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Spectrofluorimetric procedure for the determination of sulfanilamide in surface water

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Abstract
Monitoring biologically active substances have been an important one to know the impact of these substances on the environment. The development of techniques that seek to degrade or mitigate the toxicity of these substances has been of great interest. Thus, simple, low cost and accurate analytical procedures are essential for monitoring and controlling these techniques. In this work, the analytical procedure was proposed to determine the antibiotic sulfanilamide with spectrofluorimetric detection in river water samples. The procedure is based on radiation emission at 365 nm when excited at 255 nm, in acetic acid medium 0.10 mol/L. The procedure presented linearity between 1.14 to 11.2 μg mL^{-1} and a quantification limit of 1.4 μg mL. Sulfanilamide determination can be performed with a recovery ranging from 90.3 to 106%. The accuracy of determining a solution of 1.4 μg/l was 1.5% (n=10). The spectrofluorimetric procedure for determination and sulfanilamide is simple, fast (30 samples per hour), and does not require complex sample preparation.

Keywords: chemical analysis, emerging contaminants, environmental samples, molecular spectroscopy, validation

Introduction

Currently, drug residues in the environment are among the emerging pollutants that cause the most significant concern. Sulfanilamide, a bactericidal agent of the sulfonamide class, widely used in veterinary and hospital products, has been the subject of studies because there is evidence of increased bacterial resistance.

Its extensive use as an antibiotic is due to its action on bacteria acting in the folic acid metabolism cycle, acting as a competitive inhibitor of p-aminobenzoic acid [1-4].

Studies indicate the presence of sulfanilamide and other antibiotics in hospital sewers, water treatment plants, and lakes and rivers at concentrations in the range of μg/L. The reduction of concentrations of these antibiotics in effluents due to water and sewage treatment plants was more significant than 80% [3, 5].

The attenuation of the impact of contaminants on the environment has been evaluated using bioremediation processes. Sulfanilamide bioremediation was evaluated using acclimated bacteria. In this work, there was a reduction of about 79% of sulfanilamide concentration during one week of incubation [6-7].

The interaction of drugs with the natural organic matter has been evaluated as a strategy to attenuate effluent toxicity [8]. Sobrinho *et al*. (2021) developed a study involving the incorporation of sulfanilamide in natural organic matter. The authors found that natural organic matter can aid in the removal of sulfanilamide present in surface waters with an incorporation capacity of about 120 mg/g [9].

Several procedures have been described for the determination of sulfanilamide in environmental samples, such as molecular spectrophotometry [10], liquid chromatography [11-14], and capillary electrophoresis [15-16]. These analytical procedures have the advantages of being separation methods, resulting in multicomponent sample analyses, besides presenting low quantification limits. However, these methods require specialized analysts and intense sample preparation. Often the performance of this technique is superior to the need for the study, in which a technique that determines a single analysis accurately would supply the real need.

Spectro fluorimetry is an analytical technique based on the emission of energy in the form of radiation caused by the electronic transition of electron pairs excited with specific electromagnetic radiation. The technique has been used to determine species of environmental interest and characterization of organic matter of anthropogenic origin [17-18].

In this sense, it is essential to develop analytical procedures that allow the determination of chemical species of environmental interest that are simple and have the necessary accuracy to obtain the appropriate purpose. This work seeks to develop a spectrofluorimetric procedure for the determination of the antibiotic sulfanilamide in surface water samples.

Details Experimental

1. Materials and Procedures

The spectrofluorimetric procedure was developed using the spectrofluorometer Cary Eclipse (Agilent), with a Xe pulse lamp and photomultiplier as a detector. The emissions spectra were obtained using a quartz cuvette with 1.00 cm (optical path) and 0.70 mL of internal volume. The spectral bandwidth used was 5 nm for excitation and emission. The PMT voltage used in the analysis was set up as low gain.

2. Reagents and Solutions

The reagents used in this work were analytical grade. They were used without further purification. The sulphanilamide stock standard solution was prepared dissolving an appropriate mass in an aliquot of concentrated acetic acid in a 100 mL volumetric flask, and the volume was made deionized water. The concentration of acetic acid in this solution was 0.10 mol/L. The analytical curve was obtained using different solutions containing an aliquot of sulphanilamide stock standard solution. The concentration of SFN solution were: 1.14, 1.41, 1.67, 5.51, 8.78, 11.2 and 13.6 µg mL^{-1}.

The water samples were collected in an amber flask and stored in a refrigerator at 4°C. After sampling, the water samples were filtered into a polysulfone membrane filter with 0.45 µm.

