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ORIGINAL PAPER

Photochemically-Induced Fluorescence Dosage of Non-Fluorescent Pyrethroid (Etofenprox) in Natural Water Using a Cationic Micellar Medium

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Abstract An analytical method based on the use of UV-irradiation to produce fluorescent derivatives from Etofenprox a non-fluorescent pyrethroid insecticide is described. The impact of cetyltrimethylammonium chloride (CTAC) micellar medium on the Etofenprox photochemically-induced fluorescence (PIF) is reported. Parameters influencing the sensitivity and repeatability of the PIF method have been optimized. The alkaline medium (NaOH 6×10^{-2} M) + CTAC surfactant molecules (3.84 mg/ml) in acetonitrile is found to be very suitable for this pyrethroid insecticide analysis in environment matrices. Linear dynamic range is established over more than two orders of magnitude. The limit of detection is lower than 5 ng/ml. The method seems to be suitable for environmental matrices quality control. Application to the analysis of spiked natural waters gave recoveries rate ranged from 94 to 104% and 107 to 115% respectively for river and pound water.

Keywords Pyrethroid insecticide · Etofenprox · Micellar media · PIF analytical method

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Introduction

Pyrethroid insecticides are worldwide used to control malaria and others related insect diseases like chikungunya, Nile fever and dengue [1, 2]. Hence, pyrethroid insecticide-treated nets are largely used in sub-Saharan Africa for malaria prevention particularly in children under five years of age and pregnant women [3]. Because of their recent widespread uses in community health needs, pyrethroids will probably contribute significantly to the contamination of environment matrices in the next years. In appropriate environment conditions, pyrethroid insecticides are destroyed by bio-and photodegradation processes [4]. However, they have an immediate toxicity level higher than DDT (Dichloro-Diphenyl-Tricholoroethane) a prohibited persistent pesticide [5–7]. They attack insects and exposed people central nervous system [2, 5]. Human intoxication can caused severe health consequences which can lead to death in serious intoxication cases [2, 5–8]. These substances also present an important biodisponibility capacity and caused a reproduction trouble for wildlife animals [9–12]. Their indirect environmental impacts observed are horrific, they act essentially on the downstream food chain animals [2, 11]. That will influence in long term the local biodiversity and will be an important human intoxication source. So, it's very important to develop more sensitive and selective methods to monitor pyrethroid residues in order to prevent their bioaccumulation in the environment.

Gas chromatography remains the main analytical method used for pesticide residues analysis because of its excellent separation and detection potential, especially when combined with mass spectrometry [13–16]. The second most used method is high performance liquid chromatography

(HPLC), with UV-visible and photoconductivity detectors [17–20]. However, the above two techniques have some drawbacks; they are costly and relatively time-consuming. Most rapid and cheaper methods have been reported for their analytical interests. Simple capillary methods [21, 22], enzyme-linked immunosorbent assay (ELISA) [23, 24] and direct fluorescence or induced-fluorescence methods [4, 16, 25–29]. Among the rapid analysis methods mentioned above, the fluorescence ones have been widely investigated because of its high sensitivity, simplicity and versatility [25]. Direct fluorometry cannot be applied to some pesticides analysis because they are naturally non-fluorescent [4]. However, this difficulty can be overcome by photochemically-induced fluorescence (PIF). The used of simulated UV-irradiation allowed transforming non-fluorescent pesticides to their metabolites presenting in some cases a fluorescent signal. To improve the induced-fluorescence signal, tensioactives molecules are used. Micelles, cyclodextrins and liposomic vesicles are known for their ability to enhance probes luminescence intensities. Indeed, Micellar media provide a favorable microenvironment for organic molecules so that their excited single state is stabilized and the fluorescence efficiency is improved [4, 28–31].

In this work, we have developed a photochemically-induced fluorescence (PIF) method for the analysis of Etofenprox, a pyrethroid insecticide massively used to combat Malaria in sub-Saharan Africa. In order to increase the sensitivity and the selectivity of the method, cationic micellar solutions of CTAC have been used to enhance and/or stabilize the PIF signal.

