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Short communication

Determination of sulphathiazole and sulphanilamide by photochemically induced fluorescence and first-derivative fluorescence

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Abstract

This manuscript reports the usefulness of the determination of sulphathiazole (ST) using photochemically induced fluorescence (RTPF) and RTPF coupling with first derivative (D^1-RTPF), and the determination of sulphanilamide (SAN) by meaning first derivative of the emission spectrum. By irradiating 5 min, with intense UV radiation, sulphathiazole, in ethanol:water 20:80 (v/v) solutions at pH 4.5–5.0, show fluorescence emission at 342 nm (λ_{ex} = 251 nm). Under these conditions, a linear relation, fluorescence intensity–ST concentration, was found between 0.23 and 3.00 μg mL^{-1} of ST. The method was applied for determining ST in a pharmaceutical drug. ST was also determined in honey by using the D^1-RTPF signal, applying the standard addition method, and measuring at 324.8 nm. Under the same experimental conditions of pH and solvent, a fluorimetric method for determining SAN in presence of ST is proposed. Calibration graphs for SAN determination were established using the amplitude of the first derivative of the emission spectrum measured at 324.4 nm, as the analytical signal. This method has been applied to determining SAN in a pharmaceutical formulation.

Keywords: Photochemically induced fluorescence; First-derivative; Sulphathiazole; Sulphanilamide; Honey; Pharmaceuticals

1. Introduction

Sulfonamides constitute an important and one of the oldest groups of anti-microbial compounds. They are registered as antibacterials that are widely used in pharmaceutical preparations, especially in veterinary practice. As a consequence, residues of these drugs may remain in foods of animal origin [1–3]. Therefore, it is very important for the quality control of sulfonamide formulations and their trace determination in foods.

Sulphanilamide is one of the first anti-infective agents to be prepared by synthetic medicinal chemists, and has been in use for over four decades. Currently, this sulfa drug is used in the treatment of vulvovaginitis caused by *Candida albicans*.

Sulphathiazole is widely used in veterinary practice for the treatment of various bacterial infections, e.g., in diseases of honey bees.

For determining sulphanilamide, chromatographic methods have the leading position, by using mass spectrometric detection [4,5], being applied in biological fluids [6], pharmaceutical preparations [6–9] and foods [10,11]. Another techniques employed are spectrophotometry [6,12,13] with flow-injection analysis (FIA) [14] and fluorescence [15] with flow-injection analysis [16].

In the case of sulphathiazole, a few spectrophotometric [17–23] methods have been developed for its analysis. Also, flow-injection analysis methods have been proposed [24]. HPLC techniques with different detection types as photometry, mass spectrometry and fluorimetry have been proposed [6,25–28], and fluorimetric methods [29,30].

For weakly fluorescent and non-fluorescent analytes, derivatization to convert the analyte into a more detectable product (i.e., better fluorescing) constitutes a means of enhancing the analyte detectability. In addition to chemical reactions, an alternative approach is to use a photochemical reaction. In this case, intense UV–vis radiation converts non- or weakly fluorescent analytes of interest into highly fluorescent

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products. It is called photochemically induced fluorescence (RTPF) detection.

A few years ago, the photochemical decomposition of sulfonamides was reported and photochemically induced fluorescence was shown to be a precise, selective, and sensitive analytical method for determining sulfonamides [31].

In this work, new methods for determining ST using photochemically induced fluorescence and RTPF coupling with first derivative (D^1-RTPF), and SAN by first derivative of the emission spectrum are proposed. They were applied in honey (ST) and pharmaceuticals (ST and SAN).

2. Experimental

All fluorescence spectral measurements were performed on a Perkin-Elmer model LS-50 B Spectrofluorimeter, interfaced with a Pentium PC microcomputer. The analysis, data manipulation, differentiation, and graphical representation of the fluorescence data was made by the software package FL WinLab V4.00 from Perkin-Elmer.

