

SPECTROFLUORIMETRIC DETERMINATION OF BETA-BLOCKERS ATENOLOL AND BISOPROLOL FUMARATE RESIDUES IN SENEGAL NATURAL WATERS

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A spectrofluorimetric method was developed to determine residues of two β -blockers, atenolol (AT) and bisoprolol fumarate (BF), in Senegal's natural waters. The electronic absorption and fluorescence spectral properties of both β -blockers were investigated in several organic solvent mixtures [e.g., MeOH/H₂O (60/40 v/v), cyclodextrins (β -cyclodextrin, HP- β -CD)], and in the presence of surfactants (SDS, Triton X, Tween 80). After optimization, satisfactory analytical figures of merit were obtained for the determination of both β -blockers: concentration linear dynamic range of over one to two orders of magnitude, limits of detection (LODs) from 1.3 to 5.4 ng/ml for BF and from 1.2 to 3.7 ng/ml for AT, limits of quantification (LOQs) from 4.5 to 18.1 ng/ml for BF and from 4.0 to 12.5 ng/ml for AT. Relative standard deviations (RSDs) were between 2.1 and 5.3 %, according to the β -blockers. The spectrofluorimetric method was applied to the analysis of fortified river water and wastewater (effluent) collected in Senegal and France and spiked with both β -blockers. It yielded good recovery values, from 93.3 to 107.8 % for AT and from 97.4 to 108.9 % for BF. Our results demonstrated the simplicity, rapidity, and sensitivity of the spectrofluorimetric method to quantify residues of β -blockers in environmental waters.

Keywords: β -blockers; atenolol; bisoprolol fumarate; spectrofluorimetric method; environmental samples; water analysis

СПЕКТРОФЛУОРМЕТРИСКО ОПРЕДЕЛУВАЊЕ НА ОСТАТОЦИ НА БЕТА-БЛОКАТОРИТЕ АТЕНОЛОЛ И БИСОПРОЛОЛ ФУМАРАТ ВО СЕНЕГАЛСКИТЕ ПРИРОДНИ ВОДИ

Разработен е спектрофлуорметриски метод за определување остатоци на два β -блокатора, атенолол (AT) и бисопролол фумарат (BF), во природни води на Сенегал. Електронските апсорпции и флуоресцентни спектрални својства на двата β -блокатора беа испитани во неколку органски смеси на растворувачи [на пример, MeOH/H₂O (60/40 v/v), циклодекстрини (β -циклодекстрин, HP- β -CD)] и во присуство на површински активни супстанции (SDS, Triton X, Tween 80). Откако беше извршена оптимизација, беа добиени задоволителни аналитички вредности за определување на двата β -блокатора: концентрациски линеарен динамичен опсег од два реда на големина, граници на детекција (LOD) од 1,3 до 5,4 ng/ml за BF и од 1,2 до 3,7 ng/ml за AT, граници на квантификација (LOQ) од 4,5 до 18,1 ng/ml за BF и од 4,0 до 12,5 ng/ml за AT. Релативните стандардни девијации се движеа од 2,1 до 5,3 % во зависност од β -блокаторите. Спектрофлуорметрискиот метод беше применет за анализа на спајкувани со β -блокатори речни и отпадни води (ефлуенти) земени од Сенегал и Франција. Беа добиени добри аналитички приноси, од 93,3 до 107,8 % за AT и од 97,4 до 108,9 % за BF. Нашите резултати ја покажаа едноставноста, брзината и осетливоста на спектрофлуорметрискиот метод за квантитативно определување на β -блокаори во водите од животната средина.

Клучни зборови: β -блокатори; атенолол; бисопролол фумарат; спектрофлуорометриски метод; примероци од животна средина; анализа на вода

1. INTRODUCTION

For several decades, the occurrence and fate of pharmaceutical residues and their metabolites in environmental matrices have become an attractive research field.^{1–6} One of the first studies on the presence of these pharmaceutical residues in the environment was reported in 1977 by Higaite and Azarnoff,³ who quantitated chlorophenoxyisobutyrate (CPIB) and salicylic acid (metabolites of clofibrate and aspirin) by gas chromatography-mass spectrometry (GC-MS) in the effluent of a Kansas City sewage disposal plant (Missouri, USA). Over a ten-month period, the average daily output of CPIB was 2.1 kg (0.7–2.9 kg) and salicylic acid 8.6 kg (0.5–28.7 kg). More recently, Glassmeyer et al. in 2017² and Klimaszyk and Rzymiski in 2018⁴ investigated the role of pharmaceutical contaminants in pollution.

Also, a number of research groups^{7–10} have investigated the presence of pharmaceutical residues in various environmental compartments. Among these compounds, β -blocker drugs were commonly found in environmental waters. Indeed, β -blocker drugs, such as atenolol and bisoprolol, are frequently used to treat cardiovascular diseases, such as hypertension, congestive heart failure, angina, and cardiac arrhythmia.¹¹ Consequently, they were often found in environmental waters because of the relatively high incidence of cardiovascular disease worldwide. Due to the increasing occurrence of cardiovascular diseases in developing countries such as West Africa, β -blocker drugs were ranked third among pharmaceuticals most commonly present in aquatic environments.

The introduction of emerging environmental pollutants mostly resulted from inefficient wastewater treatment plants (WWTPs) and the direct discharge effluents of domestic and hospital wastewater in natural waters. Consequently, β -blocker drugs were found at low concentrations, ranging from ng/ml to μ g/ml, in various matrices including wastewater^{12–14}, surface water¹⁵, groundwater^{9,16,17}, tap water, and drinking water¹⁸. Several studies reported the presence of several β -blockers such as atenolol, bisoprolol, metoprolol, propranolol, and sotalol in the environmental waters of different countries. For example, atenolol and bisoprolol were found in influents of Canadian WWTPs at concentrations of 1650 ng/l and 38 ng/l, respectively, and in effluents at 987 ng/l and

24 ng/l, respectively¹³. Also, atenolol and bisoprolol were measured in the effluent of WWTPs of France at concentrations of 2450 ng/l and 630 ng/l, respectively.¹⁹ In the same study, atenolol and bisoprolol were quantified in surface waters at concentrations of 240 ng/l and 38 ng/l, respectively. Moreover, these β -blockers were found in tap water at low concentrations. For example, bisoprolol was detected above 15 ng/l,¹⁸ and atenolol was found at 2 ng/ml in tap water in France. However, few studies have been performed on the presence of pharmaceuticals in the environment in Africa. Most of this research has been done in South Africa. Most studies indicated that the pharmaceutical levels in water samples were higher in Africa than in the other continents due to the lack of WWTPs. For instance, atenolol was found at relatively high concentrations of 39 μ g/l and 3 μ g/l, respectively, in the Umgeni River (South Africa)²⁰ and the Lagos River (Nigeria).²¹

