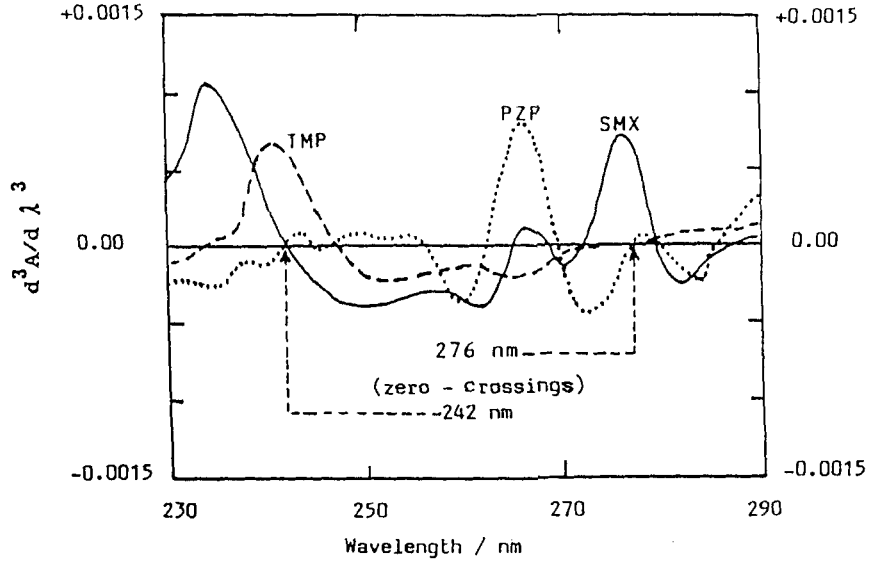

Fig.2. Third-derivative absorption spectra of 10 ug ml^{-1} TMP (dashed line), 30 ug ml^{-1} SMX (solid line) and 20 ug ml^{-1} PZP (dotted line) in 0.1 M hydrochloric acid.

The problem of closely overlapping spectra of TMP, SMX, and PZP has been circumvented by making use of the third-derivative spectra of the mixture. Fig. 2 shows the third-derivative absorption spectra of TMP, SMX, and PZP. It can be seen that, because of the closeness of the three overlapping spectra of there compounds, they are not sufficiently resolved to give distinct peaks or shoulders in the third-derivative spectrum of the mixture. In this instance, the zero-crossing method is the most appropriate for resolving mixtures of these compounds, and it was used in this work with satisfactory results. As discussed elswhere,[26-28] the zero-crossing method involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelengths of the derivative spectra of the other components. Therefore, the

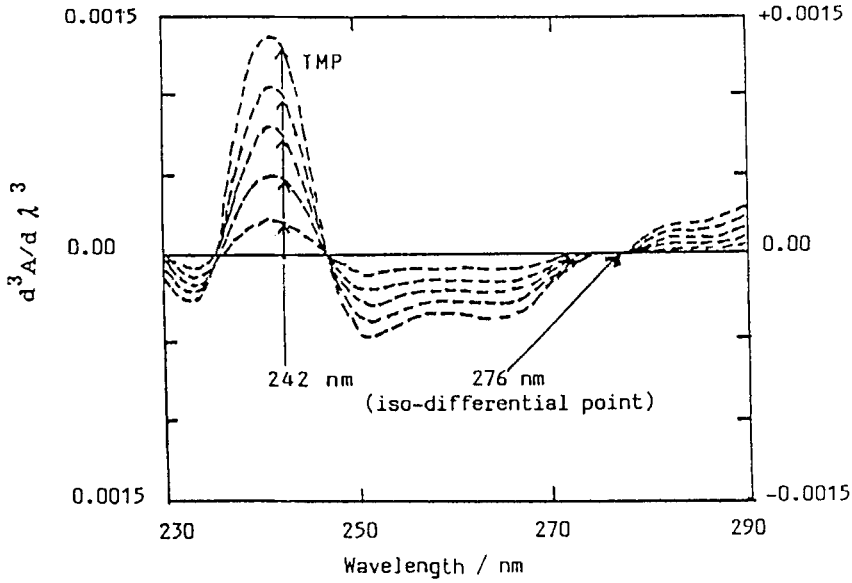

Fig.3. Third-derivative absorption spectra of 4-20 ug ml^{-1} TMP at several different concentrations in 0.1 M hydrochloric acid.

third-derivative amplitudes (^3D) at 242 nm (zero-crossing of SMX and PZP) and at 276 nm (zero-crossing of TMP and PZP) have been chosen for simultaneous determination of TMP and SMX, respectively (see Fig.2). Third-derivative spectra of TMP, SMX, and PZP at various concentrations are shown in Figures 3-5. As can be seen there is a distinct iso-differential point at 276 nm (optimum wavelength for SMX) irrespective of the concentration of TMP and PZP; and another one at 242 nm (optimum wavelength for TMP) irrespective of the concentration of SMX and PZP. Thus TMP may be determined at 242 nm (isodifferential point of SMX and PZP); SMX may be determined at 276 nm (isodifferential point of TMP and PZP) in the ternary mixture of TMP, SMX, and PZP.