3. Procedure for the Determination of SFN

A 500 µL aliquot of sample solution or a volume standard solution containing SFN was transferred to a 10.0 mL volumetric flask, and the volume was made with acetic acid 0.10 mol/L. The sample solutions containing acetic acid were centrifugated before the analysis. The solutions containing SFN were transferred to a cuvette, and the emission spectrum was obtained, varying the wavelength of excitation between 300 to 420 nm.

A plot of emission intensity of SFN standard solution in 365 nm against the concentration of standards solutions was used to obtain the analytical curve. A least-squares fit to a straight line of the obtained fluorescence intensity values versus SFN concentration was performed. The determination of SFN was done by extrapolating the emission intensity in an equation of the analytical curve obtained in the same way.

Results and Discussions

Sulfanilamide is a sulfonamide in which the sulfamoyl functional group is attached to aniline at the 4-position. The SFN presents a pKa of 10.5 [19]. An excitation matrix emission of a sulfanilamide solution was obtained and is presented in Fig.1. The emission excitation matrix (EEM) is a series of emission spectra obtained sequentially, varying the excitation wavelength. Thus, we obtain a graph in which one can verify the spectral region in which the highest intensity of electronic processes occurs in the sulfanilamide molecule. Fig. 1 finds the excitation of the SFN occurs between 220 and 280 nm. The EEM shows that the emission occurs between the wavelengths of 325 and 400 nm, with a maximum of 365 nm.

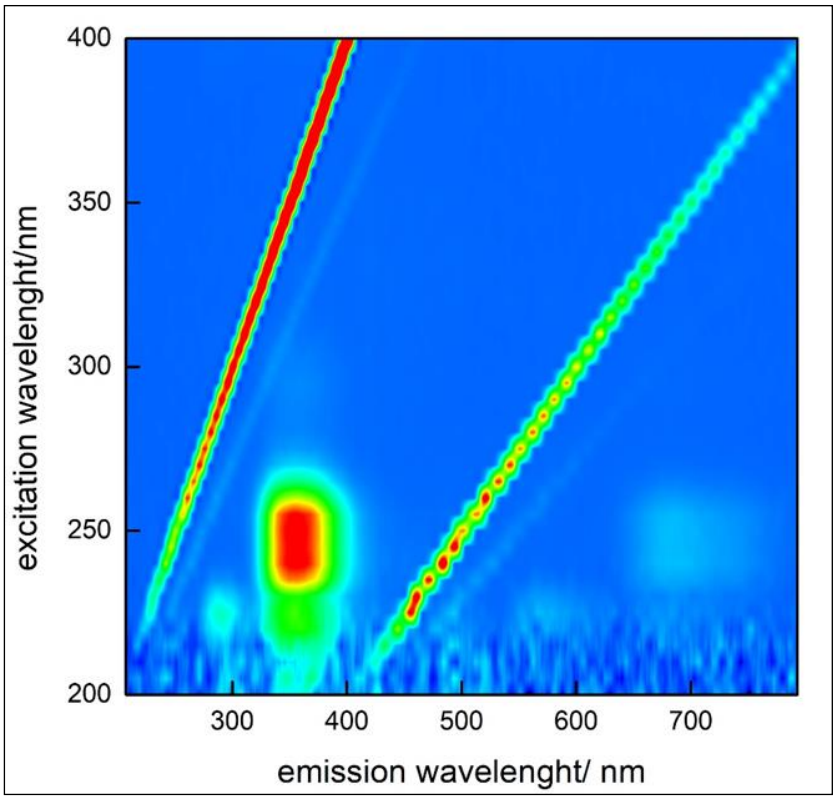

Fig 1: Excitation emission matrix of standard solution of SFN

Sulfanilamide is presented in the non-ionic form protonated in solutions with pH up to 9.6. Above this pH, The SFN begins to dissociate into the anionic form that fluoresces with lower emission intensity [20].

The effect of the pH of the solution on the emission intensity of a standard solution of SFN 21 µg mL^{-1} was evaluated using the following solutions: hydrochloric acid 0.10 mol/L (pH=1.0), acetic acid 0.10 mol/L (pH=2.9), and sodium hydroxide 0.10 mol/L (pH=10.0). In this study, the higher intensity of emission occurred at 365 nm when the acetic acid solution 0.10 mol/L was used. At this pH, SFN is presented as a non-ionic protonated form [20]. The emission intensity remains with slight variation in the pH range evaluated. At alkaline pH, the intensity decreases, probably due to the formation of the deprotonate ionic form [20]. The use of acidic solutions was proposed since SFN is covalently linked to the humate present in natural waters. Humates are a mixture of different substances that can be complex organic salts, humic and fulvic acids, which are carried from soils or sludge to the bodies of water [21]. The presence of humates can cause severe interference in the spectrofluorimetric determination of SFN in these sample types, compromising the accuracy of the spectrofluorimetric procedure. Thus, the solution of the samples in acid solution prevents the solubilization of the humates, being removed by centrifugation during sample preparation.