Therefore, the contamination of the environment by pharmaceutical residues has raised concerns in recent years about their potential risks to aquatic organisms and human health.^{10,22–24} Studies have shown that biologically active pharmaceutical residues were persistent and accumulated in natural waters. In addition, since they are very water-soluble, most β -blockers were found to exist in nature largely in their ionized form at neutral pH, leading to their enhanced availability in the environment.²⁵ Therefore, even at low concentrations, they can affect the life of aquatic species, such as fish, algae, invertebrates, and the ecosystem.¹⁵ For example, bisoprolol was classified as hazardous to the environment in the acute III category with $EC_{10} = 3.6$ mg/l for the crustacean *Daphnia similis*.²⁶ Also, the toxicity test on *Daphnia magna* showed that EC_{50} values of atenolol and bisoprolol were above 100 mg/l.²⁷ Moreover, the atenolol degradation products, such as ATE-152, ATE-238, and ATE-254, generated by photocatalysis, and ATE-301, formed by chlorination, were more toxic than the parent compounds.²⁸

Consequently, the determination of these drugs was essential for monitoring their fate in the environment. Thus, several sensitive analytical approaches have been developed to detect low levels of β -blockers. The most commonly used methods for the detection and quantification of pharmaceutical contaminants in wastewater and natural waters were chromatographic techniques combined

with mass spectrometry, including GC/MS, LC/MS, and LC/MS/MS.^{12,29–35} However, the GC analysis of β -blockers required long and tedious derivative steps. Therefore, LC/MS/MS was considered the method of choice. For example, a comparative study of GC/MS and LC/MS/MS indicated that only the latter method allowed one to analyze extremely polar β -blockers, such as sotalol, because of an incomplete derivatization of the functional groups.³⁶ Also, ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and liquid chromatography-quadrupole-linear ion trap mass spectrometry (LC-QLIT MS) were increasingly applied to identify and quantify β -blockers at the ng/l level in environment.³² However, chromatographic methods are relatively expensive and time-consuming. In addition, the determination of β -blockers in environmental water is sometimes realized by multi-residue methods with different classes of compounds, which need optimization of many parameters during the analysis.

In contrast, spectrofluorimetry presents several advantages. It is easier, less expensive, more sensitive, and less time-consuming than chromatographic methods, especially for fluorescent compounds like β -blockers. Therefore, several spectrofluorimetric methods were reported for the deter-

mination of β -blockers in pharmaceutical formulations, biological samples, and the environment.^{37–40} For example, Bakir et al. in 2018³⁸ determined atenolol in a pharmaceutical formulation by a fluorimetric method based on the atenolol quenching effect on the photoluminescence of gold nanoparticles (AuNPs). Also, Abdewelwahab et al.³⁷ studied the native fluorescence of bisoprolol and rosuvastatin in human plasma by spectrofluorimetry.

The aim of this work was to develop a simple, rapid, and sensitive spectrofluorimetric method for the quantitative analysis of two β -blockers, atenolol (AT) and bisoprolol fumarate (BF) (Figure 1), in natural waters and the effluent of WWTPs from Senegal and France. The effects of several parameters, such as solvent, pH, cyclodextrin (β -CD, HP- β -CD), and surfactant (SDS, Tween 80, Triton X) concentrations, on the fluorescence properties of both compounds were optimized in order to enhance the spectrofluorimetric response. Also, the analytical performance of spectrofluorimetry was compared with UV absorption spectrophotometry. After liquid-liquid extraction and using the standard addition procedure, we applied the spectrofluorimetric method to the quantitative analysis of AT and BF in spiked natural waters and the effluent of Senegal WWTPs.

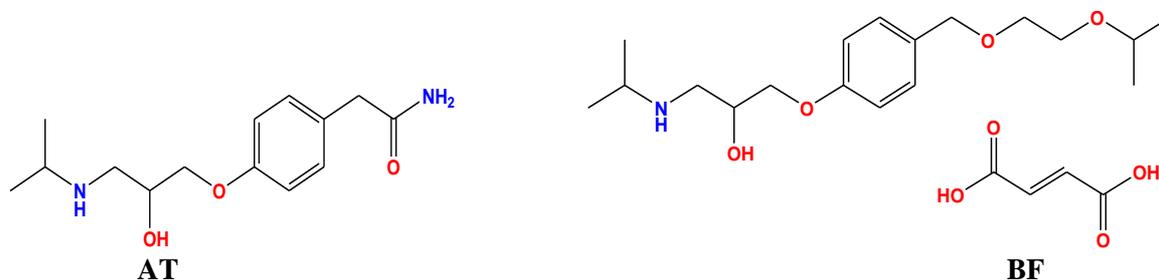


Fig 1. Chemical structure of atenolol (AT) and bisoprolol fumarate (BF)

2. EXPERIMENTAL

2.1. Reagents

Atenolol (99 %) (2-[4-[2-hydroxy-3-(propan-2-ylamino) propoxy] acetamide (AT) and bisoprolol fumarate (99 %) (*E*)-But-2-enedioic acid; 1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxy-methyl) phenoxy]propan-2-ol (BF) were purchased from Aldrich. High purity β -cyclodextrin (β -CD), hydroxypropyl- β -cyclodextrin (HP- β -CD), sodium dodecyl sulfate (SDS, 98 % m/m), Tween 80 (polyoxyethylene (20) sorbitan monooleate), and Triton X-100 (10 % by weight in water, $d = 1.01$) were purchased from Sigma-Aldrich. Spectroscopic grade solvents, including 2-propanol (2-PrOH, 99.9 %),

methanol (MeOH, 99.9 %), acetonitrile (ACN, 99.8 %), dimethyl sulfoxide (DMSO, HPLC grade), hexane (95.8 %), dichloromethane (DCM, 99.8 %), and butyl acetate (99 %) were also obtained from Sigma-Aldrich. All the other reagents (NaOH, HCl) were of analytical reagent grade. Ultra-pure water (Milli-Q Advantage A10 system, Millipore) was used for preparing all solutions.

