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Simultaneous determination of sulphathiazole and sulphanilamide in pharmaceuticals by derivative spectrophotometry

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Abstract: Third-order and fourth-order derivative spectra have been used for determining sulphathiazole, sulphanilamide and their binary mixtures. The method is suitable for 1–22 μg ml^{-1} of sulphathiazole and for 0.5–14 μg ml^{-1} of sulphanilamide and can be applied for determining the sulphonamides in pharmaceuticals.

Keywords: *Derivative spectroscopy; sulphonamides; UV-spectroscopy.*

Introduction

Antibiotics and other antimicrobial agents are used in attempts to prevent infections in a variety of situations. Sulphonamides are extensively used in medical and veterinary practice for the treatment of bacterial infections, and sulphathiazole is one of the agents most widely used in beekeeping [1, 2].

Many methods have been published for the determination of sulphonamides in body fluids [3, 4], foodstuffs and feeds [5]. On the basis of the Bratton–Marshall procedure [6] other methods have been developed and automated [7–10]. The non-specifity is the major disadvantage of the Bratton–Marshall based methods, and samples containing two sulphonamides cannot be resolved.

Derivative spectrophotometry particularly with digital processing [11], is an analytical technique of great utility for resolving some mixtures of compounds with overlapping spectra. The fundamental principles and conventions have been described in the pioneering work of O'Haver and Green [12], Fell *et al.* [13–15] and others [16]. The purpose of this paper is to develop a new method for resolving binary mixtures of sulphonamides by using third and fourth-order derivative spectrophotometry. Samples containing sulphathiazole and sulphanilamide can be resolved, and the method has been applied for the analysis of pharmaceuticals.

Experimental

Apparatus

A Beckman Instrument DU-50 spectrophotometer connected to an IBM PC-XT computer fitted with Beckman 'Data Leader' Software was used for all absorbance measurements.

Reagents

Sulphathiazole and sulphanilamide ethanolic solutions, 10^{-3} M, were prepared from Sigma R.A. products.

Sodium acetate–acetic acid buffer (pH 4.5) was prepared by dissolving 6.8 g of sodium acetate and 3.0 ml of acetic acid (37%) in water and diluting the solution of 1 l with water.

Procedure for determining sulphathiazole and sulphanilamide

Samples were prepared in 25-ml volumetric flasks containing 1–22 μg ml^{-1} of sulphathiazole or 0.5–14 μg ml^{-1} of sulphanilamide, or their binary mixtures, 5 ml of pH 4.5 buffer solution, and ethanol up to 2.5 ml, followed by dilution with water. Absorption spectra of the samples were recorded at a scan speed of

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750 nm min^{-1} between 200–400 nm. The spectra obtained were smoothed by 15-point smoothing. The third-order derivative spectra were recorded with a $\Delta\lambda$ = 28 nm; the fourth-order derivative spectra were recorded with a $\Delta\lambda$ = 54 nm. On the basis of these derivative spectra, the following methods were developed:

Sulphathiazole. Determine the sulphathiazole content from the third-order derivative spectrum by measuring the signal at $^3D_{297}$ (cf. 15 for the nomenclature convention) or from the fourth-order derivative spectrum at $^4D_{290.6}$, using the zero-crossing points with respect to sulphanilamide in both cases.

Sulphanilamide. Determine the sulphanilamide content from the third-order derivative spectrum at $^3D_{268}$ or from the fourth-order derivative spectrum at $^4D_{278}$, these being the zero-crossing points for sulphathiazole.

Procedure for determining sulphathiazole and sulphanilamide in pharmaceutical formulations. Bucodrin (Fardi): a powdered tablet was extracted with ethanol assisted with a mechanical shaker or an ultrasonic bath, filtered through common filter paper and the filtrate used for measuring. Amidrin (Fardi): 2.50 ml of sample were diluted to 100 ml with ethanol. Aliquots of these solutions were taken and the sulphathiazole and sulphanilamide were determined as described above.

Results and Discussion

Sulphathiazole and sulphanilamide solutions showed overlapping UV spectra in all pH ranges studied, making it difficult to resolve mixtures by classical spectrophotometry. However, derivative spectrophotometry can be used for resolving this problem satisfactorily.

First, the stability of the sulphonamide solutions has been tested, and the influence of solvent and changes in the UV spectra with pH have been examined.