Experimental

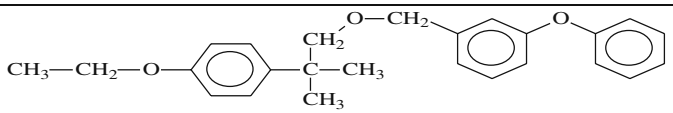
Reagents

Etofenprox (99%, m/m) was purchased from Cluzeau Info Labo (CIL, France) and used as received. Etofenprox structure and physicochemical properties are presented in Table 1 [32]. Cetyltrimethylammonium chloride (CTAC, 25%, wt. solution in water) is from Sigma-Aldrich. Stock solutions of Etofenprox were prepared in acetonitrile (CH_3CN) for HPLC grade (VWR International SAS). The used sodium chloride (NaCl, +99% m/m), hydrochloride acid (HCl, 36% m/m) and sodium hydroxide (NaOH, +99% m/m) are from Sigma-Aldrich. The prepared solutions were protected from light irradiation with aluminum foil and stored in refrigerator.

Apparatus

All fluorescence and PIF measurements were performed at room temperature with Kontron spectrofluorimeter, Model SFM-25 (Zurich, Switzerland) connected to a micro-computer and controlled by software Lcwin. A standard Hellma (Mulheim, Germany), quartz fluorescence cuvette

Table 1 Structure and physicochemical properties of pure Etofenprox

Etofenprox ($C_{25}H_{28}O_3$)		
Structural formula		
IUPAC chemical name		2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether
Characteristics		Values
Solubility in	water	1 µg/L (25°C)
	acetonitrile	640 g/L (10°C)
Hydrolysis		Hydrolysis rate > 40% in distilled water at 25°C at pH range close to environmental conditions (pH = 3-9). Negligible hydrolysis at 25°C at pH 3, 5 and 7 in natural water.
Photolysis		When exposed to high-intensity lamps (30,000 lux).
Stability to heat		No loss during at least 3 months storage at 80°C. Partial degradation at 100°C.

(1-cm pathlength) and a micropipette pipetman of 10–100 μL (Gilson, France) were used. All analytical measurements were carried out under the same conditions of voltage (high voltage=400) and sensitivity (factor=1.5) [30, 31]. An unfiltered Osram 200 W HBO high pressure mercury lamp with an Oriel Model 8500 power supply was utilized to simulate the solar UV-irradiation on the insecticide solution. Here, the photolysis cuvette was placed on an optical bench at 40 cm from the mercury lamp.

Procedures

Solutions Preparation

Standard solutions of Etofenprox (2.656×10^{-3} M) were freshly prepared by dissolving the compound in acetonitrile. Serial dilutions were performed in acetonitrile for the working solutions. NaCl (1 M), NaOH (1 M), HCl (1 M) and CTAC (CTAC, 25%, wt) were prepared in distilled water to explore the medium alkalinity, acidity and micellar molecules effects on the PIF signal. The working solutions were shaken vigorously before irradiation and/or analytical measurements.

Analytical Measurements

The Etofenprox working solution was placed in a quartz cuvette and irradiated at room temperature with UV light for a fixed time. The PIF intensity was monitored at fixed analytical excitation (λex) and emission (λem) maximum wavelengths of the Etofenprox photoproducts by measuring the spectra height signal [30]. Linear calibration curves were obtained at these λex and λem values by measuring the PIF signal corresponding to the optimized analytical conditions. Fluorescence and PIF intensity measurements were corrected for the solvent signal with the appropriate blank. All fluorescence signal measurements were carried out in triplicate and the results were expressed as mean values. Microcal Origin, version 6.00 application software was used for the statistical treatment of the data.

Results and Discussion

Photochemically-Induced Fluorescence (PIF)

Etofenprox is naturally non-fluorescent, whereas an intense fluorescence band appeared upon UV-irradiation. The fluorescence excitation and emission spectra recorded in acetonitrile after 0 and 30 min under solar simulator UV-irradiation were recorded (Fig. 1). The excitation and emission spectra obtained have similar shape in all the studied media. In acetonitrile, the excitation spectrum

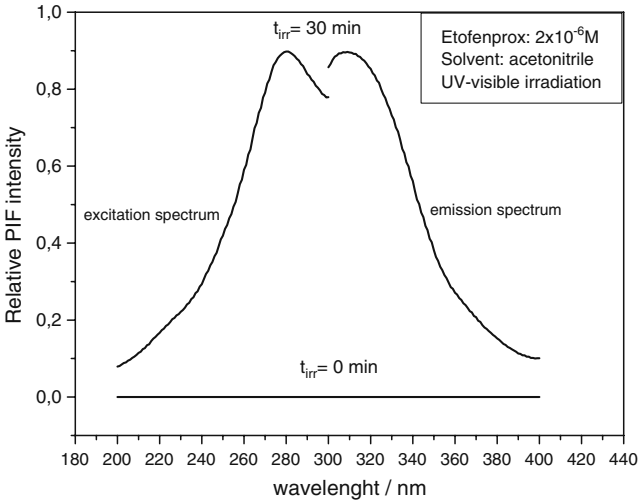


Fig. 1 Fluorescence excitation and emission spectra of Etofenprox under UV-visible irradiation

presents a maximum peak at 280 nm, while the emission spectrum maximum peak is located at 310 nm. No significant wavelengths shift of the emission band occurred on changing the solvent, whereas a red-shift (15 nm) of the excitation band occurred and took place when CTAC is used with acetonitrile. The selected analytical optimum wavelengths are (λex=280 nm; λem=310 nm) in acetonitrile and (λex=295 nm; λem=310 nm) in the CTAC-acetonitrile micellar solutions.

