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Spectrofluorimetric determination of sulphonamides in pharmaceutical compounds and foods*

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Abstract: Spectrofluorimetry and room temperature photochemically-induced fluorescence (RTPF) have been applied to the determination of sulphacetamide (SAC), sulphaguanidine (SG) and sulphamethazine (SMT) in milk and pharmaceutical formulations. The methods are suitable for determining 0.02–0.10 μg ml of SAC, 0.10–0.50 μg ml of SG, and 0.40–1.00 μg ml of SMT.

Keywords: *Spectrofluorimetry; photochemical-fluorimetry; sulphonamides.*

Introduction

Sulphonamides are currently used in pharmaceutical preparations [1] because of their antibacterial properties. They are extensively utilized in veterinary practice with the risk of the presence of residue contaminating food products [2–5]. Therefore, it is of importance to be able to determine the total content of sulphonamides in pharmaceuticals as well as in veterinary feeds.

Several methods have been proposed for the determination of sulphonamides. Molecular spectroscopic methods are the most frequently used. Photometric methods, alone or in combination with derivative techniques, based on the Bratton–Marshall reaction, have been applied in clinical chemistry, and semiautomated by implementation as a detection system in a flow injection analysis manifold [6–15].

The fluorescence of different sulphonamides has been studied [16], and proposed for the determination of several of these compounds. Thus sulphanilamide can be determined by reaction with homophthalaldehyde [17]. The analysis of sulphadiazine [18], sulphafurazol and sulphanilamide [19] has been performed in foods and pharmaceuticals, using the fluorescamine reaction. The reaction of 9-chloroacridine with sulphonamides produces a fluorescence quenching which allows the determination of sulphonamides [20].

HPLC with photometric detection has been applied for determining sulphonamides in serum and urine [21], foods [22–29], and pharmaceutical compounds [30–32]. Fluorescence has also been used as HPLC detection for determining sulphacetamide and sulphaguanidine in milk and eggs [33], and sulphadiazine, sulphapyridine, sulphamerazine and sulphametazine in meat [34].

The photochemical decomposition of sulphonamides has been investigated [35, 36]. Very recently, we developed a room temperature photochemically-induced fluorescence (RTPF) method for determining sulphamethazine, sulphamerazine, sulphadiazine, and sulphapyridine in aqueous medium [1]. Irradiation times were less than 30 min, and concentrations of 0.25–3.0 μg ml^{-1} could be measured.

In this paper, we applied RTPF to the determination of sulphamethazine (SMT) in milk and pharmaceutical preparations, and spectrofluorimetry to that of sulphacetamide (SAC) and sulphaguanidine (SG) in the same samples.

Experimental

Apparatus

A Perkin–Elmer model LS-5 spectrofluorometer was used for the fluorescence measurements (Perkin–Elmer, Norwark, CT, USA). An Osram 200-W mercury arc lamp with an

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Oriel Model 8500 power supply was utilized for the photolysis of SMT (Oriel, Stratford, CT, USA). The RTPF experimental set-up was as described previously [37].

Reagents

Stock solutions of SMT, SAC and SG (10^{-3} M) were prepared from these compounds (Sigma, St Louis, MO, USA) by dissolviing them in ethanol (Aldrich, Milwaukee, WI, USA, analytical reagent grade). More diluted solutions were prepared by diluting stock solutions with deionized water. The pharmaceutical formulations of sulphonamides were given by various manufacturers (Antebor, Lab. Biologiques Ile-de-France, Paris; Canidiarix, Thérapeutique Vétérinaire Moderne, Lempdes, France; and Sulphadimerazine 33% Noé, lab. Noé-Socopharm, Château-Thierry, France).

Procedure

For the RTPF method, an aliquot of each SMT sample solution was placed in a quartz cuvette and irradiated at room temperature with the ultraviolet (UV) light of a mercury arc lamp for a fixed time. Fluorescence intensity measurements were performed at constant excitation and emission wavelengths (see Table 1), using an optimal irradiation time of 10 min for SMT [1]. No UV irradiation was needed for SAC and SG. Linear calibration curves were established using either the photochemically-induced fluorescence intensity (for SMT) or the native fluorescence intensity (for SAC and SG) at analytical excitation and emission wavelengths (Table 1) [1].

For determining sulphonamides in milk, samples were fortified with SAC or SF, followed by dilution with deionized water at 1:1000 in convenient calibrated flasks.

For determining SMT, SAC and SG in pharmaceuticals, liquid formulations were diluted with deionized water in order to obtain a concentration within the range given in Table 1. Pharmaceutical tablets were powdered, then dissolved in ethanol–water (50:50, v/v), and diluted with deionized water at convenient concentrations (Table 1).