Ethanolic solutions of sulphathiazole and sulphanilamide (10^{-3} M) are stable for at least 10 days, and the diluted solutions in ethanol–water (1:10) were also stable for at least 1 week. When the content of ethanol was increased, a slight bathochromic effect was observed in the sulphathiazole and sulphanilamide absorption spectra.

In order to establish a suitable pH value for this work a range of values between pH 1–12 was examined. In Fig. 1 it can be observed that, in the case of these two sulphonamides, the absorption spectra remain unchanged between pH 3.5–6.0. As a result, a pH of 4.5 was considered suitably stable for an attempt to develop a quantitative method for binary mixtures.

The zero-order derivative spectra of sulphathiazole and sulphanilamide in ethanol–water (10%) at pH 4.5 are shown in Fig. 2. It can be observed that the overlapping of the spectra prevents the resolution of the mixtures by direct spectrophotometric measurement. However, derivative spectra of third-order and fourth-order can be used satisfactorily.

Owing to the extent of the noise levels observed in the derivative spectra, a smoothing function was used (Data Leader Beckman Instruments) on the basis of the Savitzky and Golay method [11]. Thus the zero-order spectra of sulphathiazole and sulphanilamide were smoothed with 5 to 25 experimental points; a 15-point smoothing algorithm was considered to be optimum for each of the two sulphonamides.

The scan rate was found not to influence the intensity or shape of the derivative spectra unduly, although the choice of scan speed was limited by the instrument used (500 and 750 nm min^{-1}). Once the operating parameters had been optimized, mixtures of sulphathiazole and sulphanilamide were studied.

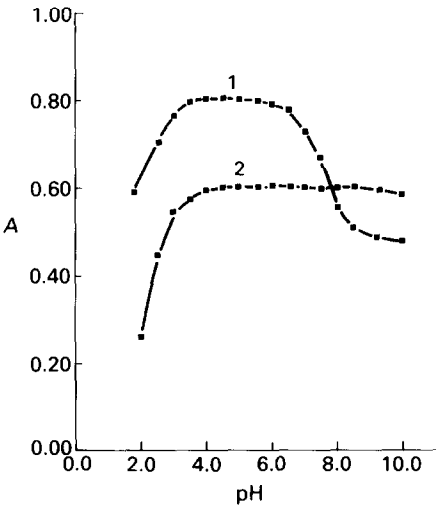

Figure 1
Influence of the pH on the absorbance of sulphathiazole (1) and sulphanilamide (2) at their λ_{max} values.

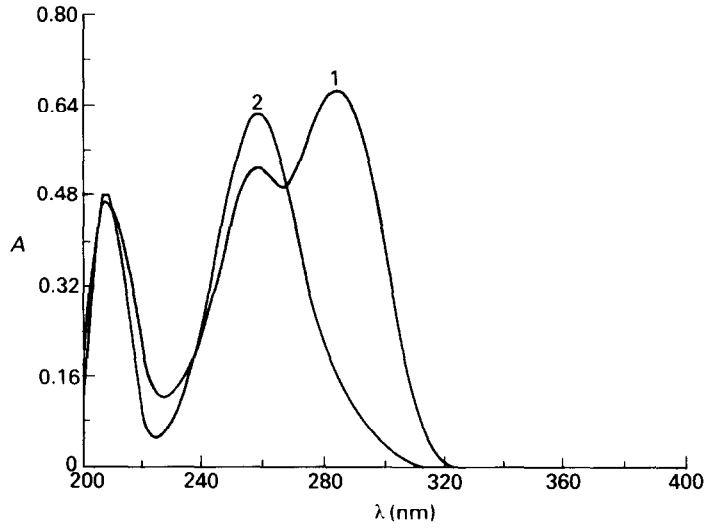

Figure 2
Absorption spectra of sulphathiazole (1) and sulphanilamide (2) solutions in ethanol–water (1:9) at pH 4.5.

Sulphathiazole and sulphanilamide mixtures

Derivative spectra of different orders were obtained using smoothed spectra of sulphathiazole, sulphanilamide and sulphathiazole–sulphanilamide mixture solutions. First-derivative and second-derivative spectra were not found to be resolved, but third-order and fourth-order derivative spectra could be used for the quantitative analysis of the resolved sulphathiazole and sulphanilamide in mixtures, as can be seen in Fig. 3. Sulphathiazole is determined by measuring the signal at $^3D_{297}$ from the third-order derivative spectrum or at $^4D_{2890.6}$ in the fourth; and is determined at $^3D_{268}$ or $^4D_{278}$ in the third-order or fourth-order derivative spectra, respectively.