2.2. Apparatus

Fluorescence spectra were obtained at room temperature (298 K) on a Shimadzu RF-6000 spectrofluorimeter equipped with a pulsed xenon lamp and interfaced to a microcomputer, processed by a software LabSolutions CS, Shimadzu (Shimadzu

Co.). Slit widths for both excitation and emission monochromators were set at 5 nm, and all measurements were performed in standard Hellma 1-cm pathlength quartz fluorescence cells. Absorption spectral measurements were also realized at room temperature with a Shimadzu UV1800 absorption spectrophotometer. An Osram 200W HBO high-pressure mercury lamp with an Oriel Model 8500 power supply was used for photolysis measurements. During photolysis, the quartz fluorescence cell was placed on an optical bench 30 cm from the mercury lamp.

2.3. Preparation of standard solutions

Standard stock solutions (10^{-3} M) of AT and BF were freshly prepared by exactly weighing and dissolving the corresponding compounds in the selected organic solvents (MeOH, 2-PrOH, ACN, DMSO, water, and DCM). Serial dilutions were performed to obtain standard working solutions. Standard stock aqueous solutions of HP- β -CD ($1 \cdot 10^{-1}$ M), β -CD ($1.5 \cdot 10^{-2}$ M), SDS ($1 \cdot 10^{-1}$ M), Triton X-100 ($1 \cdot 10^{-2}$ M), and Tween 80 ($1 \cdot 10^{-2}$ M) were prepared in ultra-pure water. The pH solutions, ranging between 1.0 and 12.0, were obtained by adding the appropriate amounts of NaOH and HCl to ultra-pure water. All solutions were protected against light with aluminum foil to avoid any photodecomposition and stored in a refrigerator.

2.4. Treatment of water samples

2.4.1. Preparation

Samples under study were collected from different places in Senegal and France. River water samples were collected in 0.5-l amber glass bottles from the Marne River at Marne-la-Vallée (France). Well water samples were collected from two cities, Medina and Parcelles-Assainies, in Dakar (Senegal). Tertiary effluent samples were obtained from the Cambérène municipal center for wastewater treatment plants (WWTPs) in Dakar. Before use, all samples were filtered through a 0.45- μ m pore, 25 mm diameter syringe filter (Sigma-Aldrich) in order to eliminate any suspended organic matter and stored at 4 °C. Tap water was obtained in our laboratory (Marne-la-Vallée University, France) during November 2019.

2.4.2. Fortification

Ten-milliliter samples of filtered water were spiked with 10 μ g/ml of an AT or BF solution in order to obtain standard solutions (1 μ g/ml).

2.5. Extraction procedure

A double liquid-liquid extraction procedure with dichloromethane was performed for both β -blockers in spiked samples in order to determine them in natural water and effluent samples. A 10-mL filtered water sample spiked with an AT or BF standard solution was placed in a separating funnel, and 10 ml of DCM was added. Then, this solution was stirred for 10 min, and after vigorously shaking it for 3 min, the organic phase was isolated. The aqueous fraction was submitted to a second extraction. After a third extraction of the aqueous fraction, all organic phases were combined and then evaporated to dryness. These concentrated samples were then dissolved in 10 ml of MeOH/Water (60/40 v/v) and used for the analyses.

2.6. Standard addition procedure

Ten milliliters of the aqueous samples were spiked with 100 ng/ml of AT or 200 ng/ml of BF standard solutions (C_i) in a 10-ml flask. Then, increasing concentrations of AT or BF were added, from 150 to 800 ng/ml for AT and from 200 to 1200 ng/l for BF (C_m), and the flasks were completed to the mark with the MeOH/Water (60/40) mixture. Afterward, the solution fluorescence signal was measured, and the standard addition procedure was applied to evaluate the recovery percentage values by means of the ratio of the measured β -blocker concentration (C_m), obtained from the calibration curves, to the initial β -blocker concentrations (C_i).

2.7. Analytical measurements and photolysis reaction

An aliquot of a working AT or BF solution was placed in a quartz cuvette, and the fluorescence emission and excitation spectra were recorded at a scanning speed of 600 nm/min. Fluorescence intensity (I_F) values were monitored at fixed analytical excitation (λ_{ex}) and emission (λ_{em}) maximum wavelengths, and the spectral peak height was measured in all solutions. All fluorescence measurements were corrected for the solvent blank signal. Fluorescence intensity measurements were carried out in triplicate and expressed as mean values to optimize the analytical results. Photolysis reactions were performed by irradiating a 3-ml volume of AT and BF working solutions, magnetically stirred at room temperature, with UV light. In order to evaluate the degree of persistence of AT

and BF in environmental waters, the AT and BF photodegradation kinetic parameters were determined.

2.8. Electronic absorption spectral properties

The UV-VIS absorption spectral properties of AT (10^{-4} M) and BF (10^{-4} M) in different organic solvents, MeOH/water, and ACN/water mixtures are presented in Table 1. Two main bands for AT and BF appeared in the 222–232 nm and 270–278 nm regions. The UV-VIS absorption spectra of both compounds were very similar, and the spectral features did not vary significantly with the solvent. The molar absorption coefficient (ϵ_{\max}) values of the short-wavelength bands were larger than 10^4 M $^{-1}$ cm $^{-1}$, indicating that these bands corresponded to π - π^* electronic transitions.

2.9. Fluorescence spectral properties

The effect of several organic solvents and of aqueous binary mixtures on the fluorescence properties of AT and BF was examined. As can be seen in Table 2, AT and BF displayed native fluorescence in all media under study. They showed similar structures in the excitation and emission fluorescence spectra (Fig. 2). A small shift in the emis-

sion wavelength occurred upon changing the solvent polarity, with a single maximum emission band located at around 296–301 nm, according to the solvent. In the case of the excitation spectra, two bands were located at 222–225 and 270–278 nm for AT and at 223–232 nm and 270–278 nm for BF. A 47 to 73 nm red shift was observed for AT and BF in aprotic solvents, including DMSO, DCM, and butyl acetate, relative to the protic solvents.

Among all solvents under study, the highest fluorescence intensity (I_F) was observed in methanol (Fig. 2). In order to optimize the AT and BF I_F values and to evaluate the influence of water in binary mixtures on I_F , different binary aqueous mixtures were tested with several solvents of different polarity (MeOH, 2-PrOH, ACN, and DMSO). It is worthwhile to mention that, for both compounds, the highest fluorescence signal in binary mixtures was found for a MeOH/H $_2$ O (60/40 v/v) mixture (Fig. 2). A similar behavior was observed in the case of other binary mixtures, such as ACN/water 70/30 v/v, and 2-PrOH/water 60/40 v/v.

Therefore, the MeOH/water 60/40 v/v mixture, which yielded the highest I_F value for AT and BF, could be considered the most convenient medium from an analytical standpoint.

