

Analytical procedure for diclofenac determination using derivative spectrofluorimetry in surface waters

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ABSTRACT

The derivative spectrofluorimetric procedure was developed for the determination of diclofenac in surface water samples. The procedure was based on the intrinsic fluorescence of diclofenac emitted at 362 nm, which was proportional to its concentration. To circumvent interferences of the samples, the second derivative of the emission spectra was used. The optimized procedure can be used for the determination of diclofenac in a concentration range of 1.48 to 22 $\mu\text{g mL}^{-1}$. The detection limit was 0.12 $\mu\text{g mL}^{-1}$. The limit of quantification obtained was 1.0 $\mu\text{g mL}^{-1}$. The diclofenac recoveries obtained a suitable for the types of samples evaluated. The spectrofluorimetric procedure with the second derivative is simple, fast, and sensitive and there is no interference of the matrix in the determination of diclofenac in surface water samples.

Keywords - validation, environmental, excitation-emission.

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I. INTRODUCTION

Emerging contaminants are a class of organic substances, which include veterinary medicines, pharmaceuticals, as well as toiletries, cosmetics, and perfumes. Due to the intense use of these substances, they end up being discarded in domestic sewage and, finally, contaminating surface water.

Some studies have been published in which these substances occur in raw sewage, groundwater, and wastewater [1,2]. Several of the emerging contaminants had been found in Water Treatment Plants; among them, the diclofenac occurs frequently. A major problem described is the low removal of some of these substances as vasodilators and lipid regulators, like clofibrac acid [3].

Some studies describe that these substances in the environment are not completely removed by treatments in sewage treatment plants contaminating surface waters[4]. The efficiency of treatment in the removal of 14 micropollutants performed was evaluated in a water treatment plant. In this study, they found the removal of more than 80% of the evaluated substances, including diclofenac [5]. The published work founded a reduction of 97% of DCF concentration after the disinfection of the sand-filtered water without the mineralization of substance [6].

Diclofenac (DCF) is a non-steroidal anti-inflammatory with analgesic, anti-inflammatory, and antipyretic action. In commercial products, it is

available in the form of sodium salt or potassium. Diclofenac has a Kow log of 4.51 and pKa of 4.15. DCF remains in the dissociated form in aqueous solutions above pH 6.0. In surface waters in which its pH ranges from 6.5 to 7.2 [7], the substance remains in the deprotonated form.

In this context, there is a need for the development of simple and rapid analytical procedures for the determination of emerging pollutants in wastewater. Spectrofluorimetry is a methodology with wide application in the determination of organic substances. The emission of electromagnetic radiation by a molecule caused by the absorption of energy originated by a radiation source confers the selectivity methodology that allows the determination of analytes in complex samples. This selectivity is mainly since the radiation emitted is different from that originated from the radiation source [8]. The application of drifting techniques in the treatment of spectrophotometric data can circumvent the problem of spectral overlap and interference of some matrices increasing the resolution of spectra, enabling the application of the procedure in the analysis of analytes in these samples [9].

The objective of this work is the development of a spectrofluorimetric procedure for the determination of diclofenac in surface water samples.

II. METHODOLOGY

2.1. Reagents and solutions

All solutions were prepared from analytical purity grade reagents and ultrapure deionized water from a Milli-Q® purification system (Millipore, Bedford, USA). The glass products and vials used were previously placed in an acid bath containing HNO₃ 10% solution (v/v) for 24 h. For the acidity study, solutions with pH were obtained from 4 to 12 with the addition of 0.10 mol L⁻¹ buffer solutions prepared from sodium acetate/acetic acid; (pH 3.8 to 5.8) phosphate/phosphoric acid (pH 6.0 to 8.0) and sodium borate/boric acid (9.2 to 12).

The standard stock solution DCF 148 µg mL⁻¹ was prepared in a volumetric flask of 100.0 mL by dissolving sodium diclofenac salt in KH₂PO₄ 0.10 mol/L buffer solution (pH=6.0), completing the volume with the same buffer.

2.2. Instrumentation

All weighing was performed using a FA104N analytical scale with an accuracy of 0.1 mg. The pH determinations of the solutions were made at

2.3. Derivative spectrofluorimetric procedure for the determination of diclofenac in surface water samples.

Standard stock solutions containing DCF and sample solutions were diluted in KH₂PO₄ 0.10 mol/L buffer solution (pH=6.0). The analytical curves were obtained by diluting aliquots of the stock solution of DCF 148.0 µg mL⁻¹ in the volumetric flask of 10.0 mL, completing the volume with buffer solution 0.10 mol L (pH 6.0). The emission spectra obtained from the solutions were pre-processed by the second derivative by the Savitzky-Golay method, using a second-order polynomial and 21-point window. The spectrum of DCF and second derivative spectrum is showed in Fig 1.

