

Short Communication

Spectral ratio derivative spectrophotometric determination of sulphaquinoxaline and pyrimethamine in veterinary formulations

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Keywords: *Spectral ratio derivative spectrophotometry; sulphaquinoxaline; pyrimethamine; veterinary formulations.*

Introduction

Veterinary and human medicines containing a sulphonamide are used to prevent infections in a variety of situations. For sulphonamide products that contain many components (e.g. four or more sulphonamides) chromatographic techniques are recommended [1–5]. However, for products formulated to contain a sulphonamide and another drug which increases the activity of the sulphonamide, simple, rapid and economic methods can be used. Spectrophotometric methods based on the Bratton–Marshall reaction are often used for determining total sulphonamides [6–9].

If the determination of the two drugs in the formulation containing the sulphonamide and another drug, e.g. sulphamethoxazole and trimethoprim, sulphadiazine and trimethoprim, is required, the derivative spectrophotometric technique is suitable, particularly when the spectra of the two drugs overlap. Recently, the first derivative of the ratio spectra [10] has been used for resolving different sulphonamides mixtures, e.g. sulphamethizole and nitrofurantoin [11] or sulphamethoxazole and trimethoprim [12].

In this paper, the simultaneous determination of sulphaquinoxaline and pyrimethamine in antibacterial formulations used in veterinary practice is described. The procedure was applied to three different veterinary formulations and the results compared with

those obtained by using the zero-crossing method in derivative spectrophotometry.

Experimental

Apparatus

A Beckman DU-70 spectrophotometer equipped with 1.0 cm quartz cells and connected to an IBM-PS 2 model 30 computer fitted with Beckman Data Leader software [13], and an Epson FX-850 printer was used for all the absorbance measurements.

A Crison microPH 2002 was used for the pH measurements.

Reagents

Standard solutions of 250 mg l⁻¹ of pyrimethamine (Sigma Chemicals) and sulphaquinoxaline sodium salt (Sigma Chemicals) were prepared, respectively, in ethanol and 50% v/v ethanol–water.

Ammonia–ammonium chloride buffer solution, 0.5 M and pH 10, was made from analytical-reagent grade reagents.

Standard solutions

Stock solutions of sulphaquinoxaline (SQX) and pyrimethamine (PMT) were added to 25-ml calibrated flasks to give final concentrations of 4–20 mg l⁻¹ of SQX and/or 4–20 mg l⁻¹ of PMT. Five millilitres of buffer solution pH 10 and sufficient water and ethanol to dilute the contents to 25 ml were added (the resulting

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final solution was 50% in ethanol). The absorption spectra of the samples were recorded, against a reagent blank between 190 and 425 nm at a scan speed of 300 nm min⁻¹, and stored in the IBM-PS 2 model 30 computer.

For the determination of SQX, the stored spectra were divided by a standard spectrum of PMT of 10 mg l⁻¹ and the ratio spectra thus obtained were smoothed by the use of 15 experimental points.

The first derivative of the ratio spectra was calculated with $\Delta\lambda = 5$ nm and the SQX content was determined by measuring at 271 nm (¹DD₂₇₁), 241 nm (¹DD₂₄₁), and peak-to-peak (¹DD_{271,241}).

For determining PMT, the spectra of the mixtures were divided by a standard spectrum of 7 mg l⁻¹ of SQX. The ratio spectra were smoothed by the use of 23 experimental points and their first derivatives was calculated with $\Delta\lambda = 5$ nm.

The content of PMT was determined by recording the first derivative of the ratio spectra between 260 and 325 nm and measuring at 304 nm (¹DD₃₀₄), 290 nm (¹DD₂₉₀) and peak-to-peak (¹DD_{304,290}).

Sample solutions

Three commercial veterinary formulations (Coccirex, Disulviar potenciado and Anticoccidiosico potenciado), containing PMT and SQX, were studied. A 1 ml volume of each preparation, was diluted with 50% v/v ethanol-buffer solution pH 10 in 100-ml calibrated flasks. Aliquots of 1 ml (Coccirex) or 1.5 ml (Disulviar potenciado and Anticoccidiosico potenciado) were transferred to 25-ml calibrated flasks, buffer solution was added and the contents were diluted to 25 ml with ethanol-water to give a solution containing 50% ethanol the absorption spectra were recorded against a reagent blank and stored in the IBM-PS 2 model 30 computer. For determining SQX and PMT, the stored spectra were handled as described for the standard solutions.