An Osram 200 W mercury lamp with an Oriel model 8500 power supply was used for the photolysis of ST.

For all experiments, analytical grade chemicals and solvents were used. Ultrapure water was obtained from a Millipore Milli-Q system.

Stock ethanolic solutions of 100 $\mu g\,mL^{-1}$ of ST and SAN (Sigma) were prepared, and standard solutions of ST and SAN were prepared by suitable dilutions.

Buffer solutions acetic acid/sodium acetate 0.5 M of pH 4.75 and 5.00 were prepared.

Pharmaceuticals: Sabañotropico (per 100 g: 0.005 g dexamethasone sodium phosphate, 5 g ST, 3.5 g menthol, 3 g titanic acid, 2.5 g basic galate of bismuth, 2 g phenol, 100 g vaseline c.s.p.); "POWDERS WILFE" (per 1 g: 0.697 g ST, 0.303 g SAN).

2.1. Procedure for determining ST in pharmaceutical formulations

A suitable amount of the formulation is accurately weighted, dissolved in ethanol until insoluble remains of vaseline (if the pharmaceutical formulation is in pomade form), filtered, washed with ethanol, and diluted to a known volume (100 mL). An aliquot of the obtained solution was placed in a 25-mL volumetric flask. Ethanol was added to obtain a final 20:80 (v/v) ethanol:water medium, 3 mL of 0.5 M acetic acid/sodium acetate buffer of pH 4.75 and MilliQ grade water to final volume were added

Samples were irradiated for 5 min, the emission spectra were recorded by using an excitation wavelength of 251 nm, and the fluorescence signals at an emission wavelength of 342 nm were measured.

The calibration graph was obtained with ST solutions of concentration between 0.23 and 3.00 $\mu g\,mL^{-1}$, treated the same way than the problem sample.

2.2. Procedure for determining ST in honey samples

Ten grams of honey fortified with ST was dissolved in 100 mL of MiliQ grade water. In a 25-mL volumetric flask the volume necessary to have a final concentration of ST between 0.23 and 0.80 $\mu g\,mL^{-1}$ was introduced. Ethanol was added to obtain a final 20:80 (v/v) ethanol:water medium, 3 mL of 0.5 M acetic acid/sodium acetate buffer of pH 4.75 and MilliQ grade water to final volume were added.

Samples were irradiated for 5 min, the emission spectra were recorded by using an excitation wavelength of 251 nm. Their first-derivative spectra were obtained, smoothing with 10 experimental points and with a $\Delta\lambda$ of 24 nm; and ST was determined by measuring the amplitude of the first-derivative photochemically induced fluorescence signal a 324.8 nm, by the standard addition method.

2.3. Procedure for determining SAN in pharmaceutical formulations in presence of ST

The pharmaceutical formulation "POWDERS WILFE" is presented in dots, which are not water soluble. A suitable amount of formulation (around 0.1 g) is accurately weighted and dissolved in ethanol to a known volume (100 mL). A dilution of 1:100 in ethanol is made. An aliquot of the sample is placed in a 25-mL volumetric flask in order to have concentrations of SAN between 0.09 and 0.80 $\mu g\,mL^{-1}$, if the concentration of ST is less than 1.00 $\mu g\,mL^{-1}$; or between 0.09 and 0.60 $\mu g\,mL^{-1}$, if ST is present between 1.00 and 2.00 $\mu g\,mL^{-1}$. Ethanol is added to obtain a final 20:80 (v/v) ethanol:water medium and 3 mL of 0.5 M acetic acid/sodium acetate buffer of pH 4.75 and deionised water to final volume are added.

Emission spectra of the samples were recorded, exciting at 262 nm. The first-derivative fluorescence spectra were obtained, smoothing with 11 experimental points and with a $\Delta\lambda$ of 25 nm, and SAN was determined by measurement of the amplitude of the first-derivative signal at 324.4 nm.

