ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 1107–1112

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Analysis of sulfamethazine in the presence of sulfamerazine or sulfadiazine by first-derivative photochemically induced fluorescence ☆

M. Sanchez Pena [a], M.J. Acedo [a], F. Salinas [a], M.C. Mahedero [a], J.J. Aaron [b,*]

[a] *Department of Analytical Chemistry, Faculty of Sciences, University of Extramadura, 06071 Badajoz, Spain*
[b] *Institut de Topologie et de Dynamique des Systèmes de l'Université Paris 7-Denis Diderot, associé au CNRS (URA 34), 1, rue Guy de la Brosse, 75005 Paris, France*

Received for review 13 February 1995

Abstract

A first-derivative photochemically-induced fluorescence method is proposed for the determination of sulfamethazine (SMTZ) in the presence of sulfamerazine (SMRZ) or sulfadiazine (SDZ). Linear calibration plots were obtained for SMTZ in SMTZ–SMRZ or SMTZ–SDZ binary mixtures with correlation coefficients larger than 0.999. For SMTZ the lower limits of detection were 7–13 ppb. Recoveries of 91–114% were obtained in the analysis of SMTZ in pharmaceutical formulations.

Keywords: Photochemically induced fluorescence; First-derivative spectroscopy; Sulfamethazine; Sulfadiazine

1. Introduction

Sulfonamides constitute a class of drugs which are frequently used in pharmaceutical preparations, especially in veterinary practice. As a consequence, residues of these drugs and their possible metabolites may remain in food of animal origin [1–5].

Several methods have been developed for the determination of sulfonamides. Ultraviolet (UV) visible spectrometric methods are the most widely used [6–14]. The fluorescence of several sulfonamides has also been investigated [15] and fluorimetric methods based on chemical derivatization have been proposed for their analysis [16,17].

However, N-1-substituted sulfonamides containing a π-electron deficient heterocycle are weakly or non-fluorescent. It has been shown in these laboratories that the fluorescence emission of these photoreactive sulfonamides can be photochemically induced by UV irradiation in aqueous solution [18,19]. Room-temperature photochemically induced fluorescence (RTPF) has been proved to be a rapid and sensitive method for the determination of these compounds [18–21]. In addition, RTPF has been coupled to a flow-injection analysis (FIA) manifold for the semi-automatic, routine determination of several sulfonamides [22].

Sulfadiazine (SDZ), sulfamerazine (SMRZ) and sulfamethazine (sulfadimidine) (SMTZ) are the pyrimidin-2-yl sulfamide and the 4-methyl and 4,6-dimethyl derivatives, respectively. They are structurally and pharmacologically very similar. Mixtures of two or three of these

☆ Presented in part at the Vth International Symposium on Quantitative Luminescence Spectrometry in Biomedical Sciences, Ghent, Belgium, May 1993.

* Corresponding author.

SSDI 0731-7085(95)01555-8

Table 1
Analytical figures of merit for the first-derivative RTPF determination of SMTZ, SMRZ and SDZ

Compound	Concentration range (ppm)	Regression equation [a]	Correlation coefficient	LOD [b] (ppb)	LOQ [c] (ppb)
SMTZ	0.05 3.0	$^1D_{325.5} = 81.26c - 1.21$	0.999	16	53
		$^1D_{368} = 36.08c - 1.38$	0.999	9	31
SMRZ	0.04 0.4	$^1D_{331} = 49.86c + 0.93$	0.998	12	42
SDZ	0.3 - 1.0	$^1D_{325} = 12.09c + 0.74$	0.994	107	356

[a] ^1D = relative first-derivative RTPF signal at a particular analytical wavelength; c = analyte concentration.
[b] LOD = lower limit of detection, defined as the concentration of solution giving a signal-to-noise (S/N) ratio of 3.
[c] LOQ = lower limit of quantification, defined as the concentration of solution giving a S/N ratio of 10.