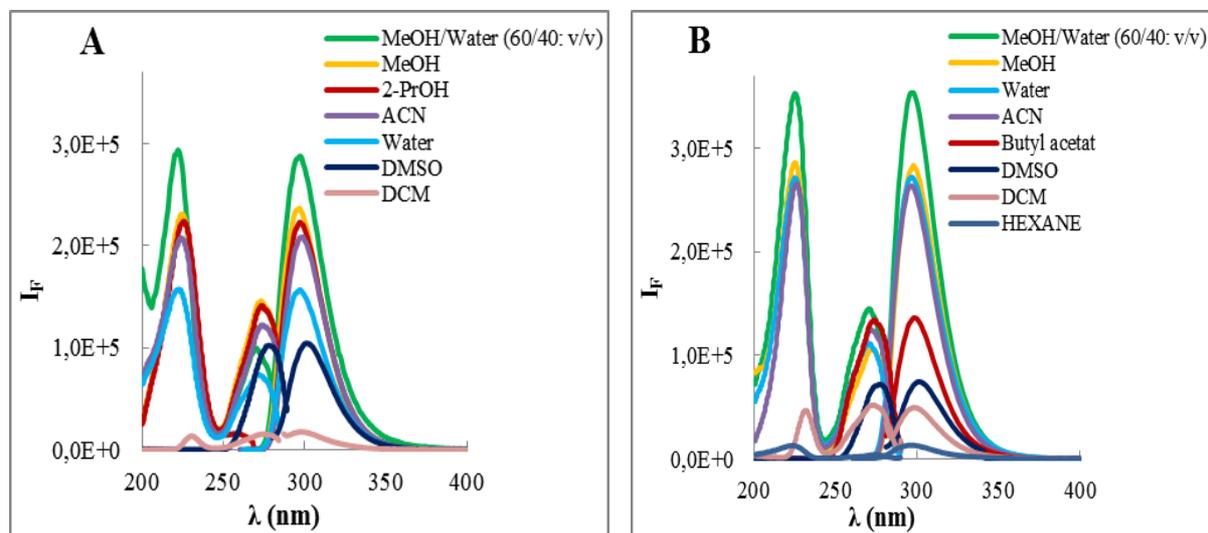


Fig. 2. Fluorescence excitation and emission spectra of 2.10^{-5} M (A) atenolol and (B) bisoprolol in different solvents and in MeOH/Water (60/40 v/v) mixture

2.10. Effect of pH on the fluorescence spectra

We investigated the effect of pH on the fluorescence signal of AT and BF in water and in the MeOH/water (60/40 v/v) mixture (Fig. 3A and 3B). Both β -blockers could be considered weak

bases ($pK_a = 9.2$). In water, at pH 7, they are protonated and positively charged.

For AT, the curves of I_F vs pH, established in the 2–12 pH range (Fig. 3A), indicated that I_F increased progressively from pH 2 to pH 5, reached a maximum value in the pH 5–6 region,

and then decreased between pH 7 and 12 in water. In MeOH/water (60/40 v/v) mixture, a rapid increase of I_F was noted in the pH range 2 to 4, a maximum I_F value was reached at pH 6, and a decrease took place until pH 12.

In the case of BF, I_F varied very little in water between pH 2 to 8 and then decreased until pH

12 (Fig. 3B). An I_F increase was observed in the MeOH/water (60/40 v/v) mixture for the pH range 2–4. Then, the I_F value became practically constant in the pH 7–11 region and decreased in stronger basic media. Therefore, a mean fixed pH value of 6.8 was selected for both β -blockers.

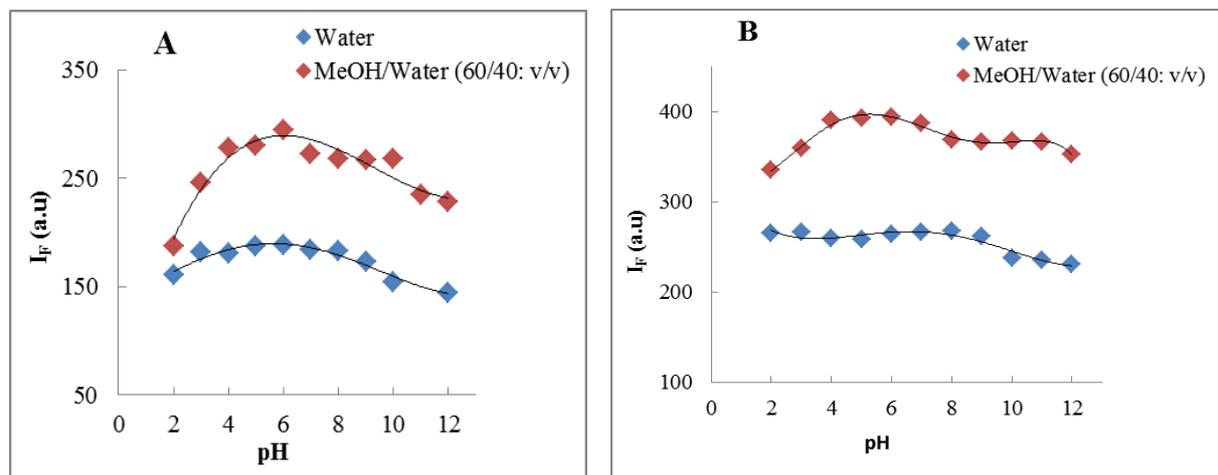


Fig. 3. pH effect on the fluorescence intensity of (A) atenolol ($3 \cdot 10^{-6}$ M) and (B) bisoprolol ($2 \cdot 10^{-6}$ M) in water and in MeOH/water (60/40 v/v) mixture

Table 1

Electronic absorption spectral characteristics of AT and BF in different media

Solvent	Atenolol		Bisoprolol furamrate	
	λ_{abs} (nm)	ϵ_{max} ($\text{l mol}^{-1} \text{cm}^{-1}$)	λ_{abs} (nm)	ϵ_{max} ($\text{l mol}^{-1} \text{cm}^{-1}$)
MeOH	222	16775	224	16620
	273	2660	273	1600
2-PrOH	222	15621	224	16445
	270	1800	270	1590
ACN	226	16200	224	17580
	276	2600	274	1860
Water	222	18825	222	16280
	272	1642	272	1500
DMSO	278	7550	275	5175
DCM	232	14350	232	14900
SDS	222	19200	224	15575
	274	1820	275	1840
MeOH/Water (60/40: v/v)	223	17075	223	19600
	273	1700	273	2100
ACN/Water (70/30: v/v)	224	15420	223	17201
	274	1610	274	1620
Butyl acetate	–	–	274	2725
Hexane	–	–	220	3751