Optimization of the UV-Irradiation Time

The Etofenprox photoproducts formation was first evaluated. We investigated the evolution of the PIF intensity with the UV-irradiation time for a fixed amount of Etofenprox (2×10^{-6} M) in acetonitrile. When increased the irradiation time, a progressive PIF signal increase is observed (Fig. 2),

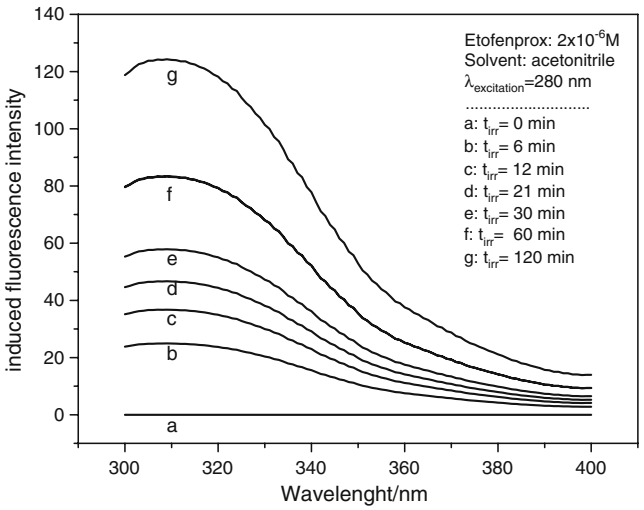


Fig. 2 Evolution of Etofenprox PIF emission spectra

resulting from the Etofenprox continuous photodegradation. Curves displaying the variation of the PIF height signal versus the UV-irradiation time (Fig. 3), showed that even after two hours irradiation the PIF maximum value is not reached. A longtime irradiation enhanced strongly the emission signal but will be a very time-consuming method. In order to overcome this difficulty, we have first irradiated the Etofenprox solution for only 30 min. Then we seek to enhance the formed photoproducts fluorescence signal with CTAC molecules. Indeed, over the critical micellar concentration (cmc), tensioactive molecules formed micelles; microcapsules which are able to encapsulate and protect the excited singlet state of many fluorophores against non-radiative de-excitation pathways thus inducing an enhancement of the fluorescence signal [29–31].

PIF Signal Optimization in Organized Media

CTAC Concentration Effect on the PIF

An increase in surfactant molecules concentration has generally a marked influence on the fluorescence intensity of analytes [28–31]. Etofenprox photochemically-induced fluorescence intensity versus the logarithm of surfactant concentration is presented in Fig. 4. To lower CTAC concentrations, the PIF intensity remains unaffected until the surfactant critical micellar concentration is reached. After this, when the CTAC concentration increased (1.40 to 3.84 mg/mL), one assisted to an important increase of the Etofenprox PIF intensity. Here, the PIF signal increased proportionally to the number of micelles formed. When the surfactant concentration increased after 3.84 mg/mL, a dynamic equilibrium is established between the insecticide molecules adsorbed at the micelles and those in the bulk.

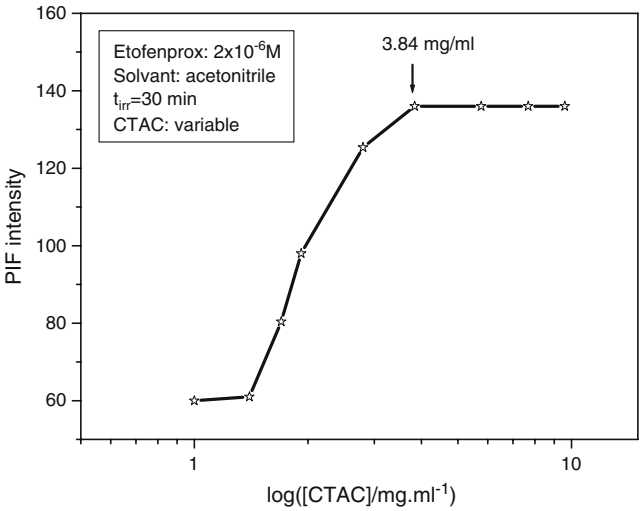


Fig. 4 Influence of CTAC concentrations on the Etofenprox PIF intensity

The ratio of bound to free molecules becomes constant. That's why the PIF signal intensity becomes constant when the saturation is reached. The selected CTAC concentration for this study was 3.84 mg/ml.