sulfonamides are prescribed to avoid alterations of renal function by formation of microcrystals because of the low solubility of these compounds. The reason is that each compound administered as a third part of the total dose acts independently with respect to its solubility. The similarity of their UV spectra and chemically derivatized or photochemically induced fluorescence spectra prevents the utilization of these spectroscopic methods for determining these compounds in mixtures. The application of HPLC is recommended for this purpose. For example, mixtures of SMTZ and SMRZ have been analyzed in meat by HPLC using the fluorescamine reaction [23], and SMTZ, SMRZ and SDZ have been determined by HPLC with spectrophotometric detection [24]. RTPF has been applied to the determination of SMTZ, SMRZ and SDZ one-component samples in aqueous media [18], and the determination of SMTZ alone in pharmaceutical formulations and food [20,22]. However, as already mentioned, the use of the latter technique for the resolution of binary mixtures is not feasible because of its lack of spectral selectivity. More sophisticated, multivariate statistical methods, such as the partial least-squares (PLS) method, combined with RTPF have recently been proposed for this purpose [25].

In this paper, first-derivative room-temperature photochemically induced fluorescence is presented as an alternative approach for the quantitative analysis of SMTZ in binary mixtures with either SMRZ or SDZ, and in pharmaceutical preparations.

2. Experimental

2.1. Apparatus

Fluorescence spectral measurements were performed on Perkin-Elmer model LS-5 and Kontron model SFM-25 spectrophotofluorimeters, interfaced with a Samsung microcomputer for acquisition of the fluorescence spectra. For the treatment of the spectral data, a Beckman Data Leader Software (Version 3.0) [26] was used; a program was developed in Basic in order to convert the SFM-25 spectra file to the BSF file format through the X.Y ASCII converter included in the software. An Osram 200 W high-pressure mercury arc lamp with an Oriel Model 8500 power supply was utilized for photolysis. The RTPF experimental set-up was as described previously [18,19].

2.2. Chemicals and reagents

Stock solutions of SMTZ, SMRZ and SDZ (10^{-3} M) were prepared from the corresponding compounds or the sodium salts (Sigma) by dissolving in ethanol (Aldrich, analytical reagent grade) or de-ionized water. Serial dilutions were prepared by diluting stock solutions with deionized water. Pharmaceutical formulations of sulfonamides were a gift from various manufacturers: Sulphamerazine 33% Noé, from Lab. Noé-Socopharm (Chateau-Thierry, France); Antrima from Doms-Adrian (Courbevoie, France); Belcosulfa from Rhône-Mérieux (Lyon, France).

2.3. Procedure

General procedure

An aliquot of each sample solution (containing SMTZ, SMRZ or SDZ at a concentration within the range given in Table 1) was placed in a quartz cuvette and irradiated at room temperature with UV light for 12 min. The fluorescence emission spectra of irradiated samples were recorded between 300 and 450 nm, using an excitation wavelength of 284 nm. The first derivative spectra were obtained with a

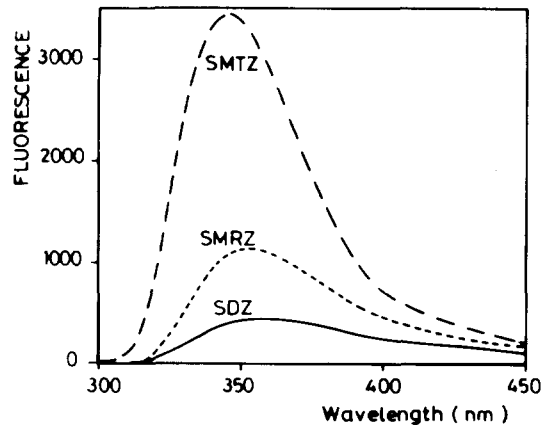

Fig. 1. Photochemically induced fluorescence emission spectra of SMTZ (1.0 ppm), SMRZ (0.45 ppm) and SDZ (0.6 ppm) in water.

bandwidth ($\Delta\lambda$) of 19 nm for SMTZ–SMRZ, and 13 nm for SMTZ–SDZ mixtures, according to the Savitzky and Golay method [27]. The SMTZ content in binary mixtures was determined by measuring the first-derivative photochemically induced fluorescence signals at 354.5 nm for SMTZ–SMRZ and 315 or 360 nm for SMTZ–SDZ mixtures.