Results and Discussion

Spectrophotometric measurements

SQX and PMT are soluble in 50% v/v ethanol-water and ethanol, respectively, and their solutions were found to be stable for 18 days at least.

The effect of pH between 1.5 and 13 on the absorption spectra of SQX and PMT was studied (Fig. 1). Both compounds exhibited significant changes in their spectra in the pH range 5.0–8.5 indicating that they may exist in solution in at least two forms arising from their respective acid-base equilibria. Their pK_a values were calculated to be 6.5 ± 0.2 and 7.0 ± 0.2, respectively. A basic medium was considered to be suitable, because a bathochromic shift occurs in an acidic medium, and pH 10 in particular was chosen because this pH is easily controlled by the use of an ammonia-ammonium chloride buffer solution. Solutions of SQX and PMT (10 mg l⁻¹) were stable for at least 2 h in 50% v/v ethanol-water and 20% buffer solution, pH 10. Figure 2 shows the

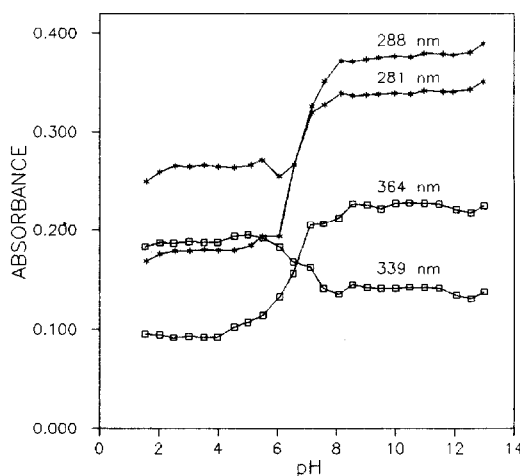


Figure 1 Effect of pH on the (□) SQX and (*) PMT absorption spectra.

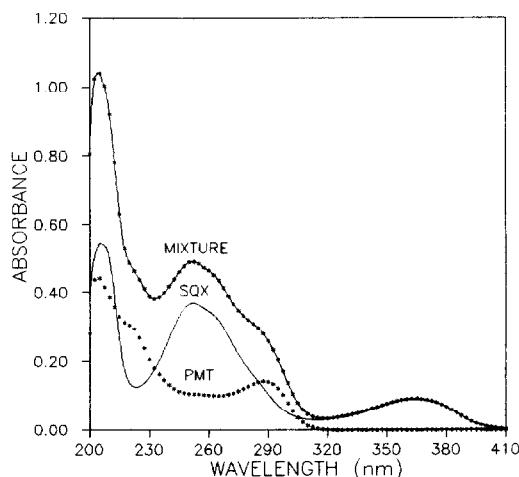


Figure 2 Absorption spectra of SQX (4 mg l⁻¹), PMT (4 mg l⁻¹) and their mixture at pH 10.

absorption spectra of SQX, PMT and their mixture at pH 10, in the wavelength range 200–410 nm. SQX can be measured at 365 nm. However, the spectra of PMT overlaps significantly and mathematical treatment of the data is recommended for determining the individual components in the mixture. The use of derivative ratio spectra is suitable for this purpose.

For the simultaneous determination of SQX and PMT by the proposed method, it was necessary to study the influence of the variables: concentration of the standard spectrum divisor, number of points for the smoothing function and the $\Delta\lambda$ for measuring the first derivative of the ratio spectra.

In the determination of SQX, the effect of varying the concentration of PMT for the divisor spectrum was tested and a concentration of 10 mg l^{-1} gave the optimum signal-to-noise ratio (SNR). Due to the noise levels in the ratio spectra, a smoothing function based on the Savitzky and Golay principle [14] was used and 15 experimental points were considered to be the optimum number. The first-derivative of the ratio spectrum was influenced by $\Delta\lambda$. When the $\Delta\lambda$ values increases, the signal amplitude decreases slightly; a $\Delta\lambda = 5 \text{ nm}$ was considered to be the optimum value.