3. Results and discussion

3.1. Determination of ST by means of RTPF

The UV absorption spectra of ST in ethanol is characterized by two maxima at 260 and 290 nm, but its excited solution at these two wavelengths is not presenting fluorescence emission. Nevertheless, after irradiation, it is observed an induced fluorescence signal (Fig. 1).

The influence of irradiation time, by using different mixtures of ethanol:water, was studied. In all cases, the fluorescence intensity increases when increasing the irradiation time. For water percentages comprised between 50 and 100%, maxima fluorescence intensities were found for an irradiating time comprised between 4 and 5 min (Fig. 2). Less water percentages produce a smaller increase of the fluorescence

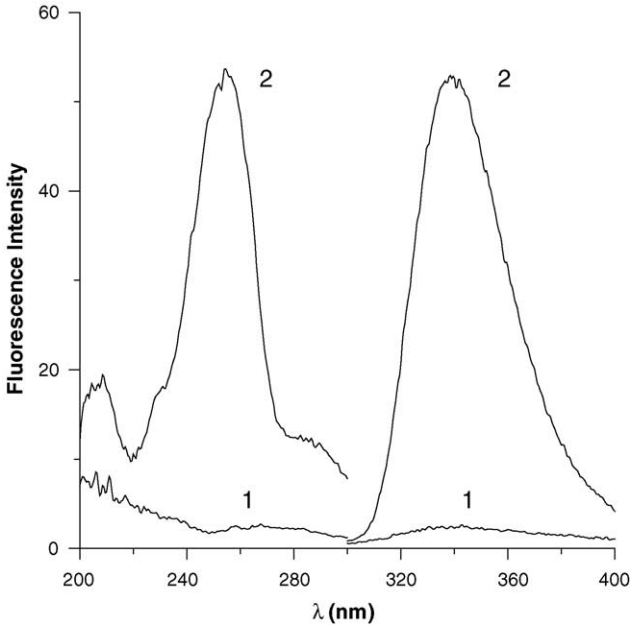

Fig. 1. Excitation and emission spectra of 5.00 μg mL^{-1} ST solution in ethanol:water 20:80 (v/v): (1) t_{irr} = 0 min; (2) t_{irr} = 4.5 min.

intensity. A 20:80 (v/v) ethanol:water medium and an irradiation time of 5 min were chosen as optima. Under these conditions, excitation and emission wavelengths of 251 and 342 nm, respectively, were found.

In Fig. 3, fluorescence intensity versus irradiation time, at several pH values, are shown. For strong acid and basic media, the increase of fluorescence intensity is small, but in a weakly acid medium (pH between 4.50 and 5.00), a maximum fluorescence intensity is obtained. Then, an apparent pH value of 4.75, obtained by addition of 0.5 M acetic acid/sodium acetate buffer solution, was selected. There is no influence of buffer concentration over fluorescence intensity.

The optimized selected conditions are: ethanol/water media (20:80, v/v), pH: 4.50–5.00, buffer HAc/NaAc 0.5 M, irradiation time: 5 min and $\lambda_{ex}/\lambda_{em}$ = 251/342 nm.

The stability of the photoproduct was studied by measuring the fluorescence intensity of 2.00 μg mL^{-1} of ST, after

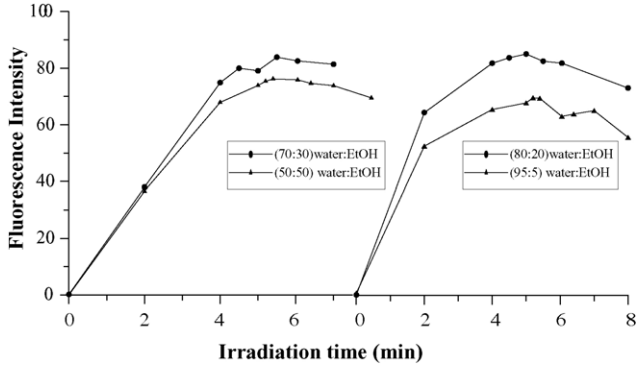

Fig. 2. Influence of irradiation time on the fluorescence intensity of 5.00 μg mL^{-1} ST in different mixtures of ethanol:water.