HCl and NaOH Effect on the PIF Signal

Acidity and alkalinity are factors affecting both fluorescence intensity and micelles-analytes binding abilities. HCl and NaOH can enhance or decrease fluorescence signal of fluorophores [30, 31]. They will also be able to assure a relative stability to the direct fluorescence or PIF signal [29–31]. Therefore, it is important to know their effects on the induced fluorescence emission in CTAC micellar

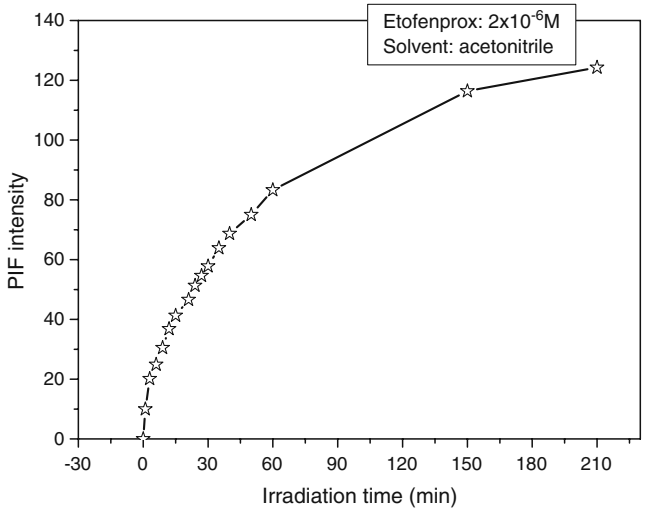


Fig. 3 Variation of the PIF intensity versus the UV-irradiation time

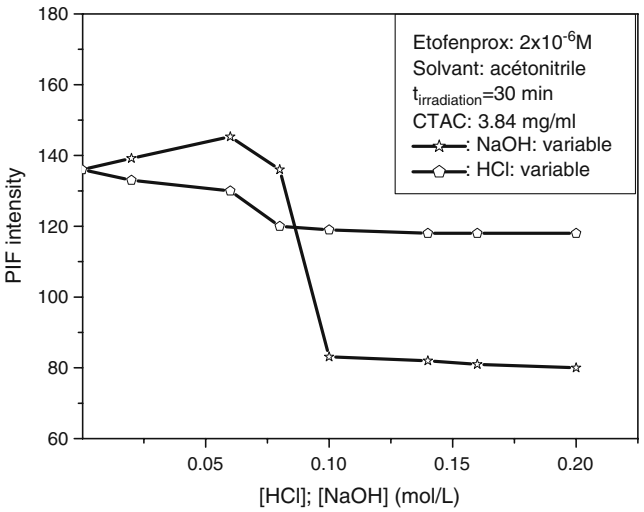


Fig. 5 Acidity and alkalinity effects on the Etofenprox PIF intensity in CTAC micellar medium

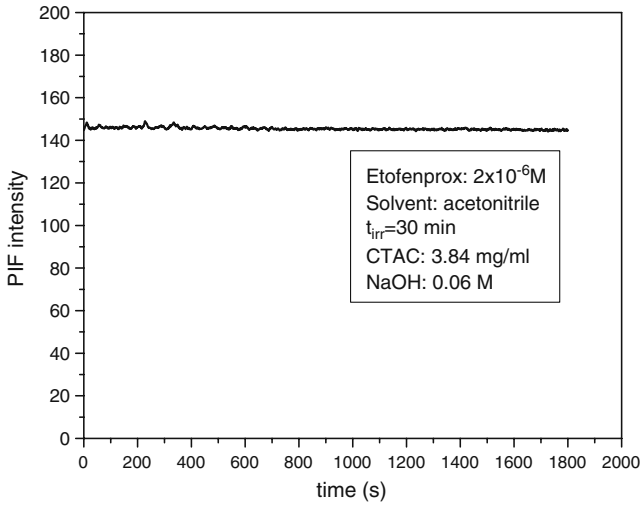


Fig. 6 Evolution of the obtained PIF emission versus time in optimized analysis conditions