1. Analytical Curve for the Determination of SFN in Water

The equation can describe the analytical curve: I=0.84(±1.20) + 16.20 (±0.04) × [SFN]; r = 0.999, where I is the emission intensity at 365 nm when excited in 255 nm, [SFN] is the concentration of sulfanilamide in µg mL^{-1}. The analytical curve was shown in Fig. 2. The linear coefficient obtained was statistically different from zero (p=0.07), indicating no systematic error. The detection limit of 0.50 µg mL^{-1} was calculated using two times the standard deviation of linear coefficient (s_y). The detection limit obtained was lesser than the spectrophotometric procedure described [22]. The quantification limit (LQ) obtained was 1.4 µg mL^{-1} ($10 \times s_y$) (8.13 µmol L^{-1}). The obtained standard deviation of the analytical curve ($s_{y/x}$) was 2.21 [23].

The significance of the angular coefficient obtained in the analytical curve equation was evaluated. The F value obtained was 5,780, in which the relationship between the concentration of the solutions and the signal obtained $F_{critical}$ (0.05;1; 5) =10.01.

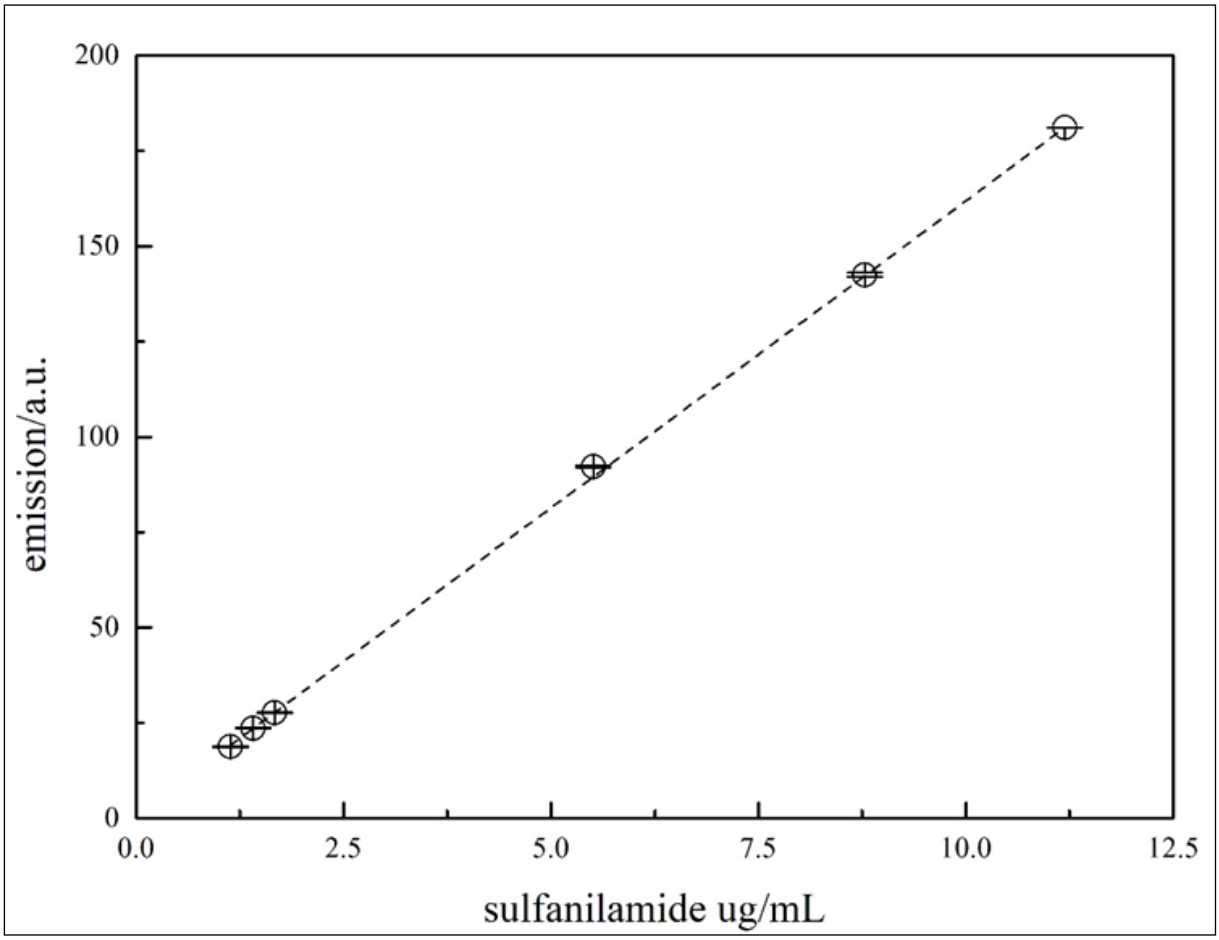

Fig 2: The analytical curve for sulphanilamide determination using the spectrofluorimetric procedure proposed. The solutions were made with acetic acid 0.10 mol/L. The standard solution of SFN using to obtain the curve were: 1.14; 1.41; 1,67; 5.51; 8.78 and 11.2 µg mL^{-1}. λ(excitation)=255 nm; λ(emission)=365 nm. Excitation and emission slit=5 nm.

The residual graphs were obtained considering the analytical curve equation, shown in Fig. 3 (a). The residual graph can verify that the residuals, the difference between the signal obtained and that predicted by the analytical curve equation, are dispersed in the figure randomly and within the range considered adequate ($2\times s_{y/x}$) [24].
The linearity of the proposed spectrofluorimetric procedure was evaluated according to [25]. The sensitivity graph of each solution used to construct the analytical curve was obtained and presented in Fig. 3 (b). It was verified or that the average sensitivity between the solutions in the analytical curve was 16.45 mL/µg. In this case, the dynamic linear range is those solutions in which the sensitivity obtained was between ±5% of the mean sensitivity, represented by the dashed lines Fig. 3(b) [25]. Thus, the linear range for determining the selected SFN was 1.14 to 11.2 µg mL^{-1}.