After optimization of the variable parameters, the linearity of the amplitude values in the first-derivative ratio spectra was measured for a range of concentrations of SQX as shown in Fig. 3. Linear calibrations graphs were obtained between 1 and 25 mg l^{-1} (Table 1).

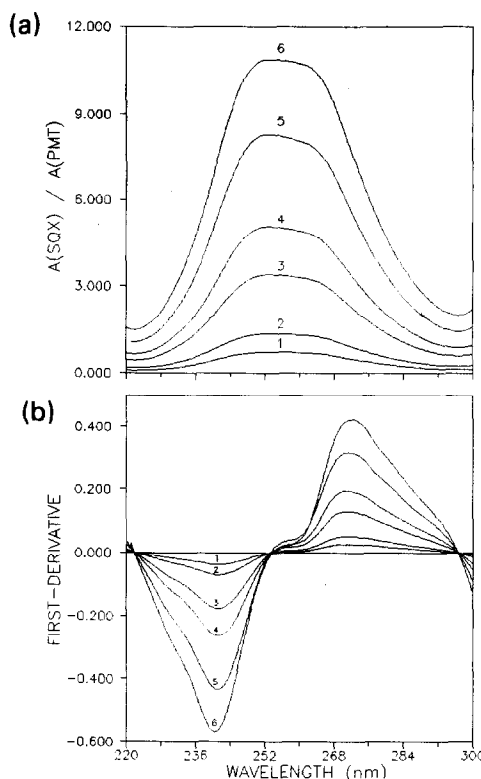


Figure 3 Ratio spectra (a) and first-derivative ratio spectra (b) of different concentrations of SQX: 1 (2 mg l^{-1}), 2 (4 mg l^{-1}), 3 (10 mg l^{-1}), 4 (15 mg l^{-1}), 5 (25 mg l^{-1}) and 6 (35 mg l^{-1}).

For determining PMT an analogous procedure was followed. The optimum concentration of spectrum divisor (SQX) was 7 mg l^{-1} (optimum SNR), and the ratio spectra were smoothed with 23 points. The first derivative of

Table 1 Calibration data in the determination of PMT and SQX

Equations	Correlation coefficient	Range (mg l^{-1})	Standard deviation	
			Slope ($\times 10^{-4}$)	Intercept ($\times 10^{-3}$)
PMT				
${}^1\text{DD}_{304} = -1.26 \times 10^{-3} + 0.01888 C^*$	0.9999	1–50	0.34	0.92
${}^1\text{DD}_{290} = -6.4 \times 10^{-4} + 0.01072 C^*$	0.9999	1–50	0.24	0.66
${}^1\text{DD}_{304,290} = -1.91 \times 10^{-3} + 0.002960 C^*$	0.9999	1–50	0.55	1.49
${}^1\text{D}_{315} = -1.2 \times 10^{-4} + 3.9 \times 10^{-4} C^*$	0.9999	1–50	0.01	0.04
${}^1\text{D}_{253.25} = 1.1 \times 10^{-4} + 1.4 \times 10^{-4} C^*$	0.9998	1–50	0.01	0.02
${}^2\text{D}_{272.25} = 0.8 \times 10^{-5} + 2.3 \times 10^{-4} C^*$	0.9999	1–35	0.01	0.02
SQX				
${}^1\text{DD}_{271} = 1.77 \times 10^{-3} + 0.01266 C^*$	0.9999	1–25	0.67	0.90
${}^1\text{DD}_{241} = 2.18 \times 10^{-3} + 0.01738 C^*$	0.9999	1–25	0.77	1.03
${}^1\text{DD}_{271,241} = 3.95 \times 10^{-3} + 0.03005 C^*$	0.9999	1–25	1.42	1.89
${}^1\text{D}_{386} = 7 \times 10^{-5} + 8.2 \times 10^{-4} C^*$	0.9999	1–35	0.01	0.02
${}^1\text{D}_{262.75} = 2.2 \times 10^{-4} + 1.53 \times 10^{-3} C^*$	0.9998	1–20	0.06	0.06
${}^2\text{D}_{396} = 4 \times 10^{-5} + 2.1 \times 10^{-4} C^*$	0.9999	1–35	0.01	0.02
${}^2\text{D}_{229} = 3.4 \times 10^{-4} + 1.31 \times 10^{-3} C^*$	0.9997	1–25	0.13	0.17

* Concentration of PMT and SQX = mg l^{-1} .

the ratio spectra was optimized with $\Delta\lambda = 5$ nm as the most suitable value.