medium in order to develop a more sensible and repeatable method for Etofenprox residues dosage in environment matrices. The results of the medium acidity or alkalinity effects showed (Fig. 5) that the Etofenprox PIF signal is enhanced in alkaline medium until NaOH concentration reached 6×10^{-2} M, and then decreased rapidly after this value. In the acidic medium, the PIF signal decreased until HCl concentration reached 7.5×10^{-2} M. After this value, the PIF signal is stabilized when the HCl concentration increased. For high sensitivity need, the selected medium for further investigation is the alkaline medium NaOH=6×10^{-2} M. However, the above studies do not reveal the major information about the relative stability of the formed complex (repeatability conditions), since these curves were recorded in a relatively short time, at the maximum emission for each NaOH value. To obtain more information on the stability of the formed fluorophores in NaOH (6×10^{-2} M), kinetic studies were performed (Fig. 6). The results showed that the photoproducts fluorescence signal is relatively stable in acetonitrile + CTAC (3.84 mg/mL) + NaOH (6×10^{-2} M). A similar effect was observed for others fluorophores previously studied in CTAC micellar medium [30, 31].

Analytical Figures of Merit

To evaluate the analytical interest of the enhanced PIF of Etofenprox in micellar medium approach, calibration graph were established under the above optimum analytical conditions. Linear calibration curve was obtained and the analytical figures of merit for the dosage of Etofenprox by PIF determined. The optimized analysis conditions, the statistical treatment of the data including the linear dynamic range (LDR), correlation coefficient (r^2), limit of detection (LOD) and quantification (LOQ) and the absolute limit of detection (ALOD) are summarized in Table 2. Linear PIF intensity versus Etofenprox concentration calibration plot is obtained with an excellent precision, as shown by the correlation coefficicient of 0.999. The obtained limit of detection is significantly lower (LOD<5 ng/mL); suitable for quantitative analysis of Etofenprox traces in natural waters.

Analytical Application

In order to verify the applicability of the proposed method to authentic samples, recovery experiments of the insecticide under study were performed on Niger River and surrounding pond water samples spiked by adding known Etofenprox amounts to the samples. Each sample was analyzed twice. Etofenprox 2×10^{-3} M was prepared as a stock solution. The river and the pond water were spiked with 2×10^{-7}, 5×10^{-7}, and 1×10^{-6} M of the above stock solution. For each sample, its Etofenprox content was extracted using an acetonitrile solution (recovery rate> 98%). Then analyzed with the proposed method: 30 min UV-irradiation + CTAC (3.84 mg/mL) + NaOH (6×10^{-2} M) in acetonitrile. It was found that the determination of Etofenprox residues in river and pond water was satisfactory. The recoveries rate for Etofenprox over three concentrations were between 94 to 104% and 107 to 115% respectively in river and pond water. Compared to river water, the important recoveries rate in pond water is probably due to interfering substances (dissolved organic maters (DOM)). Indeed, after analysis we have noticed that the pond water (DOM=25 mg/L) is five times rich

Table 2 Optimized analysis conditions of the method and its Analytical Figures of Merit

Optimized analysis conditions				Analytical figures of merit					
Solvent	CTAC (mg/mL)	NaOH (mol/L)	Tirr[a] (min)	λex/λem[b] (nm)	Range[c] (ng/mL)	(r^2)[d]	LOD[e] (ng/mL)	LOQ[f] (ng/mL)	ALOD[g] (ng)
acetonitrile	3.84	6×10^{-2}	30	295/310	22-3840	0.999	4.50	15	11.25

[a] The selected irradiation time; [b] Analytical excitation (ex) and emission (em) wavelengths; [c] Concentration linear dynamic range; [d] Correlation coefficient; [e] Limit of detection (LOD) was defined as the amount of analyte giving a signal-to-noise ratio of 3; [f] Limit of quantification (LOQ) was defined as the amount of analyte giving a signal-to-noise ratio of 10; [g] Absolute limit of detection (ALOD), calculated using 2.5 mL sample

in dissolved organic maters then the river water (DOM= 5 mg/L) [32]. According to the above obtained results, in most instances, the accuracy of our method is satisfactory for real samples analysis.

Conclusion

We have demonstrated in this work that UV-visible irradiation of Etofenprox insecticide during 30 min yields strongly fluorescent photoproducts. The obtained fluorescence signal is enhanced and stabilized in alkaline CTAC micellar medium. Using this photochemically-induced fluorescence (PIF) approach, we have developed a simple, efficient and reproducible analysis method suitable for Etofenprox residues determination in natural waters.

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