Photolysis of β-blockers in environmental waters

Anne Piram, Arnaud Salvador *, Cécilia Verne, Bernard Herbreteau, René Faure

Université de Lyon, F-69622, Lyon, France
Université Lyon 1, Villeurbanne, France
CNRS, UMR 5180, Laboratoire des Sciences Analytiques, France

ARTICLE INFO

ABSTRACT

Many drugs such as β-blockers have been shown to occur in aquatic environments. Even if adequate eco-toxicity data are not available, it is of primary importance to get informations about their fate in environmental waters, particularly about their photofate in sewage treatment plant effluents (STP). The main difficulties when studying pharmaceutical photochemical behaviour in environmental waters, are linked to the very low environmentally relevant concentrations ($ng\,L^{-1}$ to $\mu g\,L^{-1}$) which can generate problems in terms of analytical sensitivity. Moreover, the complexity of environmental matrices can modify micropollutants degradation kinetics. The photodegradation of β-blockers has been compared at two concentration levels ($10\,\mu g\,L^{-1}$ and $10\,mg\,L^{-1}$) and in two different matrices (pure water and STP effluent). It has been shown that the concentration does not influence β-blockers degradation pathways, thus allowing the identification of degradation compounds using the $10\,mg\,L^{-1}$ solutions. Although environmental waters speed up the degradation process, the same photoproducts were appeared in both matrices. Using LC–MS/MS, hydroxyl radical additions have been identified as an important degradation pathway for especially pindolol, propranolol and timolol, leading to several positional isomers, corresponding to mono-, di- or tri-hydroxylations. Kinetics of appearance/disappearance of these photoproducts have been studied in STP effluents.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Many drugs have been shown to occur in aquatic environments. Until today, most efforts have been carried out to develop sensitive analytical methods allowing their quantification in complex matrices such as sewage treatment plant (STP) waters at very low concentration (Zühlke et al., 2004; Castiglioni et al., 2005; Salvador et al., 2007; Piram et al., 2008). Some monitoring studies have also been performed and pharmaceuticals were generally detected in the $ng\,L^{-1}$ to the low $\mu g\,L^{-1}$ range (Ternes, 2001; Heberer, 2002; Kolpin et al., 2002; Yasojima et al., 2006). As an example, β-blockers which are usually prescribed as anti-hypertensive or anti-arrhythmic in human medicine, have been quantified up to $2\,\mu g\,L^{-1}$ in STP effluents (Huggett et al., 2003) and to $240\,ng\,L^{-1}$ in rivers (Zuccato et al., 2005). Even if full adequate ecotoxicity data are not available (Ankley et al., 2007), they have been shown to be ecotoxicologic substances as they can affect cardiac rhythm, generate abnormalities or reduce mobility of spermatozoids of fish (Huggett et al., 2002; Cleuvers, 2005; Dzialowski et al., 2006).

In environmental waters, organic compounds such as pharmaceuticals can undergo various degradation pathways, such as biotic and abiotic (hydrolysis, photolysis) degradation. The environmental stability of organic pollutants is important to consider in order to perform a suitable prediction on their environmental fate. However, little is known about pharmaceuticals stability in environmental waters and research efforts have to be focused on this thematic. The environmental stability of a particular compound is important in order to estimate its environmental fate. As an example, propranolol half-life determination was used in a river catchment model by Robinson et al. (2007). By means of this model, they showed that predicted environmental concentrations could decrease by 11–68% along a river. Determination of β-blockers photochemical half-lives is then of high importance in order to assess their environmental fate. Propranolol, metoprolol and atenolol half-lives under xenon arc lamp irradiation have already been investigated by Liu and Williams (2007) in pure water. This study has shown a high photolability of propranolol ($t_{1/2} < 21\,h$) whereas metoprolol and atenolol are poorly degraded under UV exposure (half lives of several hundred hours). In their study, a particular interest has been carried out on propranolol photodegradation at environmentally relevant concentrations. A large number of photoproducts were observed and some of them have been characterised. Photoproducts structures have also been reported by Andrisano et al. (1999) and Andrisano et al. (2001) for labetalol and atenolol and by Sortino et al. (2002) and Uwai et al. (2005) for propranolol. However, these experiments were

* Corresponding author. Address: Université de Lyon 1, France. Tel.: +33 4 72 43 11 52; fax: +33 4 72 44 62 02.
E-mail address: arnaud.salvador@univ-lyon1.fr (A. Salvador).

not environmentally relevant as they have been performed in organic solvent, solid state or in concentrated media about 1 g L^{-1}. Thus, undoubtedly, the proposed structures could be formed, but more experiments would be necessary to assess the possibility of their appearance under environmental conditions.

In fact, all published studies were performed in pure water or solvent. However, environmental matrices components are known to influence the photodegradation of micropollutants. For example, it is known that dissolved organic matter (DOM) such as humic acids act as inner filters towards some pharmaceuticals and as photosensitizers towards some others (Zepp et al., 1985; Andreozzi et al., 2003). Inorganic ions, such as nitrite or nitrate favour free hydroxyl radical production which are likely to oxidise organic pollutants (Mack and Bolton, 1999). So, indirect photolysis can take place in environmental matrices and acquisition of data in such matrices is important.

The aim of this work, was to obtain information about β-blockers photochemical stability in environmental waters: (1) to predict β-blockers photofate in the environment and (2) to identify the main β-blockers photoproducts initially appeared in the photodegradation process. When photolysis experiments are performed in laboratory scale and at environmentally realistic concentrations, some problems arise since high concentrations are necessary for photoproduct characterisation and low concentrations are required to be environmentally relevant. Besides, in environmental matrices, indirect photolysis could result in a different photochemical behaviour of target analyte, compared to direct photolysis experiments conducted in pure water. Consequently, it is necessary to ensure that neither the concentration, nor the matrix, affect β-blockers photodegradation prior to attempt the characterisation of photoproducts. In this paper, the photochemical behaviour of several β-blockers is compared: (1) in pure and environmental water and (2) in concentrated and diluted solution.