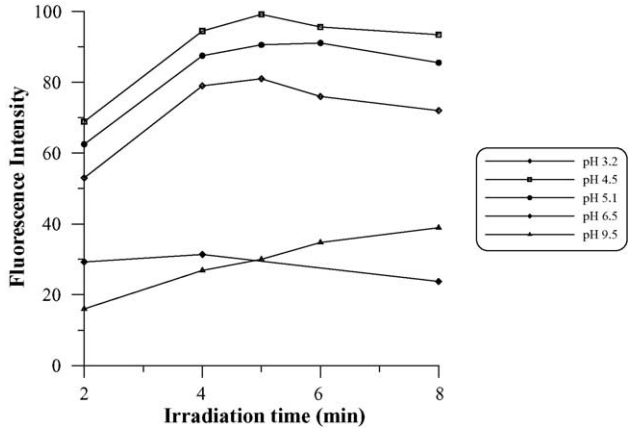

Fig. 3. Influence of irradiation time on the fluorescence intensity of ST in ethanol:water 20:80 (v/v) at several pH values; λ_{ex} = 251 nm.

irradiation of 5 min, with time. It was found that the photoproduct is stable at least 1 h. In the same way, it was found that the working solutions are stable at least 2 weeks.

Under these conditions, a linear relation fluorescence intensity–ST concentration was found. Using the fluorescence intensity after the irradiation process as the analytical signal, a calibration graph was obtained. The statistical calibration data and figures of merit of the method are: intercept ± S.D., 1.6 ± 0.2; slope ± S.D., 18.3 ± 0.1; analytical sensitivity (γ^{-1}), 0.03 μg mL^{-1}; limits of detection: Clayton's method ($\alpha = \beta = 0.05$) [32] and Winefordner–Long's method [33] 0.08 and 0.06 μg mL^{-1}, respectively.

3.1.1. Determination of ST in pharmaceutical formulations

The proposed method has been applied to the determination of ST in the pharmaceutical formulation Sabañotrópico. The procedure followed has been previously described, and the results obtained are good (Table 1) with recoveries near 100%. This pharmaceutical formulation was analysed by using an spectrophotometric reference method [20] and measuring the amplitude of the first derivative signal at 307 nm. A recovery of 95% and a relative standard deviation (R.S.D.) of 5%, were obtained.

3.2. Determination of ST by means of D^1-RTPF

In order to determine ST in foods, which in general present an appreciable background signal, the following step was the optimization of the instrumental conditions to obtain the

Table 1
ST determination in pharmaceutical formulation

Formulation weight (g)	ST* present (μg mL^{-1})	% Recovery
0.1229	2.45	96
0.1095	2.19	95
0.1016	2.03	93

* Concentrations calculated in accordance with the pharmaceutical amount indicated. Mean values (n = 3).

first derivative photochemically induced fluorescence emission spectra.

Taking into account the noise levels on the derivative spectra, a smoothing function, on the basis of the Savitzky–Golay method [34] was used. The zero-order spectra finally was smoothed by using 10 points.

In order to obtain the first-derivative photochemically induced fluorescence emission spectra, it was necessary to optimize the bandwidth ($\Delta\lambda$). The values assayed were between 14 and 24 nm, and a bandwidth of 24 nm was selected, because the derivative signal remains practically constant but the noise level decreases. This first derivative signal presents a maximum at 324.8 nm.