UV-VIS (concentration = 10^{-4} M), λ_{abs} = absorption maximum wavelength, ϵ = molar absorption coefficient

Table 2

Fluorescence spectral characteristics of AT and BF in different media

Solvent	Atenolol			Bisoprolol fumarate		
	λ_{ex} (nm)	λ_{em} (nm)	I_{F}	λ_{ex} (nm)	λ_{em} (nm)	I_{F}
MeOH	224	297	13.4	224	297	6.9
	273		9.5	274		2.0
2-PrOH	222	297	12.7	226	298	5.8
	273		9.2	275		1.8
CAN	225	300	11.6	225	297	6.2
	274		8.0	272		2.4
Water	222	297	8.9	226	298	6.6
	271		4.5	271		2.1
DMSO	277	301	5.9	278	300	1.6
DCM	273	297	1.0	273	297	1.0
	232		0.8	232		0.9
SDS	225	297	14.6	226	278	7.4
	273		8.2	274		2.9
MeOH/Water (60/40: v/v)	224	297	18.0	225	298	8.2
	270		6.5	275		2.7
ACN/Water (70/30: v/v)	225	300	16.4	226	298	7.0
	272		5.3	274		2.3
Butyl acetate		-		274	298	3.0
Hexane		-		223	296	0.3

AT and BF (concentrations = $2 \cdot 10^{-5}$ M), λ_{ex} = excitation wavelength, λ_{em} = emission wavelength, I_{F} = relative fluorescence intensity normalized to dichloromethane

2.11. Fluorescence in organized media (cyclodextrins and surfactants)

In order to optimize the fluorescence intensity of AT and BF, the effect of concentrations of cyclodextrins (HP- β -CD = $2 \cdot 10^{-2}$ M and β -CD = $1 \cdot 10^{-2}$ M) and of surfactants [SDS = $2 \cdot 10^{-2}$ M, Tween 80 = $2 \cdot 10^{-2}$ M and Triton X = $5 \cdot 10^{-4}$ M] was studied.

The addition of fixed cyclodextrin and surfactant concentrations to the β -blocker solutions ($2 \cdot 10^{-5}$ M) produced, in most cases, an enhancement of their fluorescence intensity relative to water (Fig. 4). However, no significant spectral change was noted in all media under study. In all organized media, the fluorescence intensity of both β -blockers was the highest in the presence of SDS relative to the other organized media. As shown in Figure 4, the fluorescence intensity of both β -blockers was maximum for an optimal SDS concentration of $2 \cdot 10^{-2}$ M. The fluorescence emission spectra characteristics of both β -blockers were not affected, and the fluorescence intensity gradually increased with the SDS concentration in the range 0 – $2 \cdot 10^{-2}$ M in water (1.7 fold and 1.4 fold relative

to water for AT and for BF, respectively). However, in the case of Tween 80, the fluorescence signal of both β -blockers was drastically quenched, and Triton X exhibited an important natural fluorescence at the same emission wavelength as both β -blockers (about 300 nm), which prevented the use of these surfactants for the spectrofluorimetric study of both β -blockers. As shown in Figure 5, the AT and BF fluorescence intensity increased in the presence of SDS, in agreement with the literature data.⁴¹ The increased fluorescence in micellar media was attributed to the stabilization/protection of the excited singlet state, which prevented the fluorescence decay and non-radiative quenching processes. The critical micellar concentration (CMC) values were determined from Figure 5. As can be seen, the curve describing the evolution of the fluorescence intensity of β -blockers as a function of SDS concentration comprised three parts. The CMC corresponded to the intersection between the trend curve (Fig. 5, part I, residual phase in water) and the micellization phase curve (Fig. 5, part II). Therefore, the CMC values were $8 \cdot 10^{-3}$ mol l⁻¹ for atenolol and $6 \cdot 10^{-3}$ mol l⁻¹ for bisoprolol.

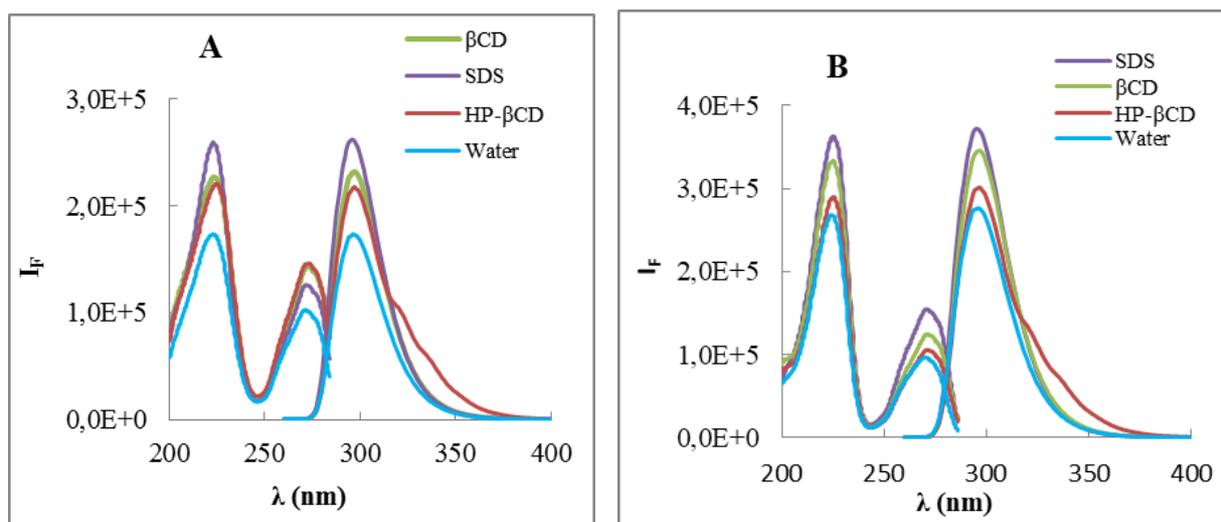


Fig 4. Fluorescence excitation and emission spectra of ($2 \cdot 10^{-5}$ M) atenolol (A) and bisoprolol (B) solutions in pure water, in HP- β -CD (0.01M), in β -CD (0.01M), and in SDS (0.02M) $\lambda_{\text{ex}} = 224$ nm and $\lambda_{\text{em}} = 297$ nm