Surface water samples were collected in streams in the city of Dourados, Mato Grosso do Sul, Brazil, filtered with nylon filter® 0.45 µm, and the filtrate was stored in amber bottles at 4°C until the time of analysis.

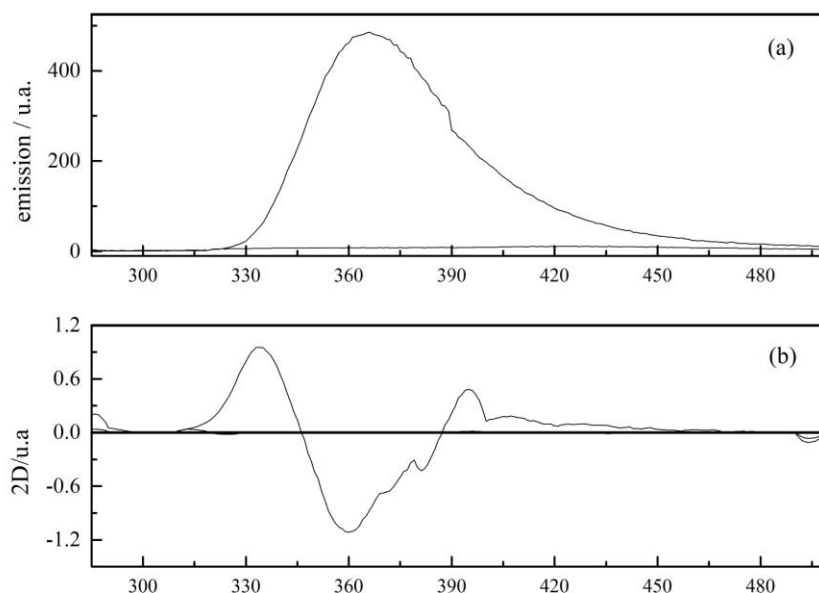


Figure 1. (a) Standard solution emission spectrum of DCF 29 µg mL⁻¹; $\lambda_{\text{excitation}}=244$ nm. (b) second derivative of the emission spectrum of the standard solution of DCF 29 µg mL⁻¹. Excitation slit=5 nm; emission slit=10 nm.

a HANNA-branded pHmeter, connected to a combined glass electrode and stainless-steel temperature sensor. The spectrofluorimetric measurements were performed in a spectrofluorometer (Varian®, Cary Eclipse) equipped with a Xenon lamp, using a quartz bucket, with four polished faces (10 mm optical path) and volume of 3.5 mL.

2.4 Derivative spectrofluorimetric procedure for the determination of diclofenac

The analyses of the samples were performed by diluting about 1,000 µL of the filtered solution of the samples in a 10.0 mL flask, completing the volume with KH₂PO₄ solution 0.10 mol L⁻¹ (pH 6.0). The emission spectra were obtained with the excitation window with an opening of 5 nm and the emission window with an

opening of 10 nm. The voltage of the detector used was 600 V, with filters selected in an automatic mode both in excitation and emission. The spectral scan performed was between 250 and 500 nm.

III. RESULTS AND DISCUSSION

The proposed procedure is based on the intrinsic fluorescence of electromagnetic radiation by diclofenac, in which it promotes radiation emission at 366 nm when excited at 244 nm.

Initially, the influence of the pH of the solution on the emission intensity of the standard Solution of DCF was evaluated. In this study emission spectra of different solutions containing DCF $29 \mu\text{g mL}^{-1}$ were obtained at different pH. The effect of pHs 3.8 to 12 were evaluated. There was a marked decrease in emission at 366 nm (excitation=245 nm) when the pH was above 7.0. This is due to the pKa of DCF, the main substance

present in the solution above pH 7.0 is the anionic form deprotonated DCF. This substance has low quantum efficiency, i.e., it has a lower fluorescence than a protonated substance. Thus, pH 6.0 was selected for the proposed procedure.

The emission spectrum of the standard Solution of DCF $29 \mu\text{g mL}^{-1}$ is shown in Fig. 1(a). The sample solutions in which the quantification of DCF has proposed present an emission spectrum with broadband close to this region of the electromagnetic spectrum, ranging from 380 to 500 nm. To overcome matrix interference problems, we chose to use the construction of the analytical curve using the second derivative of the emission intensity spectrum, as shown in Fig. 1(b).