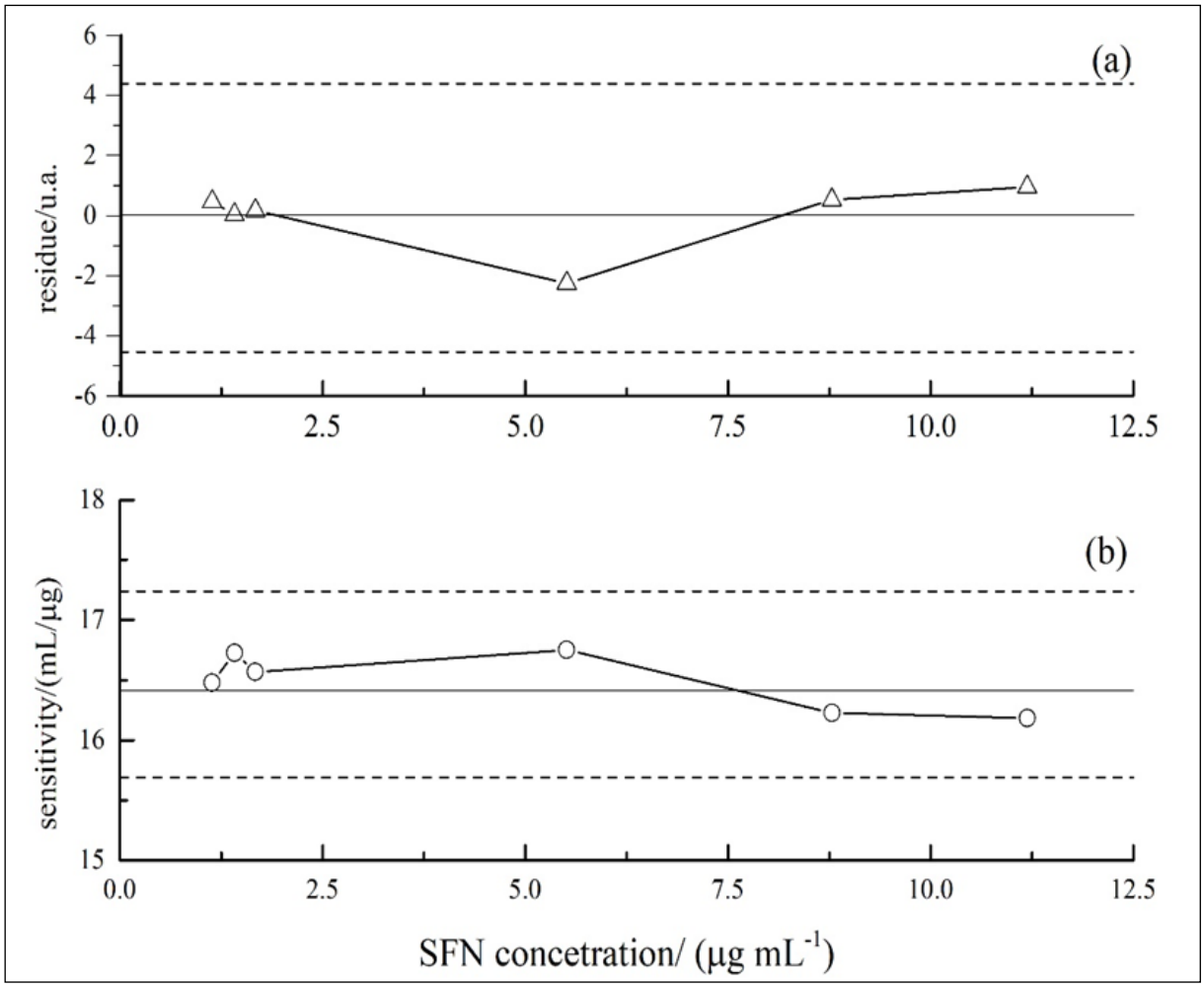

Fig 3: (a) Residual graph of the spectrofluorimetric procedure. The dashed line represents the allowed limits ($2\times s_{y/x}$); (b) Evaluation of the dynamic linear range of the proposed spectrofluorimetric procedure. The dashed line represents the allowed limits ±5% of the mean sensitivity

The precision of the proposed procedure was evaluated by obtaining different analytical curves intra-day and inter-days, evaluating the sensitivity (in mL/µg) of the proposed spectrofluorimetric procedure. The coefficient of variation of the sensitivity of analytical curves obtained intra-day was 5.6% (n=6). Thus, it was verified that the coefficient of variation of sensitivity inter-days was 5.5 % (n=11). These studies indicate that the proposed spectrofluorimetric procedure presents adequate robustness to be implemented in environmental control laboratories.

2. Recovery Tests for Determination of SFN Using the Proposed Procedure

The effect of the sample matrix on the determination of SFN was evaluated by adding an aliquot of the standard solution of SFN to surface water samples. Then, the concentration of SFN was obtained using the proposed spectrofluorimetric procedure. In this study, four different concentrations were added (1.29, 1.43, and 1.60 µg mL^{-1}) were added to two different samples, and the determination was done using the proposed procedure. The obtained concentrations were compared with those concentrations added. The recoveries of SFN in these samples varied from 90.3 to 106%. The recoveries