3.2.1. Determination of ST in honey

First of all, the matrix effect was studied, by preparing different samples of honey: by no addition of ST, by adding 1.00 $\mu g\,mL^{-1}$ and 2.00 $\mu g\,mL^{-1}$; and two standard solutions of ST 1.00 $\mu g\,mL^{-1}$ and 2.00 $\mu g\,mL^{-1}$ in order to compare the results obtained. For that reason, all the samples were irradiated 5 min, their emission spectra were registered, and after that, their first derivative spectra were obtained. The honey presents a wide band in all the wavelength interval, and in the case of the 2.00 $\mu g\,mL^{-1}$ samples, there is no addition of the signals with an inversion of fluorescence (then, the standard of 2.00 $\mu g\,mL^{-1}$ presents higher fluorescence signal in comparison with this concentration of ST in honey). It means that, in presence of high concentration of ST, part of it remains in honey. This behaviour is explained by Schwaiger and Schuch [30] due to the reaction between ST and glucose of honey.

In consequence, it is not possible to determine of ST in all its concentration range, by the standard addition method. The derivative spectra of honey show almost no background signal. It was found the possibility of its determination up to 0.80 $\mu g\,mL^{-1}$, then, it was obtained as a new calibration graph (by using D^1-RTPF as the analytical signal); the statistical calibration data and figures of merit of the method are: linear calibration range, 0.23–0.80 $\mu g\,mL^{1}$; intercept ± S.D., 0.2 ± 0.1; slope ± S.D., 19.9 ± 0.4; analytical sensitivity (γ^{-1}), 0.02 $\mu g\,mL^{-1}$; limits of detection: Clayton's method ($\alpha = \beta = 0.05$) and Winerfordner–Long's method 0.04 and 0.02 $\mu g\,mL^{-1}$, respectively.

The proposed method was applied to different types of honey by using the standard addition method, and three independent determinations in each case. Good results, as summarized in Table 2, were obtained.

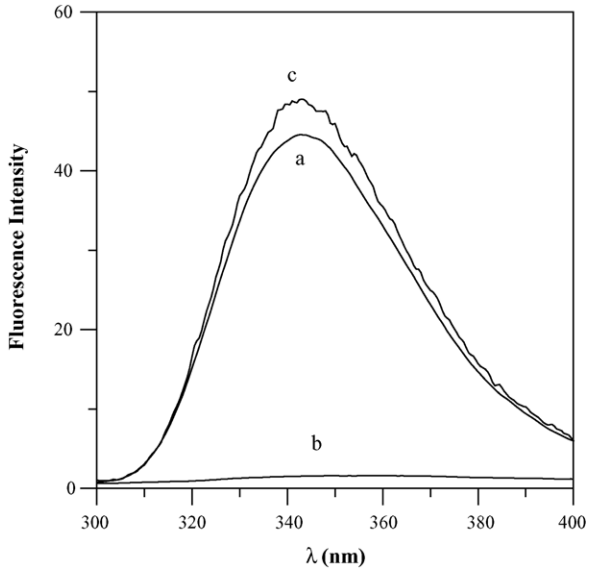

Fig. 4. Emission spectra: (a) 0.50 $\mu g\,mL^{-1}$ SAN; (b) 1.00 $\mu g\,mL^{-1}$ ST; (c) mixture.

3.3. Determination of SAN by D^1-fluorescence

Sulphanilamide shows a native fluorescence signal that decreases with the irradiation time, but sulphathiazole has not native fluorescence signal. Then, we try to propose a method for determining SAN in presence of ST.

The excitation and emission spectra of a SAN solution under the same experimental conditions, pH, and solvents, selected for ST is obtained. Maxima at 262 and 342 nm, respectively, were found.

A linear relationship between fluorescence intensity and SAN concentration up to 0.80 $\mu g\,mL^{-1}$ was found. By using synthetic samples of SAN, ST, and a mixture of both analytes, their fluorescence emission spectra were obtained. The fluorescence intensity measured at 342 nm shows a small signal of ST, but it is not possible to determine SAN in its presence (Fig. 4).