The influence of the $\Delta\lambda$ value on the derivative spectra was tested in a range of values between 4–44 nm (third-derivative spectrum) and between 6–120 nm (fourth-derivative spectrum). As a result, a $\Delta\lambda$ values of 28 and 54 nm were considered suitable for the third and fourth-derivative spectra, respectively.

When all the instrumental parameters had been optimized, the linearity of the selected derivative measures for both sulphonamides was examined. A range of concentrations was studied for sulphathiazole (1–22 μg ml^{-1}) and for sulphanilamide (0.5–14 μg ml^{-1}), as shown in Figs 4 and 5, respectively. The statistical data from the calibration graphs obtained from these spectra are given in Table 1. To check the

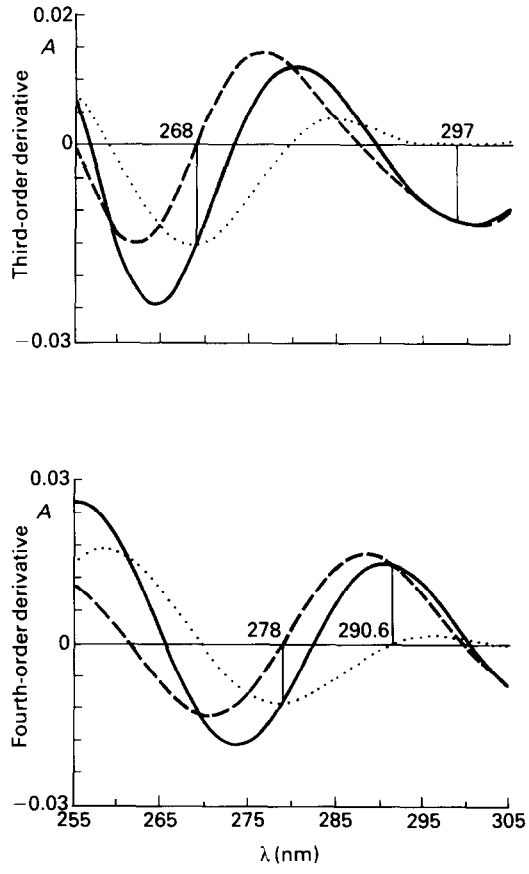

Figure 3
Derivative spectra of the sulphathiazole, sulphanilamide and sulphathiazole–sulphanilamide mixture solutions in ethanol–water (1:9), at pH 4.5. Key: (--) sulphathiazole; (···) sulphanilamide; (——) mixture.

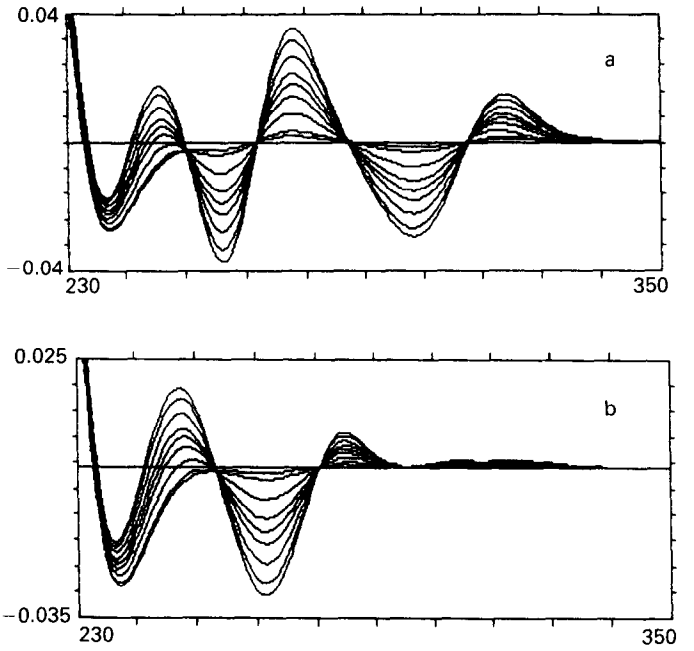

Figure 4
Influence of sulphathiazole concentration on the derivative spectrum: (a) third-order derivative; (b) fourth-order derivative.