Pharmaceutical formulations

For Belcosulfa (composition per tablet: colistine sulfate 10^6 IU and sulfamethazine 2 g) and Antrima formulations (composition per tablet: sulfadiazine 400 mg and trimethoprim 80 mg), one tablet was powdered, dissolved in 250 ml of ethanol–water (50:50, v/v) and diluted to the required concentration with deionized water. For the Noé preparation, the liquid sample was diluted 1000 times with deionized water. All sulfonamide concentrations of diluted solutions were within the range given in Table 1.

3. Results and discussion

The effect of different variables (excitation and emission wavelengths, irradiation time, solvent) on the photochemically induced fluorescence intensity of SMTZ, SMRZ and SDZ has been investigated in previous papers [18,20,25]. These results enabled a validation procedure for the present first-derivative RTPF method to be established. According to this approach, optimal UV-irradiation times of SMTZ, SMRZ and SDZ in an aqueous medium were 10, 12 and 18 min, respectively [18]. For the present determination of SMTZ in binary mixtures with SMRZ or SDZ, an irradiation time of 12 min was selected as a compromise value [25]. A photochemically induced fluorescence excitation wavelength of 284 nm was chosen, corresponding approximately to the excitation maximum for the three compounds under study.

The photochemically induced fluorescence emission spectra of SMTZ, SMRZ and SDZ solutions presented only one band, with maxima located at 345.5, 353 and 358 nm, respectively (Fig. 1). As already mentioned, the strong overlapping of these zero-order spectra requires the use of a derivative spectroscopic approach for determining SMTZ in the presence of SMRZ or SDZ.

3.1. First-derivative photochemically induced fluorescence spectra

In order to obtain first-derivative photochemically induced fluorescence emission spectra, it was necessary to optimize the bandwidth ($\Delta\lambda$) used in the differentiation of the RTPF spectra of SMTZ, SMRZ, SDZ and their binary mixtures. The $\Delta\lambda$ values assayed were 7, 9, 11, 13, 15, 17 and 19 nm. Optimal values of $\Delta\lambda = 19$ and 13 nm, respectively, were selected as giving the best spectral resolution for SMTZ–SMRZ and SMTZ–SDZ binary mixtures.

First-derivative RTPF emission spectra of SMTZ, SMRZ, SDZ, SMTZ–SMRZ and SMTZ–SDZ mixtures, recorded after optimization of spectral parameters, are shown in Figs. 2 and 3. It can be seen that first-derivative spectra exhibit maxima at 325.5, 331 and 325 nm for SMTZ, SMRZ and SDZ, respectively, and a minimum at 368 nm for SMTZ. No significant minimum could be found for SMRZ and SDZ.

Calibration graphs were constructed by plotting the amplitude of the signals at 325.5 ($^1D_{325.5}$) and 368 nm ($^1D_{368}$) for SMTZ, 331 nm ($^1D_{331}$) for SMRZ and 325 nm ($^1D_{325}$) for SDZ against the respective sulfonamide concentration. Linear relationships were established over a concentration range of one to two orders of magnitude. The analytical figures of merit are given in Table 1. The correlation coefficients were very close to unity, indicating that the precision of analytical curves is excellent. The limits of detection (LODs)