Under these conditions, the proposed procedure presented an analytical curve with second-order polynomial regression between DCF concentrations from 1.48 to $22.2 \mu\text{g mL}^{-1}$. The

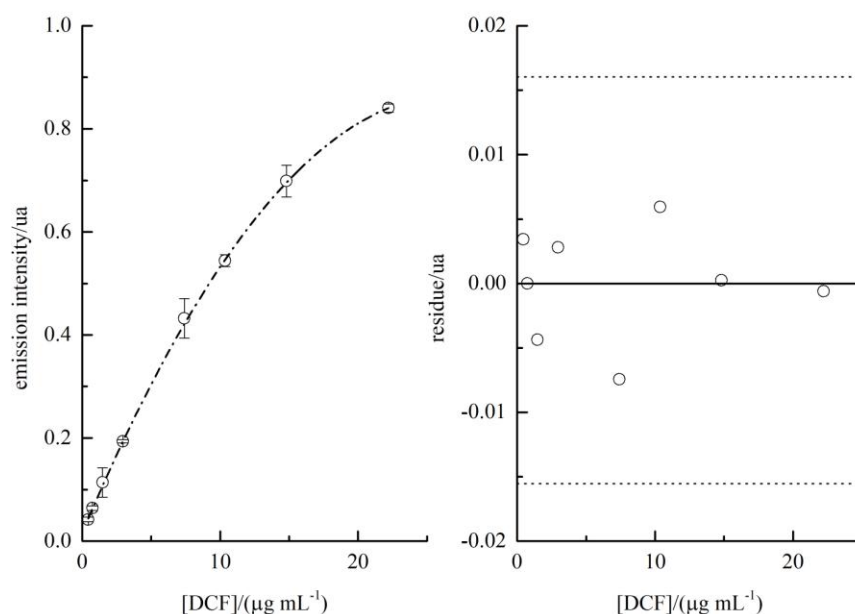


Figure 2 Spectrofluorimetric procedure for the determination of diclofenac in surface water samples. (a) Analytical curve employing the second derivative (2D) of the spectrum at 334 nm. (b) Residual graph of the analytical curve; the chart limits were established as $2 \times s_y/x$.

analytical curve is presented in Fig. 2(a). The analytical curve was described by the polynomial equation $2D = 0.016 (\pm 0.002) + 0.063 (\pm 0.001) \times [DCF] - 1.19 \times 10^{-3} (\pm 2.3 \times 10^{-5}) \times [DCF]^2$; $r=0.9999$, in which 2D is the second derivative at 334 nm ($\lambda_{\text{excitation}}=245 \text{ nm}$) and [DCF] is the concentration of diclofenac in $\mu\text{g mL}^{-1}$. All coefficients of the polynomial equation presented statistical significance at the level of 95%. The residues of the analytical curve, the difference between the value of

the second derivative of the emission intensity predicted by the analytical curve and that 2D value obtained experimentally at 334 nm of the standard Solution of DCF, are presented in Fig. 2(b). In this figure, it is verified that the residues of the analytical curve used are randomly distributed throughout the DCF concentration range evaluated and remain among the stipulated values, i.e., twice the standard deviation of the analytical curve ($sy/x=0.0080$ a.u.). This indicates that the second-order polynomial regression of the analytical curve adequately describes the dataset of the analytical curve.

The detection limit of the procedure was calculated using the standard deviation of the analytical curve (sy/x) [10]. The detection limit found was $0.12 \mu\text{g mL}^{-1}$ ($3 \times sy/x$). The limit of quantification obtained was $1.00 \mu\text{g mL}^{-1}$ ($10 \times sy/x$).

To evaluate the effect of the sample matrix on the determination of the DCF using the proposed spectrofluorimetric procedure, we chose to evaluate the recovery of the DCF added to different surface water samples [11]. In the study of DCF recovery, three different concentrations of DCF reference solution (1.48 , 10.4 , and $29.6 \mu\text{g mL}^{-1}$) were added separately to surface water sample solutions from the city of Dourados/MS. The results of the DCF concentration obtained in the samples, discounted from those originally without the addition of the reference solution, were compared with the previously added DCF concentrations. DCF recovery ranged from 90 to 111%. To evaluate the accuracy of the spectrofluorimetric procedure, the hypothesis test was used to confirm the absence of interference from the proposed spectrofluorimetric procedure in the determination of DCF for surface water samples. In this case, it was established as the null hypothesis (H_0): $\text{Rec} = 100\%$ and as an alternative hypothesis (H_1): $\text{Rec} = 100\%$ (bilateral test). The values of the applied t-test found, with 95% confidence, presented lower calculated values than those established by the tabulated value (2.02). This indicates that the mean of the recoveries in this concentration range studied was statistically equal to 100%, that is, it does not present interference of the sample matrices.

The accuracy obtained and expressed by the coefficient of variation in the determinations of DCF in the recovery study ranged from 0.7% to 5.3%, indicating the high precision in the determination of DCF in the analyzed samples [12].

The proposed spectrofluorimetric procedure is a low cost, simple and easy to perform. It does not require complex sample preparation as well as a specialized analyst. Under the conditions presented, the determination of DCF consumes about 140 mg of KH_2PO_4 per determination. It is estimated that the proposed procedure allows the determination of 20

determinations per hour, which is about 10 samples processed per hour.

IV. CONCLUSIONS

The proposed procedure is easy to perform, requires simple sample preparation and has the advantage of not requiring specialized analysts. The proposed spectrofluorimetric procedure allows the determination of diclofenac in water samples without the need for the use of organic solvents.

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