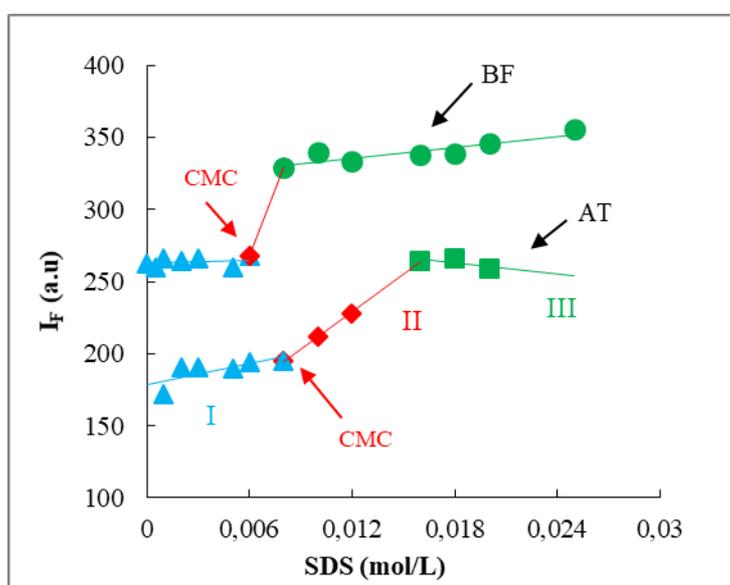


Fig. 5. Effect of SDS concentration on the AT ($3 \cdot 10^{-6}$ M) and BF ($2 \cdot 10^{-6}$ M) fluorescence intensity at pH = 6.8 ($\lambda_{\text{ex}} = 224$ nm and $\lambda_{\text{em}} = 297$ nm)

2.12. Photodegradation kinetic study

In order to evaluate the possible persistence of AT and BF in the environment, we studied their photodegradation kinetics in water by following the evolution of the fluorescence signal (I_F) with the UV irradiation time (t_{irr}) at the analytical excitation and emission wavelengths. Curves of I_F for AT ($3 \cdot 10^{-6}$ M) and BF ($2 \cdot 10^{-6}$ M) vs t_{irr} in water were characterized by a slow decrease of the fluorescence signal, indicating the progressive degradation of both β -blockers, leading to weakly fluorescent product(s). As can be seen in Figure 6, only about 20 % of AT and 40 % of BF were degraded

in water for $t_{\text{irr}} = 120$ min. A linear plot of the curve of $\ln(I_0/I)$ vs t_{irr} was obtained, with correlation coefficients near unity for both β -blockers. Our results indicated that the degradation reactions obeyed first-order kinetics, with rate constant (k) values of $2.1 \cdot 10^{-3} \text{ min}^{-1}$ and $4.2 \cdot 10^{-3} \text{ min}^{-1}$ for AT and BF, respectively. A significantly faster photodegradation rate in water was found for BF (half-life time ($t_{1/2}$) = 165 min) than for AT ($t_{1/2} = 330$ min) (Table 3). In addition, our results indicated the relative stability and persistence of AT and BF and showed the relatively slow photodegradation of both β -blockers in water under UV irradiation.

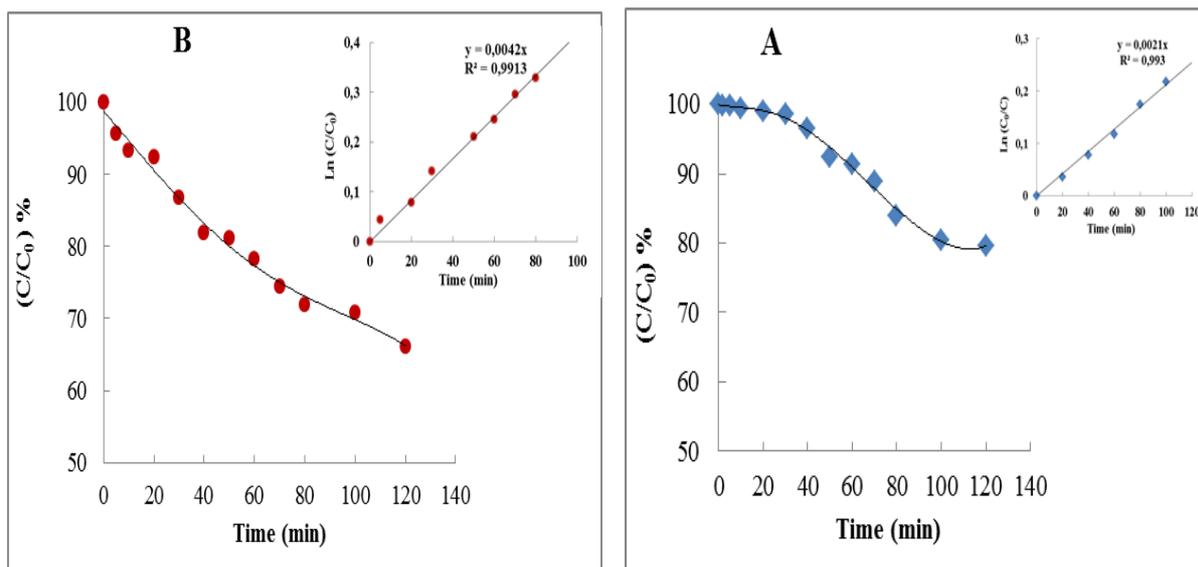


Fig. 6. Effect of t_{irr} on the photodegradation of $3 \cdot 10^{-6}$ M AT (A) and $2 \cdot 10^{-6}$ M BF (B) in water. Insert: kinetic curves for $\log C/C_0 = f(t)$

Table 3

Kinetic parameters of the β -blocker photolysis reactions

Compound	λ_{em} (nm)	Order	r^2	$t_{1/2}$ (min)	$k \cdot 10^{-3}$ (min^{-1})
AT (3×10^{-6} M)	297	1	0.993	330	2.1
BF (2×10^{-6} M)	297	1	0.991	165	4.2

2.13. Analytical figures of merit

In order to select the optimal medium for the analytical study of AT and BF, the analytical figures of merit for the determination of AT and BF by the spectrophotometric UV-VIS and fluorescence methods were compared in various media (Table 4).