obtained were compared with those obtained using capillary electrophoresis [15] and spectrophotometric procedure using solid-phase extraction [22]. The recovery results obtained were better than those obtained by chromatographic procedures [12, 13].

The accuracy of the proposed procedure for determining SFN was evaluated employing the hypothesis test. The null hypothesis H_0: Rec=100% and the alternative H_1: Rec ≠ 100% (two tailed test). Therefore, since the experimental *t* value is lower than the critical one, both recoveries results are not statistically different of 100%. The recoveries obtained indicate the absence of a matrix effect in the determination of SFN.

Table 1: Recovery test in the determination of SFN using the spectrofluorimetric procedure in surface water samples

SFN/ μg mL^{-1}		CV/%	Recovery/%	T(calculated)[(b)]
Added	Founded[(a)]			
1.29	1.21±0.02	1.6	93.8	−2.85
1.43	1.41±0.02	1.4	98.6	p=0.052
1.60	1.53 ±0.03	2.0	95.6	
1.29	1.24 ±0.01	.08	96.1	0.36
1.43	1.45 ±0.01	0.7	10.	p=0.376
1.60	1.70 ±0.02	1.2	106	

[(a)]Average of three determinations ± standard deviation;
H_0:Rec=100% and the alternative H_1: Rec≠100% (bilateral test)
[(b)] $t_{critical}$=4.31 (α=0.05, ν=2)

Conclusions

The analytical procedure with proposed spectrofluorimetric detection presented a robust procedure, which presents low interference of the samples of the evaluated river water. The procedure is fast, safe, requires no complex sample preparation, and does not require a specialized analyst.

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