Then, the first derivative fluorescence spectra for the three samples were obtained, and it was observed that the ST signal

Table 2
Results obtained for ST determination by standard addition in honeys

ST^{added} (μg/g)	D^1-RTPF			
	% R[a] ± R.S.D.	% R[a] ± R.S.D.	% R[b] ± R.S.D.	% R[c] ± R.S.D.
10	112 ± 7	111 ± 4	112 ± 9	111 ± 7
30	103 ± 4	103 ± 6	91 ± 4	90 ± 4
50	91 ± 4	90 ± 4	91 ± 7	89 ± 3

[a] Spanish honey.
[b] Swiss honey.
[c] Belgian honey.

Table 3
Results obtained for SAN determination in a pharmaceutical formulation

Formulation weight (g)	SAN present ($\mu g\,mL^{-1}$)*	% Recovery
0.1049	0.13	104
	0.32	97
	0.51	93
0.0949	0.12	96
	0.29	98
	0.46	94
0.1013	0.12	99
	0.31	95
	0.49	90

* Mean values ($n = 3$).

is practically zero, and it is possible to determine SAN, by using the signal of the mixture.

The instrumental conditions were optimized to obtain the first derivative spectra, and it was found as optimum: smooth, 11 experimental points; $\Delta\lambda$, 25 nm; λ, 324.4 nm

A calibration graph for SAN determination was established, using the amplitude of the first-derivative fluorescence spectra measured at 324.4 nm without irradiation, as the analytical signal; the statistical calibration data and figures of merit of the method are: intercept ± S.D., 0.8 ± 0.3; slope ± S.D., 38.1 ± 0.6; analytical sensitivity (γ^{-1}), $0.01\,\mu g\,mL^{-1}$; limits of detection: Clayton's method ($\alpha = \beta = 0.05$) and Winefordner–Long's method 0.03 and $0.02\,\mu g\,mL^{-1}$, respectively.

After that, the possibility of determining SAN when ST is present, in concentrations between 0.22 and $3.00\,\mu g\,mL^{-1}$, was studied. Four series of solutions containing a fixed concentration of ST with values of 1.00, 1.50, 2.00, and $3.00\,\mu g\,mL^{-1}$, and increasing concentrations of SAN between 0.09 and $0.80\,\mu g\,mL^{-1}$ were prepared. After obtaining the first-derivative signals, the equations were compared with the equation obtained for SAN without presence of ST (their slopes). The results obtained show that the determination of SAN is possible between 0.09 and $0.80\,\mu g\,mL^{-1}$, if the concentration of ST is in the range between 0.22 and $1.00\,\mu g\,mL^{-1}$ and, if ST is present between 1.00 and $2.00\,\mu g\,mL^{-1}$, the determination of SAN is possible only in a shorter range of concentrations comprised between 0.09 and $0.60\,\mu g\,mL^{-1}$.

3.3.1. Determination of SAN in pharmaceutical formulations in presence of ST

Before applying the proposed method for determining SAN in presence of ST, the possible matrix effect was studied. For that purpose, the standard addition method was applied and no matrix effect was found. Then, the proposed method was directly applied.

The pharmaceutical formulation analysed was "POWDERS WILFE" following the procedure described previously, and recoveries near 100% were obtained as it is shown in Table 3.

4. Conclusions

It has been proposed as a method for determining sulphathiazole in pharmaceutical formulations by using photochemically induced fluorescence; and for its determination in honey by RTPF coupling with first derivative. In this case, a very weakly fluorescent analyte has been converted into a highly fluorescent product.

Besides that, the determination of sulphanilamide in pharmaceutical formulations in presence of sulphathiazole is proposed by using the first derivative of the emission spectrum as the analytical signal.

The proposed methods are simple and inexpensive, and do not need any complex pre-treatment of samples containing these sulfonamides. In comparison with other procedures existing in literature, the proposed methods are rapid, because it is not necessarily a derivatization; and the selectivity is good, taking into account the utilization of fluorescence signal, and even more, in the cases where we employ the enhancing fluorescence signal due to the use of the photochemical reaction.

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