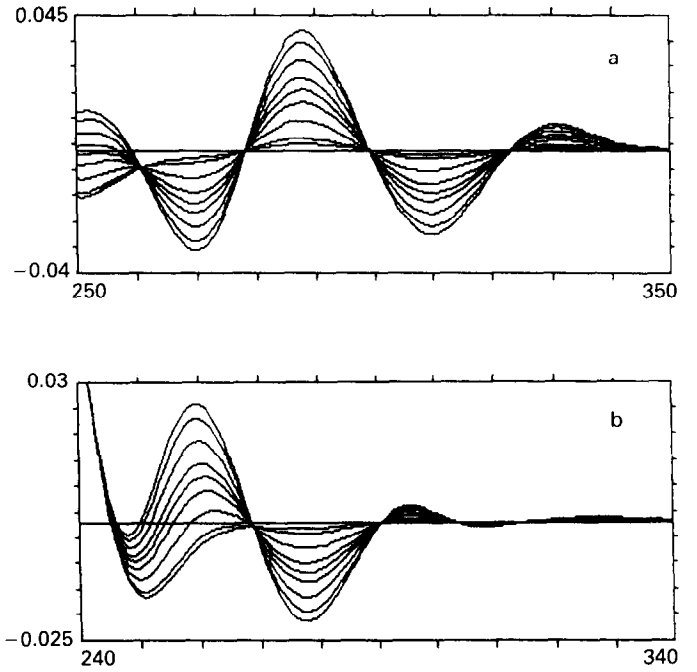

Figure 5
Influence of sulphanilamide concentration on the derivative spectrum: (a) third-order derivative; (b) fourth-order derivative.

precision of the methods, the signals for replicate samples (n = 9) containing sulphathiazole (8.9 μg ml^{-1}) and sulphanilamide (7.0 μg ml^{-1}) were measured individually (Table 2).

Application

The simultaneous determination of sulphathiazole and sulphanilamide in synthetic mixtures was performed using the zero-crossing measurement method [12, 16] at the wave-

Table 1
Calibration data for sulphathiazole and sulphanilamide

Compound	Equation	Correlation coefficient (r)*
Sulphathiazole	$^3D_{297} = 0.2 \times 10^{-4} + 12.5 \times 10^{-4}\,C$ (7.5×10^{-5})† (5.6×10^{-6})‡	0.9999
	$^4D_{290.6} = 0.6 \times 10^{-4} + 16.2 \times 10^{-4}\,C$ (7.9×10^{-5})† (5.9×10^{-6})‡	1.0000
Sulphanilamide	$^3D_{268} = 1.0 \times 10^{-4} + 21.4 \times 10^{-4}\,C$ (6.5×10^{-5})† (7.8×10^{-6})‡	1.0000
	$^4D_{278} = 1.6 \times 10^{-4} + 15.0 \times 10^{-4}\,C$ (2.6×10^{-5})† (3.2×10^{-6})‡	1.0000

*n = 5.
C = concentration of sulphathiazole or sulphanilamide (μg ml^{-1}).
†Standard deviation of intercept.
‡Standard deviation of slope.

Table 2
Statistical parameters for the determination of sulphathiazole and sulphanilamide

Compound	Signal measured	Standard deviation (μg ml^{-1})	RSD* (%)	Detection limit (μg ml^{-1})	Determination limit (μg ml^{-1})
Sulphathiazole	$^3D_{297}$	5.0×10^{-5}	0.46	0.106	0.35
	$^4D_{290.6}$	7.0×10^{-5}	0.46	0.093	0.31
Sulphanilamide	$^3D_{268}$	12×10^{-5}	0.82	0.074	0.25
	$^4D_{278}$	9.7×10^{-5}	0.92	0.066	0.22

*n = 5.