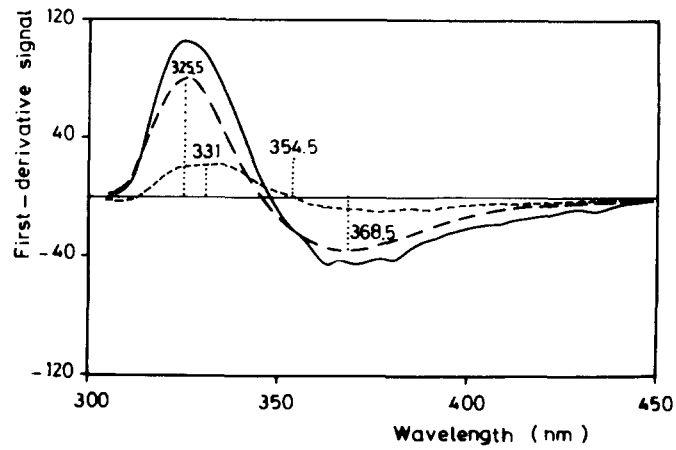

Fig. 2. First-derivative photochemically induced fluorescence spectra of SMTZ (1.0 ppm) (– – –), SMRZ (0.45) (---), and SMTZ–SMRZ (1–0.45 ppm) (——).

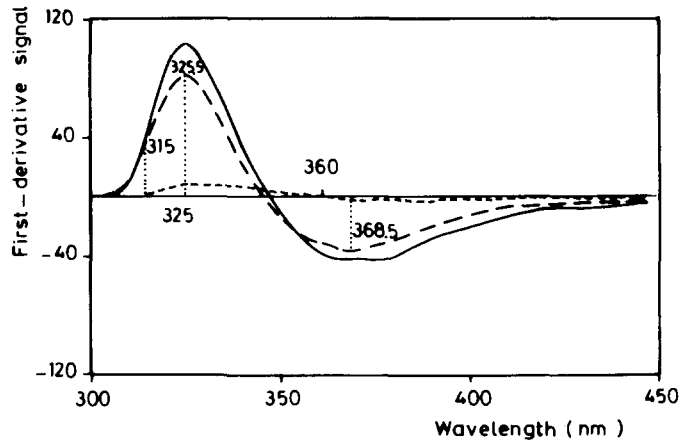

Fig. 3. First-derivative photochemically induced fluorescence spectra of SMTZ (1.0 ppm) (– – –), SDZ (0.6 ppm) (---), and SMTZ–SDZ (——) (1.0–0.6 ppm).

Table 2
Analytical figures of merit for the first-derivative RTPF determination of SMTZ in binary mixtures with SMRZ or SDZ

Mixture	Regression equation [a]	Correlation coefficient	LOD [b] (ppb)	LOQ [c] (ppb)
SMTZ–SMRZ	$^1D_{354.5} = 22.63c + 1.68$	0.999	13	43
SMTZ–SDZ	$^1D_{315} = 25.04c + 0.34$	0.999	41	139
SMTZ–SDZ	$^1D_{360} = 31.89c + 0.65$	0.999	7	24

[a] See footnote to Table 1.
[b] LOD = lower limit of detection; see definition in Table 1.
[c] LOQ = lower limit of quantification; see definition in Table 1.

were low, ranging between 9 and 107 ppb, according to the compound.

3.2. First-derivative RTPF spectra of binary mixtures

The first-derivative RTPF emission spectra of SMTZ–SMRZ and SMTZ–SDZ mixtures are also presented in Figs 2 and 3. Comparison of the first-derivative spectra of the three sulfonamides indicates that there are several wavelengths at which the first-derivative signals of SMRZ and SDZ are zero (354.5 nm for SMRZ; 315 and 360 nm for SDZ), whereas that of SMTZ has a relatively high value at these wavelengths. This feature allows the de-

termination of SMTZ in the presence of one of the other compounds at these particular wavelengths. It is also worthwhile to note that the first-derivative signal of SMTZ is zero at 345.5 nm but, at this same wavelength, the respective signals of SMRZ and SDZ are very weak, preventing the detection of the latter compounds in the presence of SMTZ.

3.3. Analytical figures of merit for binary mixtures

The analytical figures of merit for the determination of SMTZ in binary mixtures are summarized in Table 2. Calibration graphs were obtained by plotting the amplitude of the signal at 354.5 nm ($^1D_{354.5}$) for SMTZ–SMRZ mixtures, and at 315 and 360 nm ($^1D_{315}$ and $^1D_{360}$) for SMTZ–SDZ mixtures against the SMTZ concentration. The precision of the analytical curves is excellent, as shown by the correlation coefficients that are close to unity. LOD and limit of quantification (LOQ) values obtained for SMTZ in binary mixtures were close to those obtained when determining SMTZ alone. For SMTZ–SDZ mixtures, LOD and LOQ values for SMTZ were significantly lower when the derivative signal was measured at 360 nm than when the signal was measured at 315 nm.

