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Notes

Multicomponent derivative spectroscopic analysis of sulfamethoxazole and trimethoprim

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Summary

Sulfamethoxazole and trimethoprim, frequently combined in chemotherapeutic practice, are analysed by a variety of chromatographic and conventional methods. In this investigation, a multicomponent derivative spectroscopic method was employed. Determination of the two substances in mixtures of varying concentrations was effected by first derivative measurements at 280 and 294 nm. The calibration curves demonstrated correlation coefficients greater than 0.99. Quality control data representing concentrations of artificial mixtures of both components at low and high levels in alternating fashion exhibited coefficients of variation consistently below 3% and sensitivities well below 5 mg/100 ml.

Various methods have been reported for the determination of co-trimoxazole in both pharmaceutical formulations and bulk form. Other methods have dealt with their analysis in different biological fluids. While some investigators studied the analysis of sulfamethoxazole (Umagat et al., 1979) and trimethoprim (Watson et al., 1980) separately, others developed various analytical procedures for their combination. These methods include liquid (Singletary and Sancillio, 1980) and gas chromatographic (Gyllenhaal et al., 1978), spectrophotometric (Koczian-Foldvari et al., 1986), fluorometric (Lichtenwalner et al., 1979), colorimetric (Sangal and Laha, 1983), and other procedures (Rao et al., 1980; Verma, 1980).

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It has recently been shown that the application of derivative techniques to spectrophotometry is very useful in resolving spectral overlap and in cancelling irrelevant absorption (Korany et al., 1984). However, in certain circumstances spectral overlap is also observed in the derivative spectra, albeit to a lesser extent. In such cases, multicomponent analysis can be applied to account mathematically for each absorption band.

In the present investigation, the combination of sulfamethoxazole and trimethoprim was analysed via first derivative UV spectroscopy using a multicomponent mathematical approach.

All reagents were used as received by the manufacturers or suppliers. Sulfamethoxazole and trimethoprim conformed to pharmacopoeial specifications and were obtained from E. Merck (F.R.G.). Water was double distilled in glass apparatus. Stock solutions of sulfamethoxazole and

trimethoprim were prepared in methanol (100 mg/100 ml). Subsequent dilutions for working solutions were made in distilled water. First derivative UV spectrophotometric measurements were carried out using a Kontron spectrophotometer (Model Urikon 810, Kontron, Switzerland). Recording parameters: response time, 0.2 s; bandwidth, 2.0 nm; data point interval, 0.2 nm. All determinations were measured against a blank at 280 and 294 nm.

The concentrations of sulfamethoxazole and trimethoprim in the unknown mixtures were calculated using the equations:

$$A^{280} = K_s^{280} C_s\, d + K_T^{280}\, C_T\, d \qquad (1)$$

$$A^{294} = K_s^{294}\, C_s\, d + K_T^{294}\, C_T\, d \qquad (2)$$

Where A^{280} and A^{294} denote the absorbance of the mixture at 280 and 294 nm, respectively; K_s^{280}, K_s^{294} and K_T^{280}, K_T^{294} represent the absorptivity constants of sulfamethoxazole and trimethoprim at 280 and 294 nm, respectively, C_S and C_T correspond to their concentrations in the unknown mixture and d is the path length (1 cm).

Substituting for C_T in Eqn 1, C_S in Eqn 2 and zero for K^{280} and rearranging:

$$C_s = \frac{K_T^{280}\, A^{294} - K_T^{294}\, A^{280}}{K_T^{280}\, K_s^{294}} \qquad (3)$$

$$C_T = \frac{A^{280}}{K_T^{280}} \qquad (4)$$

The spectrum of trimethoprim showed a zero crossing at a wavelength of 280 nm which allowed direct measurement of sulfamethoxazole at this wavelength without significant interference. Determination of trimethoprim was effected at 294 nm where the absorbance of both substances was found to be additive. The overlapping spectra were mathematically resolved using multicomponent analysis. The heights of the bands were measured at two wavelengths, viz., 280 and 294 nm (Fig. 1). One advantage of such wavelengths is the zero crossing of the trimethoprim band at 280 nm, thus facilitating direct measurement of sulfamethoxazole at this wavelength. Since the height of the absorption band at 294 nm was additive, the contribution due to trimethoprim absorbance is consequently directly related to its concentration in the bulk mixture. The amount is trimethoprim in such a mixture is therefore calculated at 294 nm after the direct measurement of sulfamethoxazole at 280 nm and making the necessary allowance for the latter in the equation at 294 nm.

Determination of the amounts of the two drugs in the mixture was only possible after calculation of the absorptivity constants of both substances at the two pre-selected wavelengths (Table 1). For these constants to be of analytical value a true estimate had to be determined, hence it was necessary to carry out the calculations in triplicate and at various concentration levels, viz, low, medium and high corresponding to 2, 4 and 8 mg/100 ml, respectively. The constants were subsequently used to calculate the amounts of sulfamethoxazole and trimethoprim in the bulk mixtures.

To establish the linearity of absorbance at the above-mentioned wavelengths for both drugs, a range of concentrations was plotted against peak heights and no attempts were made to force the lines through the origin. In the three plots obtained, correlation coefficients greater than 0.99 were obtained, however, the intercepts of the lines were slightly below zero (Table 1). This may be related, at least in part, to the tendency of the absorbance to diminish at lower concentration levels, due to the decrease in the response of the photomultiplier tube detector to low drug concentrations. In this respect, minimum detection levels, defined as detector response of at least twice base noise, were determined and were invariably shown to be slightly greater than 0.1 mg/100 ml. These levels compare favourably even with values from chromatographic procedures (0.01–0.1 mg/100 ml) previously reported for both drugs (Bury and Mashford, 1979; McIntosh et al., 1983).