Table 3
Recovery of sulphathiazole (ST) and sulphanilamide (SA) in mixtures

ST–SA ratio	ST (μg ml^{-1}) Theoretical	Found	Recovery (%)	SA (μg ml^{-1}) Theoretical	Found	Recovery (%)
^3D						
1.4:1.0	2.2	2.2	100.0	1.4	1.1	78.6
1.0:3.2	2.2	2.2	100.0	7.0	7.1	101.4
1.0:6.4	2.2	2.0	90.9	14.0	14.0	100.0
6.4:1.0	8.9	9.0	101.1	1.4	1.5	107.1
1.6:1.0	8.9	8.9	100.0	5.6	5.7	101.8
1.0:1.4	8.9	8.9	100.0	12.6	12.5	99.2
11.9:1.0	16.7	17.0	101.8	1.4	1.7	121.4
4.8:1.0	16.7	16.9	101.2	3.5	3.6	102.9
2.4:1.0	16.7	16.6	99.4	7.0	7.2	102.9
^4D						
1.4:1.0	2.2	2.2	100.0	1.4	1.4	100.0
1.0:3.2	2.2	2.2	100.0	7.0	7.0	100.0
1.0:6.4	2.2	2.1	95.5	14.0	14.0	100.0
6.4:1.0	8.9	9.0	101.1	1.4	1.4	100.0
1.6:1.0	8.9	8.9	100.0	5.6	5.6	100.0
1.0:1.4	8.9	8.9	100.0	12.6	12.5	99.2
11.9:1.0	16.7	16.9	101.2	1.4	1.4	114.3
4.8:1.0	16.7	16.7	100.0	3.5	3.5	100.0
2.4:1.0	16.7	16.5	98.8	7.0	7.0	100.0

lengths selected in the procedure. Table 3 presents the results of the determination of different mixtures. As can be seen, satisfactory results were obtained with a mean recovery of 99.3 and 101.7% for sulphathiazole and sulphanilamide in the third-derivative mode, and 99.6 and 101.5% for sulphathiazole and sulphanilamide in the fourth-derivative mode, respectively.

Sulphathiazole and sulphanilamide were

Table 4
Analysis of sulphathiazole and sulphanilamide in formulations

Proprietary name	Composition	Signal	Found (g)	Recovery (%)	HPLC*
Bucodrin (Fardi)	Per tablet Sulphathiazole, 0.10 g 2-Ethoxy-6,9-diamino acridine lactate, 0.002 g ephedrine ester, 0.003 g	$^3D_{297.0}$ $^4D_{290.6}$	0.081 0.086	81.0 86.0	0.082
Amidrin (Fardi)	Sulphanilamide, 0.40 per 100 ml		0.44	110.0	0.42

*Using a method developed in the laboratory (n = 5).

determined in pharmaceutical preparations. Because of difficulties encountered in obtaining dosage forms containing both of the sulphonamides tested, the proposed method was applied to the determination of these drugs in commercial formulation mixtures. Samples were prepared and analysed as described above. The results obtained are in good agreement with those obtained by HPLC (Table 4).

References

[1] M.M.L. Aerts, W.M.J. Beek and U.A.Th. Brinkman, *J. Chromatogr.* **435**, 87 (1988).
[2] C.P. Barry and G.M. MacEachern, *J. Assoc. Off. Anal. Chem.* **66**, 4 (1983).
[3] L.S. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics*, p. 1113. Macmillan, New York (1975).
[4] W. Sadee and G.C.M. Beelen, *Drug Level Monitoring*, p. 631. Wiley, New York (1980).
[5] H. Horwitz, *J. Assoc. Off. Anal. Chem.* **64**, 104 (1981).
[6] A.C. Bratton and E.K. Marshall, *J. Biol. Chem.* **128**, 537 (1939).
[7] J. Rieder, *Chemotherapy (Basel)* **17**, 1 (1972).
[8] H.B. Falk and R.G. Kelly, *Clin. Chem.* **11**, 1045 (1965).
[9] A. Bye and A.F.J. Fox, *Clin. Chem.* **20**, 288 (1974).
[10] M.A. Koupparis and P.I. Anagnostopoulou, *Anal. Chim. Acta* **204**, 271 (1988).
[11] A. Savitzky and M.J.E. Golay, *Anal. Chem.* **36**, 1627 (1964).
[12] T.C. O'Haver and G.L. Green, *Anal. Chem.* **48**, 312 (1976).
[13] A.F. Fell, *Proc. Anal. Div. Chem. Soc.* **15**, 260–267 (1978).
[14] A.F. Fell, D.R. Jarvie and M.J. Stewart, *Clin. Chem. (Winston-Salem, NC)* **27**, 286–292 (1981).
[15] A.A. Fasanmade and A.F. Fell, *Analyst* **110**, 1307–1312 (1985).
[16] B. Morelli, *Analyst* **108**, 870 (1983).

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