Figure 4 shows the ratio spectra and their first-derivative spectra for different concentrations of PMT. Linear calibration graphs were obtained (Table 1) for the first derivative

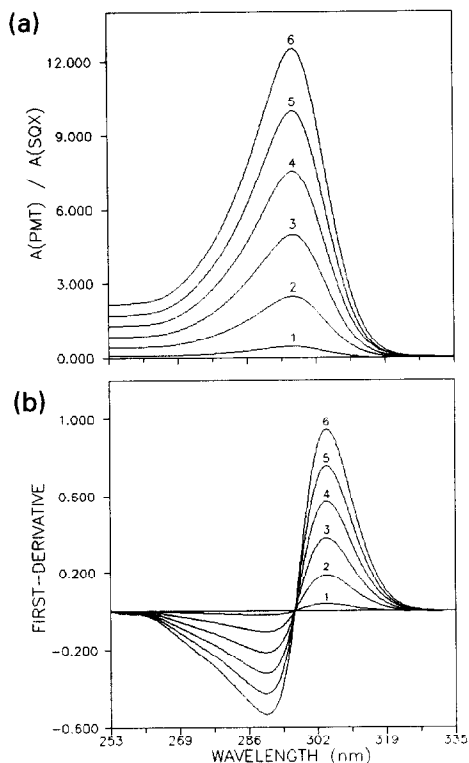


Figure 4
Ratio spectra (a) and first-derivative ratio spectra (b) of different concentrations of PMT: 1 (2 mg l⁻¹), 2 (10 mg l⁻¹), 3 (20 mg l⁻¹), 4 (30 mg l⁻¹), 5 (40 mg l⁻¹) and 6 (50 mg l⁻¹).

of the ratio spectra for concentrations between 1 and 50 mg l⁻¹. In all cases, linear coefficients were 0.9999.

Table 2 summarizes the validation data obtained from 10 replicate measurements or the reagent-blank and standard solutions of 10 mg l⁻¹ of SQX and PMT. The best quantification limits for SQX and PMT were given by, respectively, measuring at 271 nm (¹DD₂₇₁) and the peak-to-peak amplitude (¹DD_{304,290}). The smallest relative errors (%) were obtained for SQX and PMT, respectively, at 241 nm (¹DD₂₄₁) and the peak-to-peak amplitude (¹DD_{304,290}).

The proposed method has been compared with classical derivative spectrophotometry by using the zero-crossing wavelengths in the first and second derivative spectra of the PMT and SQX. The zero-crossing method involves measurement of the absolute value of the derivative spectrum of the mixture at wavelengths corresponding to the zero-crossing wavelengths in the derivative spectra of the individual components. First-derivative spectra of SQX (4 mg l⁻¹), PMT (4 mg l⁻¹) and their mixture are shown in Fig. 5. PMT can be determined by measuring at 315 nm (¹D₃₁₅) and at 253.25 nm (¹D_{253.25}); and SQX can be determined at wavelengths 386 nm (¹D₃₈₆), 286.75 nm (¹D_{286.75}) and 262.75 nm (¹D_{262.75}). In the second derivative mode (Fig. 6), PMT can be determined at 272.5 nm (²D_{272.5}) and at 239 nm (²D₂₃₉) and SQX can be determined at 396 nm (²D₃₉₆), 368 nm (²D₃₆₈) and 229 nm (²D₂₂₉).