In order to establish the range of applicability of the proposed approach, several synthetic binary mixtures containing SMTZ and SMRZ or SDZ in different proportions were prepared and analyzed. The results are presented in Table 3. It can be seen that the recoveries of SMTZ in the presence of SMRZ or SDZ are satisfactory, indicating that no significant interference occurs in the determination of SMTZ in binary mixtures.

Table 3
Determination of SMTZ in various binary mixtures containing SMRZ or SDZ

SMTZ–SMRZ mixtures[a]

SMTZ added (ppm)	Found (ppm)	SMRZ added (ppm)	Recovery (%)
0.5	0.55	0.20	110
1.0	0.96	0.20	96
1.0	0.98	0.45	98
1.0	1.04	0.35	104
1.0	1.10	0.30	110
1.0	0.98	0.25	98
1.0	1.06	0.15	106
2.0	1.99	0.20	100
2.5	2.27	0.20	91

SMTZ–SDZ mixtures[b]

SMTZ added (ppm)	Found (ppm)	SMRZ added (ppm)	Recovery (%)
0.5	0.57	1.0	114
1.0	1.06	0.3	106
1.0	1.10	0.4	110
1.0	1.07	1.0	107
1.5	1.38	0.4	92
2.0	1.87	0.4	93
2.5	2.38	0.4	95

[a] First-derivative RTPF signals were measured at λ_{em} = 354.5 nm.
[b] First-derivative RTPF signals were measured at λ_{em} = 360 nm.

3.4. Analytical applications

The first-derivative RTPF method was applied to the analysis of SMTZ in the presence of SMRZ or SDZ in pharmaceutical formulations. First-derivative signals were measured at 354.5 and 315 nm, respectively. The standard addition and the direct measurement procedures were used (Table 4).

Satisfactory results were obtained by the standard addition procedure, with recoveries of 104–115% and 94–97.5%, respectively, for pharmaceutical formulations containing SMTZ with SMRZ and with SDZ.

The recoveries obtained with the direct measurement procedure were 101 and 107.5%, respectively, for the same pharmaceutical formulations (Table 4). This demonstrates that the first-derivative RTPF approach is not affected significantly by the pharmaceutical matrix. No significant interference due to the presence of trimethoprim in Antrima tablets was observed.

4. Conclusions

It has been shown for the samples examined that first-derivative photochemically induced fluorescence at room temperature can be applied satisfactorily to the determination of SMTZ in the presence of SMRZ or SDZ in pharmaceutical formulations without significant interference. Moreover, this method is simple, precise and inexpensive and does not need any complex pretreatment or chromatographic separation of samples containing these sulfonamides.

Table 4
Recovery of SMTZ in pharmaceutical formulations by first-derivative RTPF

Mixture	SMTZ [a]		
	Added (ppm)	Found (ppm)	Recovery (%)
Standard addition procedure			
SMTZ–SMRZ [b]	–	0.267	–
	0.10	0.424	115.5
	0.15	0.433	103.8
	0.20	0.514	110.1
SMTZ–SDZ [c]	–	0.219	–
	0.10	0.311	97.5
	0.20	0.393	93.8
	0.25	0.446	95.1
Direct measurement procedure			
SMTZ–SMRZ [b]	33.0 [d]	33.39 [d] (8.9%) [e]	101.0
SMTZ-SDZ [c]	0.2	0.215 (14.9%) [e]	107.5

[a] SMTZ concentration in ppm, unless otherwise stated.
[b] Composition: 0.2 ml Belcosulfa diluted solution +0.2 ml Noé diluted solution. First-derivative RTPF signals were measured at $\lambda_{em} = 354.5$.
[c] Composition: 0.4 ml Antrima diluted solution +2 ml of a 2.5 ppm SMTZ solution. First-derivative RTPF signals were measured at 315 nm.
[d] SMTZ concentration in g/100 ml.
[e] Relative standard deviation (%) of three to five determinations.