Artificial mixtures of known concentrations of both substances were employed in the determination of the quality control and statistical data. The amounts of drugs in such mixtures were chosen in order to obtain high and low levels of both substances in alternating fashion to account for any

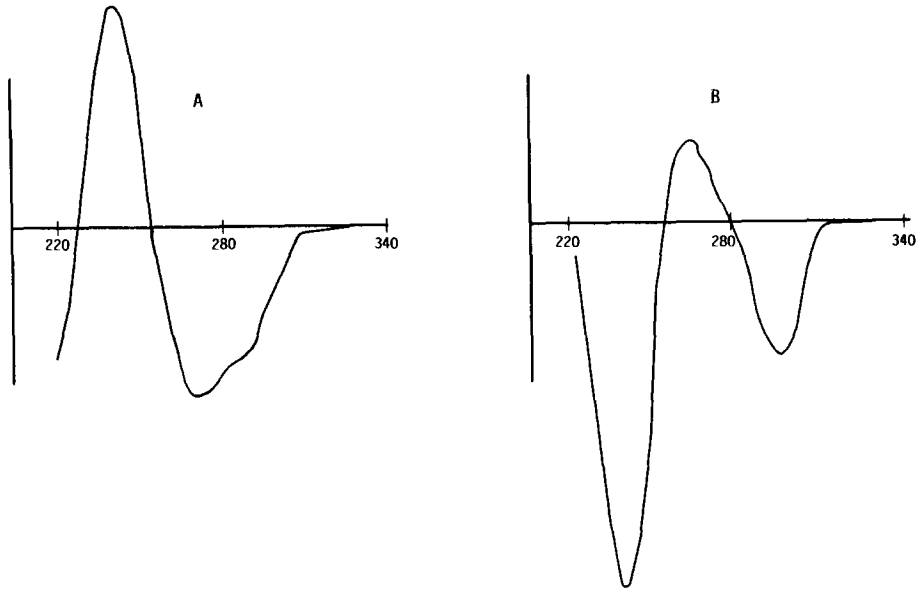

Fig. 1. Representative spectra of (A) sulfamethoxazole and (B) trimethoprim in distilled water.

TABLE 1

Absorptivity and calibration data of sulfamethoxazole and trimethoprim at 280 and 249 nm

	λ (nm)	*m*	*c*	*r*	$K \pm SD$	MDL (mg/100 ml)
Trimethoprim	280	0	0	0	0	0
	294	3.07	−1.0	0.993	2.84 ± 0.28	0.25
Sulfamethoxazole	280	4.06	−1.0	0.996	3.83 ± 0.29	0.16
	294	2.39	−0.5	0.992	2.30 ± 0.26	0.19

λ, wavelength (nm); m, slope; *c*, intercept; *r*, correlation coefficient; *K*, absorptivity constant; MDL, minimum detection level.

TABLE 2

Statistical and quality control data for the determination of sulfamethoxazole and trimethoprim

	Mixture 1		Mixture 2		Mixture 3		Mixture 4	
	C_S = 2.00	C_T = 2.00	C_S = 2.00	C_T = 8.00	C_S = 8.00	C_T = 2.00	C_S = 8.00	C_T = 8.00
Mean	1.90	1.91	1.90	8.30	8.34	1.92	8.35	8.38
± SD	0.035	0.04	0.027	0.106	0.107	0.036	0.129	0.188
CV	1.84	2.15	1.42	1.28	1.28	1.88	1.55	2.24
Bias	5.00	4.50	5.00	3.75	4.75	4.00	4.38	4.75
Sensitivity ($\times 10^{-2}$)	0.21	0.25	0.16	2.54	2.57	0.22	3.10	4.51

C_S and C_T denote the concentrations (in mg/100 ml) of sulfamethoxazole and trimethoprim, respectively.

[a] Bias = 100 × [(measured concn − theoretical concn)/theoretical concn].

[b] Sensitivity = $\frac{\pm 3 \times SD \times Concn}{100}$.

possible interference due to a greater proportion of one component compared to the other. For an unknown reason, the results clearly demonstrated a consistent underestimation of the lower concentrations and an overestimation of the higher concentrations of the two components. This may be explained, in part, by the fact that the mean values of the absorptivity constants were incorporated in the equation and used in subsequent calculations. These constants, in fact, exhibited standard deviations in the range of 0.26–0.29 (Table 2).

The quality control data, however, demonstrated good accuracy, reproducibility and sensitivity attributable to this method. Coefficients of variation were consistently lower than 2.5% and the sensitivity ranged between 0.16 and 4.5 mg/100 ml. In comparison, similar coefficients of variation and sensitivity levels were reported for a single component direct spectrophotometric assay (Klimowicz, 1981) and for chromatographic procedures (Bury and Mashford, 1979).

Finally, despite the great number of methods described in the literature for the analysis of sulfamethoxazole and trimethoprim, a UV spectroscopic method remains a simple, readily accessible and relatively inexpensive procedure for routine analysis. The procedure described in this report utilized these advantages for the analysis of a mixture of commonly combined substances.

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