Calibration graphs were drawn for AT and BF under the optimal conditions, using the optimal signal at the λ_{abs} or λ_{em} maximum (Table 4). Analytical figures of merit were obtained from 3-5 measurements performed on at least six concentrations for each compound. Linear calibration graphs were established by plotting the UV-VIS absorbance or the fluorescence intensity vs. the AT or BF concentration [$Abs = f(C)$ or $I_F \propto (C)$]. The correlation coefficient (R^2) values (> 0.99) suggested excellent precision of measurements. Our results, summarized in Table 4, indicated linear dynamic ranges (LDR) of about one to two orders of magnitude. The reproducibility of measurements was satisfactory, as shown by the small relative standard deviation (RSD) values ranging from 0.7 to 2.0% for the UV-VIS absorption method and from

0.6 to 5.3% for the fluorescence method. The limit of detection (LOD) and limit of quantification (LOQ) values were calculated on the basis of a β -blocker concentration giving a signal-to-noise ratio (S/N) of 3 and 10 (IUPAC criterium), respectively. The LOD values for the UV-VIS absorption spectrophotometry ranged from 36.7 to 51.1 ng/ml for BF and from 18.9 to 28.2 ng/mL for AT. It is worthwhile to note that the LOD and LOQ values obtained by the fluorescence method were much lower than those measured by the UV-VIS absorption method, which were between 1.3 and 5.4 ng/ml for BF and 1.2 and 3.7 ng/ml for AT. The LOQ values ranged from 4.5 to 18.1 ng/ml for BF and from 4.0 to 12.5 ng/ml for AT, according to the medium.

Our results confirmed that the fluorescence method was much more sensitive than UV-VIS absorption spectrophotometry. Moreover, the MeOH/water (60/40 v/v) medium gave the lowest LOD values for both compounds, increasing the sensitivity and precision of the fluorescence method for the determination of AT and BF. For these reasons, the MeOH/water (60/40 v/v) mixture appeared to be the most convenient medium for de-

termining AT and BF concentrations in fortified or real water samples.

We compared also our results of the fluorescence method for the determination of β -blockers with literature data (Table 5). We found that our spectrofluorimetric method gave, in most cases, lower LOD and LOQ values than literature values. For example, higher LOD values of 870 ng/ml and 40 ng/ml were obtained, respectively, by Bakir et al. (2018)³⁸ for the determination of AT in urine, using the quenching effect of AT on the photoluminescence of gold nanoparticles, and by Bavili

Tabrizi et al. (2019)⁴² for the determination of AT in pharmaceutical preparations. Also, Abdelwahab (2018)³⁷ reported the analysis of BF in human plasma by spectrofluorimetry with a LOD value of 6 ng/ml. Moreover, LOD values of 2.7 ng/ml for AT and 0.1 ng/ml for BF, close to ours, were obtained by Gil-Garcia et al. (2011)²⁵ using the LC-DAD method. It is worthwhile to note that the LOD values obtained by LC-MS/MS for the analysis of AT and BF in the water samples were better than ours.¹²

Table 4

Analytical figure of merit for the spectrophotometric and spectrofluorimetric methods of determination of AT (A) and BF (B)

A	Spectrophotometric method						Spectrofluorimetric method					
	Solvent	λ_{Abs} (nm)	LOD (ng/ml)	LOQ (ng/ml)	R^2	LDR (ng/ml)	RSD (%)	λ_{em} (nm)	LOD (ng/ml)	LOQ (ng/ml)	R^2	LDR (ng/ml)
MeOH	222	25.1	83.7	0.99	540–13500	0.9	297	1.6	5.4	0.99	13.5–800	2.2
2-PrOH	222	25.2	84.1	0.99	540–13500	0.8	297	2.2	7.2	0.99	13.5–800	2.3
ACN	226	28.2	94.1	0.99	540–13500	0.7	300	2.0	6.8	0.99	13.5–800	0.6
Water	222	21.7	72.3	0.99	540–13500	1.0	297	3.3	11.0	0.99	25–1000	0.8
DMSO	278	–	–	–	–	–	301	3.7	12.5	0.98	135–1600	4.6
SDS	222	18.9	68.1	0.99	135–10600	1.9	297	3.0	10.2	0.99	13.5–800	4.0
MeOH/Water (60/40 v/v)	222	23.9	73.6	0.99	540–13500	1.6	297	1.2	4.0	0.99	13.5–800	2.5
ACN/Water (70/30 v/v)	223	23.4	78.0	0.99	540–13500	0.6	300	1.9	6.4	0.99	13.5–800	1.0

B	Spectrophotometric method						Spectrofluorimetric method					
	Solvent	λ_{Abs} (nm)	LOD (ng/ml)	LOQ (ng/ml)	R^2	LDR (ng/ml)	RSD (%)	λ_{em} (nm)	LOD (ng/ml)	LOQ (ng/ml)	R^2 (%)	LDR (ng/ml)
MeOH	224	41.3	137.3	0.99	880–22000	2.0	297	1.5	5.0	0.99	15–1200	3.94
ACN	224	40.5	134.9	0.99	880–22000	1.3	297	1.9	6.2	0.99	15–1200	3.12
Water	222	43.1	143.5	0.99	880–22000	1.7	298	1.7	5.6	0.99	15–1200	4.40
DMSO	275	–	–	–	–	–	300	5.4	18.1	0.99	220–4400	5.32
SDS	224	51.1	171.1	0.99	880–22000	1.9	298	1.4	4.8	0.99	15–1200	2.35
MeOH/Water (60/40 v/v)	223	36.7	122.5	0.99	880–22000	2.0	297	1.3	4.5	0.99	15–1200	2.36
ACN/Water (70/30 v/v)	223	45.0	150.1	0.99	880–22000	1.6	298	1.4	4.7	0.99	15–1200	2.84

LOD = Limit of detection, defined as the analyte concentration giving a signal-to-noise (S/N) ratio of 3.

LOQ = Limit of quantification, defined as the analyte concentration giving an S/N ratio of 10. R = correlation coefficient.

LDR = Linear dynamic range. RSD = Relative standard deviation (in %).

Table 5

Comparison of literature methods of determination of AT and BF

β -blocker	Sample	Method	Protocole	Mean recovery (%)	Linear range (ng/ml)	LOD/ LOQ (ng/ml)
Atenolol Bisoprolol This work	Pure water	Spectrofluorimetry	LLE ^a	101.5	25–1000	3.3
			$\lambda_{\text{ex}}=224\text{nm}$; $\lambda_{\text{em}}=297\text{nm}$	100.4	15–1200	1.7
Atenolol Bisoprolol +5 β -blockers ¹²	Influent and Effluent	LC/MS/MS ^c	SPE ^b	103.8	1–100	0.0008
				82.3	1–100	0.00034
Atenolol, Bisoprolol ⁴³	Human bones	CG-MS ^d	SPE	106	0.1–15	0.1ng/mg
				60	0.3–150	0.3ng/mg
Atenolol Bisoprolol +5 β -blockers ²⁵	River water	LC-DAD ^e	SPE-LC-LC	88.5	5.0–30	2.7
				103.3	1.0–30	0.1
Atenolol ⁴⁴	Human plasma	HPLC-FLD ^f	LLE $\lambda_{\text{ex}}=276\text{ nm}$; $\lambda_{\text{em}}=297\text{nm}$	98.4	5–150	1.5
Atenolol ³⁸	Urine	Spectrofluorimetry	Quenching by AuNPs $\lambda_{\text{em}}=705\text{ nm}$	99.1	2500–10000	870
Atenolol Carvedilol ⁴²	Pharmaceutical preparations	Spectrofluorimetry	SDS $\lambda_{\text{ex}}=274\text{ nm}$; $\lambda_{\text{em}}=302\text{ nm}$	101.8	130–750	40
Bisoprolol Rosuvastatin ³⁷	Plasma	Spectrofluorimetry	$\lambda_{\text{ex}}=227\text{ nm}$; $\lambda_{\text{em}}=297\text{nm}$	99.7	20–500	6
Bisoprolol ⁴⁵	Plasma	HPLC-FLD	Microextrac tion $\lambda_{\text{ex}}=275\text{ nm}$ $\lambda_{\text{em}}=305\text{ nm}$	61.4	10–100	3