Table 2
Validation data*

Signal measured	Standard deviation (mg l ⁻¹)	Relative error (%)	Limit of detection (mg l ⁻¹)	Limit of quantification (mg l ⁻¹)
PMT†				
¹ DD ₃₀₄	0.107	0.74	0.20	0.67
¹ DD ₂₉₀	0.084	0.58	0.20	0.68
¹ DD _{304,290}	0.071	0.49	0.06	0.21
¹ D ₃₁₅	0.125	0.38	0.31	1.03
¹ D _{253.25}	0.325	2.61	>0.01	>0.03
² D _{272.25}	>0.071	0.08	>0.01	>0.03
SQX†				
¹ DD ₂₇₁	0.048	0.37	0.01	0.03
¹ DD ₂₄₁	>0.029	0.07	0.04	0.14
¹ DD _{271,241}	0.048	0.37	0.02	0.06
¹ D ₃₈₆	0.061	0.46	>0.01	>0.03
¹ D _{262.75}	0.029	0.23	0.06	0.21
² D ₃₉₆	0.145	1.13	>0.01	>0.03
² D ₂₂₉	0.059	0.43	0.37	1.23

* $n = 10$.

† Concentration of PMT and SQX = 10 mg l⁻¹.

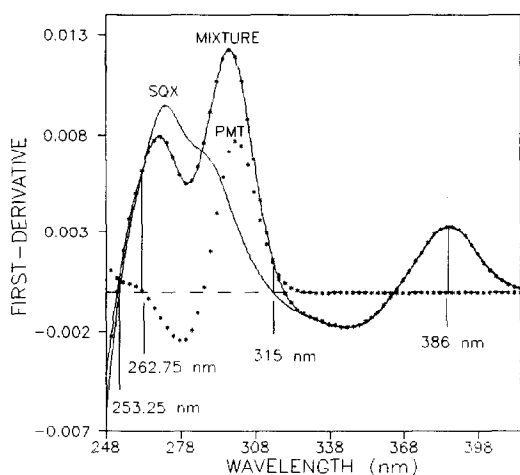


Figure 5
First-derivative spectra of 4 mg l^{-1} of SQX, PMT and their mixture.

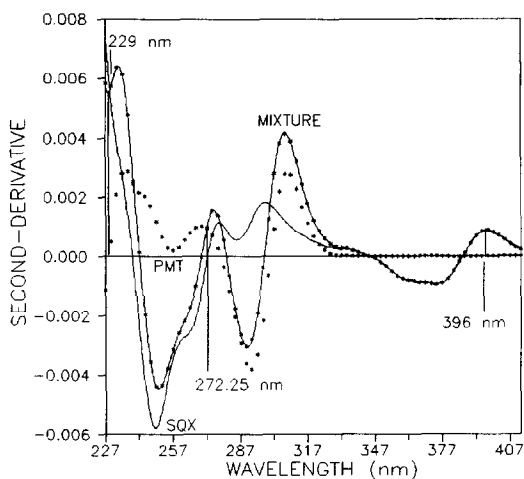


Figure 6
Second derivative spectra of 4 mg l^{-1} of SQX, PMT and their mixture.

The first- and second-derivative spectra (Figs 5 and 6), were obtained by the Savitzky and Golay method, and the effect of different values of $\Delta\lambda$ was investigated, because this parameter can affect the shape of the derivative spectra. Generally, the noise levels decrease when the $\Delta\lambda$ value is increased. However, if the value of $\Delta\lambda$ is too large, the spectral resolution deteriorates. A value of $\Delta\lambda = 15 \text{ nm}$ was considered to be optimum for first derivative measurements. In the second derivative mode a $\Delta\lambda = 20$ was considered to be optimum. However owing to the magnitude of the noise in the derivative spectra, a

smoothing function was used and 15 experimental points were considered to be the optimum number.

Calibration graphs were obtained at some of the zero-crossing wavelengths selected (Table 1). In the first-derivative mode, PMT gave straight line plots up to 50 mg l^{-1} at 315 and 253.25 nm. SQX gave straight line plots up to 35 mg l^{-1} at 386 nm and up to 20 mg l^{-1} at 262.75 nm. The correlation coefficients were between 0.9998 and 0.9999. In the second-derivative mode, linear calibration graphs were obtained for PMT between 1 and 35 mg l^{-1} at 272.25 nm and for SQX between 1 and 35 mg l^{-1} at 396 nm and between 1 and 25 mg l^{-1} at 229 nm.