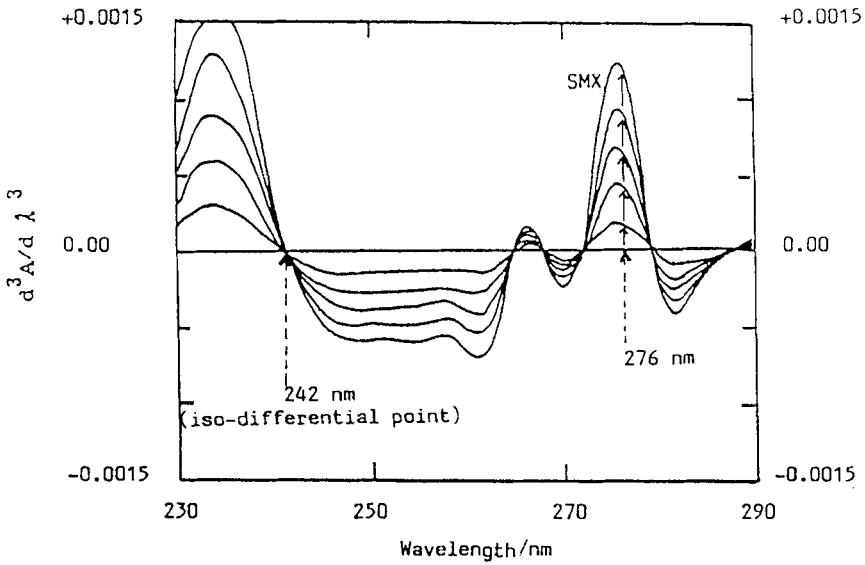

Fig.4. Third-derivative absorption spectra of 10-50 ug ml^{-1} SMX at several different concentrations in 0.1 M hydrochloric acid.

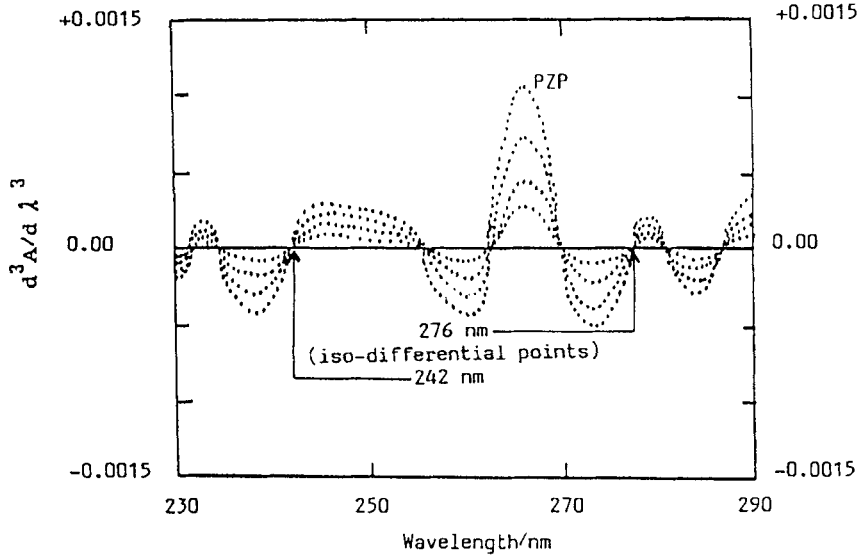

Fig.5. Third-derivative absorption spectra of 5-40 ug ml^{-1} PZP at several different concentrations in 0.1 M hydrochloric acid.

TABLE (1)

Analytical Data for the Calibration Curves (n=6) for the Determination of Trimethoprim, Sulphamethoxazole and Phenazopyridine Hydrochloride by Third-derivative and Zero-order UV Spectrophotometry.

Drug	Method	Linearity Range ($\mu g\ ml^{-1}$)	Regression equation		Correlation Coefficient, r	RSD,%*
			Slope	Intercept		
Trimethoprim	$^3D_{242}$**	4 - 20	1.549	0.722	0.9996	1.72
Sulphamethoxazole	$^3D_{276}$**	10 - 50	0.567	-0.164	0.9999	1.06
Phenazopyridine Hydrochloride	A_{448}	2.5-12.5	0.086	0.004	0.9998	0.84

* Percent relative standard deviation.

** Third derivative amplitude measured at 242 or 276 nm for trimethoprim and sulphamethoxazole, respectively.

Linearity and Precision:

Graphs obtained by plotting ^3D values at 242 nm and 276 nm for aqueous acidic solutions of TMP and SMX over the concentration ranges 4-20 and 10-50 ug ml^{-1}, respectively, show linear relationships. Analogously, the plot of absorbance at 450 nm versus concentration of PZP over the range 2.5 - 12.5 ug ml^{-1} shows that Beer's law was obeyed. Least square regression analysis was carried out for the slope (b), the intercept (a) and the correlation coefficinet (r). The results are presented in Table 1. The coefficient of variation (RSD,%) calculated for separate determination of each drug was less than 1.72% which indicates good precision and reproducibility of the proposed method.