^a LLE = liquid-liquid extraction, ^b SPE = solid-phase extraction, ^c LC/MS/MS = liquid chromatography-tandem mass spectrometry, GC-MS = gas chromatography-mass spectrometry, ^e LC-DAD = liquid chromatography-diode array detector, ^f HPLC-FLD = high performance liquid chromatography-fluorescence detector

2.14. Analytical applications

In order to test the analytical applicability and the efficiency of our spectrofluorimetric method, both β -blockers AT and BF were analyzed in authentic water samples collected in Senegal and in France. Water samples collected from the effluents of the wastewater treatment plants and rivers were probably the most impacted by AT and BF residues.

The water samples, including natural water (river and well water) and wastewater (tertiary effluents), were first spiked with 100 ng/ml of AT and 200 ng/ml of BF, then purified by the above-described liquid-liquid extraction procedure. The spectrofluorimetric method, applied to the various AT- and BF-containing water samples, was carried out in a MeOH/water (60/40 v/v) mixture. The standard addition procedure was performed by adding increasing β -blocker concentrations to the AT or BF standard solutions and by realizing AT and BF recovery experiments on the fortified tap water, natural water, and wastewater samples. In order to eliminate any possible interfering species, all water samples under study were checked to be

free of fluorescent dissolved species. The standard addition plots were linear over the concentration range under study, and the slope values of the calibration curves and standard addition curves were practically identical for all water samples, as shown in Figures 7a and 7b (see: Supplementary Material). Analytical results are presented in Table 6.

Satisfactory mean recovery values were obtained for both β -blockers, ranging from 98.1 to 105.2 % in tap water, 97.5 to 109.0 % in well water, 96.0 to 104.7 % in river water, and 93.3 to 107.1 % in tertiary effluent. RSD values were rather small, ranging from 1.1 % to 2.6 % for AT and from 2.0 % to 3.1 % for BF, which indicated a good reproducibility of the direct spectrofluorimetric method for analytical applications. The study of the AT and BF degradation kinetics in water demonstrated the persistence of both β -blockers up to 120 min. To show the analytical applicability of the proposed spectrofluorimetric method to the selected authentic samples, recovery experiments of both β -blockers were performed on spiked tap water samples free of possible fluorescent dissolved species.

Table 6a

Analytical features of AT determination and recovery values in spiked tap water, well water (Senegal), river water, and wastewater effluent samples by the standard addition procedure

Water sample	Added	Found	Recovery (%)	Mean recovery	RSD (%)
Tap water	100	100.7	100.7	100.9	1.2
	200	198.3	99.1		
	300	314.2	104.7		
	500	497.8	99.6		
	700	708.3	101.2		
Well water	900	900.7	100.1	102.3	1.1
	100	107.7	107.7		
	200	209.2	104.6		
	300	306.8	102.2		
	500	492.2	98.4		
River water	700	702.0	100.2	99.2	2.6
	900	903.2	100.3		
	100	103.0	103.0		
	200	192.0	96.0		
	300	298.1	99.4		
Effluent (WWTP)	500	497.8	99.5	96.3	2.5
	700	680.9	97.2		
	900	900.2	100.0		
	100	101.00	101.0		
	200	186.6	93.3		
Effluent (WWTP)	300	285.8	95.3	96.3	2.5
	500	472.7	94.5		
	700	684.3	97.7		
	900	903.2	100.3		

RSD: Relative standard deviation

3. CONCLUSION

In this work, we developed a simple, rapid, and sensitive spectrofluorimetric method suitable for analysis of the β -blockers AT and BF in environmental waters. The analytical usefulness of this method was significantly improved in a MeOH/water (60/40 v/v) mixture, which confirmed its good sensitivity and precision for both β -blockers. The analytical figures of merit demonstrated the simplicity and the rapidity of the method. Low-cost equipment was needed, and no complicated pretreatment was required. Also, we demonstrated that spectrofluorimetry was more sensitive than UV-VIS absorption spectrophotometry. Moreover, the rather small decrease of fluorescence intensity observed under UV irradiation showed that both β -blockers were relatively stable with rather long half-life times of 330 min for AT and 165 min for BF, which explained their presence in natural waters. Satisfactory applications were developed in tap water, river water, well water, and effluent water by using the standard addition method. According to the water sample, we

Table 6b

Analytical features of BF determination and recovery values in spiked tap water, well water (Senegal), river water, and wastewater effluent samples by the standard addition procedure

Water sample	Added (ng/ml)	Found (ng/ml)	Recovery (%)	Mean recovery	RSD (%)
Tap water	200	201.1	100.5	102.0	2.0
	350	368.2	105.2		
	500	523.92	104.8		
	800	820.1	102.5		
	1100	1107.2	100.6		
	1400	1374.0	98.1		
Well water	200	217.7	108.8	100.6	3.0
	350	358.5	97.5		
	500	542.2	104.7		
	800	833.1	101.9		
	1100	1109.5	99.2		
	1400	1415.8	99.8		
River water	200	198.4	99.2	100.9	2.9
	350	366.5	104.7		
	500	510.08	102.0		
	800	809.0	101.1		
	1100	1113.0	101.2		
	1400	1364.0	97.4		
Effluent (WWTP)	130	139.4	107.2	101.9	3.1
	280	284.6	101.6		
	430	442.4	102.9		
	730	745.0	102.0		
	1030	1028.1	99.8		
	1030	1028.1	99.8		

obtained good recovery percentage values, ranging between 93.3 and 107.8 % for AT and between 97.4 and 108.9 % for BF. The viability of the spectrofluorimetric method demonstrated that it was a suitable technique for the analysis of AT and BF in environmental waters.

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