Acknowledgments

M. Sanchez Pena is grateful to the Spanish Government (Ministerio de Educacion y Ciencia) for a FPPI Scholarship. This work was supported by Spanish–French integrated action (HF-41) of the Spanish and French governments.

References

[1] T. Galeano Diaz, A. Guiberteau Cabanillas and F. Salinas, Anal. Lett., 23 (1990) 607–616.
[2] C.W. Sigel, J.L. Wools and C.A. Nichol, J. Pharm. Sci., 64 (1975) 973–976.
[3] J. Unruh, E. Piotowski, D.P. Schwartz and R. Barford, J. Chromatogr., 519 (1990) 179–187.
[4] M. Rychener, A.E. Mooser and H. Koch-Mitt, Geb. Lebensmittelunters. Hyg., 81 (1990) 522–543.
[5] I.M. Barwick, P. Warwick and N.T. Crosby, Anal. Proc., 30 (1993) 233–234.
[6] A.C. Bratton Marshall, J. Biol. Chem., 128 (1939) 537–550.
[7] F. Salinas, A. Espinosa Mansilla and J.J. Berzas Nevado, Anal. Chim. Acta, 233 (1990) 289–294.
[8] J. Rieder, Chemotherapy (Basel), 17 (1972) 1–21.
[9] A.G. Xanakis and M.I. Karayannis, Anal. Chim. Acta, 159 (1984) 343–347.
[10] H.B. Falk and R.G. Kelly, Clin. Chem., 11 (1965) 1045–1050.
[11] A. Bye and A.F.J. Fox, Clin. Chem., 20 (1974) 288–293.
[12] M.E. Abdellatef, M.N. Elbakiny and A.A. Boulkeir, J. Pharm. Biomed. Anal., 7 (1989) 571–576.
[13] S. Othman, Int. J. Pharm., 63 (1990) 173–176.
[14] K.K. Verma and K.K. Steward, Anal. Chim. Acta, 214 (1988) 207–216.
[15] J.W. Bridges, L.A. Gifford, W.P. Hayes, J.N. Miller and D. Thorburn Burns, Anal. Chem., 46 (1974) 1010–1017.
[16] T. Sakano and T. Amano, Yakugaku Zasshi, 96 (1976) 1114–1121.
[17] J. Arthur, F. de Silva and N. Strojny, Anal. Chem., 47 (1975) 714–718.
[18] M.C. Mahedero and J.J. Aaron, Analusis, 20 (1992) 53–57.
[19] J.J. Aaron, in S.G. Schulman (Ed.), Molecular Luminescence Spectroscopy, Methods and Applications, Part 3, Wiley, New York, 1993, pp. 85–131.
[20] M. Sanchez Peña, F. Salinas, M.C. Mahedero and J.J. Aaron, Talanta, 41 (1994) 233–236.
[21] M. Sanchez Peña, F. Salinas, M.C. Mahedero and J.J. Aaron, J. Pharm. Biomed. Anal., 10 (1992) 805–808.
[22] M.C. Mahedero and J.J. Aaron, Anal. Chim. Acta, 269 (1992) 193–198.
[23] N. Takeda and Y. Akiyama, J. Chromatogr., 558 (1991) 175–180.
[24] A-E.H.N. Ahmed and S.M. El-Gizawy, Analyst (London), 114 (1989) 571–573.
[25] M. Sanchez Peña, A. Muñoz de la Peña, F. Salinas, M.C. Mahedero and J.J. Aaron, Analyst, 119 (1994) 1177–1181.
[26] Data Leader Software Pakage, Beckman Instruments Inc., Fullterton, CA, 1987.
[27] A. Savitzky and M. Golay, Anal. Chem., 36 (1964) 1627–1639.