TABLE (2)

Determination of Trimethoprim, and Sulphamethoxazole in Laboratory Prepared Mixtures by Third-derivative UV Spectrophotometry.

TMP : SMX : PZP ratio, m / m / m	Added (mg)	Recovered (mg)	% Recovery
		Trimethoprim	
0.4 : 2 : 1	0.400	0.382	95.58
0.6 : 2 : 1	0.600	0.598	98.15
0.8 : 2 : 1	0.800	0.783	97.82
1.2 : 2 : 1	1.200	1.222	101.80
1.6 : 2 : 1	1.600	1.628	101.77
2.0 : 2 : 1	2.000	1.983	99.17
Mean recovery (% ± SD) :			99.05 ± 2.21
		Sulphamethoxazole	
0.8 : 1.0 : 1	1.0	0.963	96.33
0.8 : 1.5 : 1	1.5	1.470	98.31
0.8 : 2.0 : 1	2.0	1.968	98.40
0.8 : 3.0 : 1	3.0	2.938	97.94
0.8 : 4.0 : 1	4.0	3.978	99.50
0.8 : 5.0 : 1	5.0	4.966	99.31
Mean recovery (% ± SD) :			98.29 ± 1.04

TABLE (3)

Determination of Phenazopyridine Hydrochloride in Laboratory Prepared Mixtures by Zero-order Absorption Spectophotometry.

TMP : SMX : PZP ratio, m / m / m	Added (mg)	Recovered (mg)	% recovery
0.8 : 2 : 0.25	0.25	0.250	100.09
0.8 : 2 : 0.50	0.50	0.495	99.00
0.8 : 2 : 0.75	0.75	0.761	101.42
0.8 : 2 : 1.00	1.00	0.993	99.30
0.8 : 2 : 1.25	1.25	1.251	100.11
Mean recovery (% ± SD)			99.98 ± 0.84

TABLE (4)

Assay Results for the Analysis of Trimethoprim, Sulphamethoxazole and Phenazopyridine Hydrochloride in Tablets by Third-derivatives and Zero-order UV Spectrophotometry.

Sample no.	Declared content (mg/ tablet)			Found % of the declared content		
	TMP	SMX	PZP	TMP	SMX	PZP
1	80	200	100	99.58	99.50	100.09
2	80	200	100	98.15	100.00	99.00
3	80	200	100	97.82	99.94	101.42
4	80	200	100	101.86	99.50	99.30
5	80	200	100	101.77	99.31	100.11
6	80	200	100	99.17	99.30	100.20
Mean recovery (% ± SD)				99.72±1.73	99.76±0.65	100.02±0.84

Selectivity :

In order to check the validity of the proposed method, TMP, SMX, and PZP were determined in several synthetic mixtures prepared by varying the concentration of one component, while the concentrations of the remaining two components were held constant. The concentrations were determined using third-order absorption spectra (for TMP and SMX) and zero-order absorption spectra (for PZP). Good agreement between the theoretical concentrations and the measured concentrations was obtained indicating that the proposed method is effective for the analysis of such ternary mixture (see Tables 2 & 3) and no interference occurs in the determination of each substance in the precence of the other two.

Accuracy:

The utility of this method was verified by means of a recovery assay in the presence of blank excipients containing starch, lactose, talc, and magnesium stearate in appropriate amounts. Tablets containing 80 mg TMP, 400 mg SMX and 100 mg PZP were prepared and processed according to the proposed method. Recovery were determined by comparison with the corresponding standard solution. The values of 99.72%$\pm$ 1.73 for TMP, 99.76 %$\pm$ 0.65 for SMX, and 100.02 $\pm$ 0.84 for PZP, were obtained (Table 4). The average recoveries obtained in each instance were compared with the theoretical value of 100% by means of Student's t-test. The t values were 0.40 for TMP, 1.02 for SMX, and 0.10 for PZP. As t (0.05,6) = 2.45, we can conclude that the recoveries obtained do not differ significantly from 100% for each drug.

Conclusions:

It has been demonstrated that the third-derivative and zero-order UV spectrophotometry are useful and reliable techniques for the

simultaneous determination of TMP, SMX and PZP in a ternary mixture either in a pure from or in tablets without previous separation. THe proposed method is simple and rapid, and shows good precision, accuracy and selectivity. Also, it has many advantages over conventional multicomponent analysis which require a separation technique, such as HPLC or GC. With this method, one can gain the advantages of speed and reduced cost without sacrificing accuracy. It could also be applied to the uniformity of contents assay. These advantages make it especially suitable for routine quality control.

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