Table 1
Experimental conditions for determination of sulphonamides

Compound	$\lambda_{ex}/\lambda_{em}$* (nm)	t_{irr}^{opt}† (min)	Application‡ interval (ppm)
SAC	260/345	—	0.003–0.09
SG	260/348	—	0.210–0.85
SMT	284/350	10	0.300–3.00

*λ_{ex} and λ_{em} = analytical excitation and emission wavelengths.
†t_{irr}^{opt} = optimal irradiation time corresponding to the maximum fluorescence signal.
‡Concentration range analytically useful.

Results and Discussion

Determination of sulphonamides in milk

SAC and SG were determined in milk, using a method previously described [1]. Table 2 presents the results obtained for several SAC and SG concentrations by the standard addition procedure. As can be seen the mean recoveries (R) ranged from 90 to 102%, which shows that the spectrofluorimetric method is suitable for these sample concentrations.

In the case of SAC, the regression equations (1) and (2) were found for the linear calibration and standard addition curves, respectively:

$$I_F = 481.5c + 0.86 \quad (1)$$

$$I_F = 487.0c + 20.0, \quad (2)$$

with I_F and c, fluorescence intensity and concentration of sulphonamide, respectively.

In the case of SG, the corresponding regression equations (3) and (4) were obtained, for calibration and standard addition curves, respectively.

$$I_F = 82.7c + 4.9 \quad (3)$$

$$I_F = 88.7c + 1.8. \quad (4)$$

Table 2
Determination of sulphacetamide and sulphaguanidine in milk

Sulphamide	Added (ppm)	Found (ppm)	%R*
	—	0.052	—
	0.02	0.054	100
SAC	0.04	0.094	102
	0.08	0.129	98
	0.10	0.150	99
	—	—	—
	0.20	0.18	90
SG	0.40	0.40	100
	0.60	0.60	100
	0.80	0.82	102

*R = recoveries values, measured using the standard addition procedure.

In both cases, strictly parallel straight lines were found, with correlation coefficients larger than 0.998.

Interferences

Because of the possible simultaneous presence of other sulphonamides in milk, we evaluated the effect of selected sulphonamides (acting as foreign species) on the spectrofluorimetric determination of SAC and SG (Table 3). The measurements were performed by adding specific amounts of interferent to definite concentrations of analyte. The tolerated interference level is defined as the concentration (in $\mu g\ ml^{-1}$) of interferent at which the measured fluorescence signal variation is ±5%. Our results show that the strongest interferences (corresponding to the lowest tolerated levels) are due to the non-heterocyclic sulphonamides such as sulphacetamide and sulphanilamide, and to sulphapyridine.

Table 3
Interferences with sulphacetamide and sulphaguanidine determination in milk

Interferent compound	Interference level (ppm)*	
	SAC	SG
SMT	1.0	0.6
SMR	0.6	0.6
SDZ	1.0	0.6
SPY	0.6	<0.4
SAC	—	<0.4
SAN	<0.2	<0.4
SG	<0.2	—

[SAC] = 0.20 ppm; [SG] = 0.40 ppm. SPY = sulphapyridine; SMR = sulphamerazine; SDZ = sulphadiazine; SAN = sulphanilamide.
*Defined as the concentration of foreign species at which the variation of measured fluorescence signal is larger than ±5%.

Analysis of pharmaceuticals

SMT, SAC and SG were determined in several pharmaceutical formulations, by means of RTPF or spectrofluorimetry, and using the standard addition procedure. The results are given in Table 4 for several concentrations of these sulphonamides. The mean recoveries (R) ranged from 99.0 to 104%.

For all pharmaceuticals under study, we found parallel, linear calibration and standard addition curves, with slopes equal to 714.0 and 731.0 for SAC, 73.9 and 74.6 for SG, and 20.3 and 20.5 for SMT, respectively. In all cases, correlation coefficients were larger than 0.998.

Table 4
Determination of sulphacetamide, sulphaguanidine and sulphametazine in pharmaceutical compounds

Sulphamide	Added (ppm)	Found (ppm)	%R*
	—	0.027	—
	0.020	0.049	104
SAC†	0.040	0.066	99.9
	0.060	0.089	102
	0.070	0.100	103
	—	0.41	—
	0.10	0.51	100
SG‡	0.20	0.62	101
	0.30	0.71	100
	0.50	0.92	101
	—	2.62	—
	0.40	3.05	101
SMT§	0.60	3.21	99.7
	0.80	3.41	99.7
	1.00	3.65	99.0

*R = recoveries values, measured using the standard addition procedure.
†In Antebor formulation.
‡In Canidiarix formulation.
§In Sulphadimerazine 33% Noé formulation.

Conclusion

The proposed RTPF and spectrofluorimetric methods can be applied to the determination of sulphonamides in milk and pharmaceutical formulations without significant interference of the other constituents present in the samples studied. Moreover, these methods are simple, precise and inexpensive, and they do not need any complex pretreatment of the biological or pharmaceutical samples containing the sulphonamides.

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