2. Experimental

2.1. Chemicals

Acebutolol hydrochloride (100%) (Ac), alprenolol hydrochloride (99%) (Al), atenolol (99%) (At), metoprolol tartrate salt (100%) (M), nadolol (100%) (N), propranolol hydrochloride (99%) (Pr), pindolol (99%) (Pi), sotalol hydrochloride (99%) (S) and timolol maleate salt (99.9%) (Ti) were provided by Sigma–Aldrich (Saint Quentin Fallavier, France). Bisoprolol fumarate (100%) (Bi) was provided by Sequoia research (Pangbourne, United Kingdom). Methanol HPLC grade (MeOH) was provided by SDS-Carlo-Erba (Peypin, France). Acetonitrile LC–MS grade (AcN), ammonium formate (99%) and formic acid (FOA) were obtained from Fisher Scientific (Val de Rueil, France). Ultra-pure water was generated by an Elgastat UHQ PS from Elga LabWater (High Wycombe Bucks, United Kingdom).

Each β-blocker stock solution was prepared in MeOH at 1000 mg L^{-1}. A 50 mg L^{-1} standard working (WS) solution containing the 10 β-blockers was prepared in MeOH. These solutions were stored at 4 °C, in the dark.

Low concentration (10 μg L^{-1}) experiments were performed by spiking 1 L of water with 200 μL of WS in order to estimate matrix influence on β-blockers degradation kinetics.

The photoproduct kinetics of four β-blockers (Ac, Pr, Pi and Ti) were studied separately from 10 mg L^{-1} solutions prepared by dissolving 10 mg of the required compound in 1 L of water.

UV spectra have been acquired for 10 mg L^{-1} aqueous solutions of β-blockers on a UV–visible spectrometer UV-1605 from Shimadzu (Champs-sur-Marne, France).

2.2. Sampling

Environmental matrices samples were collected from the outlet effluent of Pierre Benite STP (downstream from Lyon, France). These effluent samples were filtered up to 0.45 μm on membrane filters provided by Millipore (Molsheim, France) and stored at 4 °C. They have been used as photodegradation matrix within 1 week.

2.3. Photodegradation under UV light

Irradiation was carried out in a 3 L Pyrex glass immersion photochemical reactor, cutting out wavelengths shorter than 280 nm. The photoreactor was charged with 1 L of aqueous solution prepared as mentioned above. A high-pressure vapour mercury lamp (HPK 125 W, Cathodeon, Cambridge, England), emitting in the 250–600 nm range with a maximum emission at 365.5 nm, was placed in a water-jacked Pyrex tube (diameter 3 cm) centred in the reactor. The progress of the reaction was followed by successive samplings (1 mL).

In order to ensure that observed degradations were only due to photochemical processes, β-blockers stability in aqueous solution was previously verified, by storing 10 mg L^{-1} individual solutions and 10 μg L^{-1} mixtures in the dark and at room temperature. Dark controls in STP effluent were performed for each β-blocker which were proved to be stable during three months. As no hydrolysis was observed on control samples, it was considered that β-blockers degradation in aqueous solution under UV exposure was exclusively initiated by photochemical process.

2.4. Liquid chromatography–tandem mass spectrometry

The HPLC device consisted of Agilent 1100 series pump and autosampler (Massy, France). For each analytical method, a gradient elution was performed using solvent A which was formate buffer (10 mM ammonium formate acidified to pH 3.8 by FOA addition) and solvent B which was AcN.

The HPLC device was coupled to a Sciex API 300 triple quadrupole mass spectrometer from MDS Sciex (Toronto, Canada) equipped with a TurboIonspray Source (TIS) operating in positive ion mode. Instrument control, data analysis and processing were performed using the associated Analyst 1.4.1 software.

The nebulizer (zero air) and the curtain gas flow (nitrogen) were set at 10 (arbitrary units). The TIS source was operating at 500 °C with a flow of auxiliary gas (zero air) set at 8 l min^{-1}. The TIS voltage was set at 5 kV and the orifice and ring voltage were set at 21 and 150 V, respectively for Ac, At and M; 16 and 140 V for Al, Pr and Ti; 21 and 140 V for Bi and N; 16 and 130 V for Pi; and 6 and 120 V for S.

Degradation solutions containing all the ten β-blockers at 10 μg L^{-1} were separated by liquid chromatography on a 5-μm Hibar Purospher Star C$_{18}$ column (250 × 4.6 mm i.d., pore size 120 Å, endcapped) from Merck (Darmstadt, Germany) and detected by the mass spectrometer in the selected reaction monitoring (SRM) mode. This analytical method has been fully described in a previous article (Piram et al., 2008).

Degradation solutions of each separate β-blocker at 10 mg L^{-1} were separated by liquid chromatography on a 5-μm Aquasil C$_{18}$ column (100 × 2.1 mm i.d., pore size 100 Å, hydrophilic endcapped) operating at 0.2 ml min^{-1}, for Ac, Pi and Ti. For analysis of Pr degradation, a better chromatogram was obtained by using a 3.5-μm X-Terra MS C$_{18}$ column (50 × 2.1 mm i.d., pore size 125 Å, endcapped) operating at 0.2 ml min^{-1}. These solutions were analysed by the mass spectrometer in single ion monitoring mode (SIM), following molecular ion [M + H]$^+$ of the degraded molecule and masses corresponding to multiple additions of 16 (hydroxyl