Table 2 summarizes the validation data for different measured values of SQX and PMT in the conventional first- and second-derivative spectra.

The best limits of quantification for PMT were found at 253.25 and 272.25 nm in the first- and second-derivative spectra, respectively, and for SQX at 386 nm in the first-derivative mode and at 396 nm in the second-derivative mode.

Applications

Standard mixtures

Several mixtures of SQX and PMT were prepared from stock solution, in concentration ratios PMT:SQX from 1:5 to 5:1, and analysed by the proposed method and by conventional derivative spectrophotometry. The results obtained are shown in Table 3. Recovery values obtained by the first-derivative ratio method were between 97 and 102 for PMT and between 98 and 102 for SQX.

Veterinary preparations

The proposed method was used for determining the concentrations of SQX and PMT in three different commercial formulations and the results were compared with those given by conventional derivative spectrophotometry in the first and second mode. The results obtained are summarized in Table 4. The best recovery values for PMT were found when the derivative ratio method was used. In the case of the Disulviar preparation which showed values for PMT in excess of the label claim, the standard addition method was used in an attempt to eliminate interference, but identical results were obtained. It would appear that

Table 3
Recovery data obtained for the determination of SQX and PMT in standard mixtures

Signal measured	Recovery (%)* for ratios PMT:SQX								
	4:20	4:16	4:12	4:8	4:4	8:4	12:4	16:4	20:4
PMT									
¹ DD ₃₀₄	102	99	100	100	100	101	100	101	101
¹ DD ₂₉₀	101	101	97	98	98	100	100	100	100
¹ DD _{304,290}	101	100	98	100	100	101	100	100	100
¹ D ₃₁₅	99	99	99	99	99	98	96	96	96
¹ D _{253.25}	101	93	93	93	93	94	95	95	94
² D _{272.25}	—	—	—	109	109	103	101	103	101
SQX									
¹ DD ₂₇₁	100	101	101	102	99	98	99	98	98
¹ DD ₂₄₁	101	101	101	101	99	98	99	98	98
¹ DD _{271,241}	101	101	101	101	99	98	99	98	98
¹ D ₃₈₆	101	100	100	100	99	99	97	97	97
¹ D _{262.75}	100	101	102	101	99	97	96	93	92
² D ₃₉₆	103	102	102	103	100	100	100	100	100
² D ₂₂₉	101	101	102	103	99	103	107	102	88

* Average for two determinations.

Table 4
Results obtained for the determination of SQX and PMT in veterinary preparations

	SQX and PMT recovery (%)*		
	Coccirex	Disulviar potenciado	Anticoccidioso potenciado
PMT			
¹ DD ₃₀₄	101	108	107
¹ DD ₂₉₀	107	113	82
¹ DD _{304,290}	103	111	98
¹ D ₃₁₅	117	—	—
¹ D _{253.25}	—	—	—
² D _{272.25}	—	—	—
SQX			
¹ DD ₂₇₁	98	103	91
¹ DD ₂₄₁	97	100	87
¹ DD _{271,241}	98	101	88
¹ D ₃₈₆	98	100	95
¹ D _{262.75}	95	93	—
² D ₃₉₆	96	96	92
² D ₂₂₉	86	108	—

* Average for two determinations.

incorrect information about the concentration is given on the label.

SQX can be determined with satisfactory accuracy, based upon the declared content, for Coccirex and Disulviar, except at ²D₂₂₉ and ¹D_{262.75}.

In the case of Anticoccidioso, low recovery values were obtained by all measurements as a consequence of the presence of one or more excipients in the product.

Conclusion

The use first-derivative ratio spectra for resolving mixtures of PMT and SQX compares

favourably with the zero-crossing method in conventional derivative spectrophotometry as shown by the resolution of synthetic mixtures and veterinary formulations.

Acknowledgement — The authors gratefully acknowledge financial support from the DGICYT of the Ministerio de Educación y Ciencia of Spain (Project No. PB 90-0397).

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[Received in final revised form 29 January 1992]