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Photolytic and photocatalytic transformation of an antipsychotic drug asenapine: Comparison of kinetics, identification of transformation products, and *in silico* estimation of their properties

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ABSTRACT

The photolytic and photocatalytic transformation of an antipsychotic drug asenapine with the use of H_2O_2 and TiO_2 was studied. A method employing irradiation with a simulated full solar spectrum in the photostability chamber was applied, then the reverse-phase ultra high performance liquid chromatography with diode array detector, coupled with electrospray quadrupole time-of-flight mass spectrometer (RP-UHPLC-DAD – ESI-Q-TOF) was used for the quantitative and qualitative analysis of the processes. The developed quantitative method was fully validated, according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines, and the kinetic parameters of asenapine photodecomposition were compared. Nineteen phototransformation products were detected, and their probable structures – mainly hydroxylated and oxidized asenapine derivatives – were suggested. On the basis of the elucidated structures the computational prediction of their toxicity at the various endpoints, as well as bioconcentration factors and biodegradability was performed. The obtained results were then subjected to the principal component analysis (PCA). This chemometric technique facilitated comparison of the applied models, calculated properties of the TPs, and enabled visualization of relationships between them.

1. Introduction

Consumption of pharmaceuticals is still increasing, and according to the data published by Norwegian Institute of Public Health, sales of human medicines in Norway in 2015 were 8.6% higher than the year before (Norwegian Institute of Public Health, 2015). This conjuncture induces significant release of the drug substances, as well as their metabolites to the environment via variety of routes, for instance medical and pharmaceutical industry waste, agriculture, humans or animals excretions (Evgenidou et al., 2015; Silva et al., 2015). Obvious consequence of this state of things is occurrence of pharmaceuticals in the environmental samples, such as river and coastal waters or even drinking water (Azuma et al., 2015; Moreno-González et al., 2014; Petrović et al., 2014), and in the tissues of aquatic organisms (Brooks et al., 2005; Ramirez et al., 2007), which is particularly crucial due to proven ecotoxicity of this group of contaminants, for example to invertebrates (crustaceans, mollusca) (Henry et al., 2004; Lilius et al., 1995) and fish (Abreu et al., 2015; Brodin et al., 2013). Various drugs are also frequently detected in the waste samples, for instance wastewater (WW), wastewater effluents or sludge, and municipal solid wastes (Musson and Townsend, 2009; Peysson and Vulliet, 2013; Prieto-Rodriguez et al., 2012; Subedi and Kannan, 2015).

The Lack of regulations concerning monitoring of pharmaceuticals environmental fate and removal e.g. from WW is a well-known and still unsolved problem. Therefore drug active substances are classified as the emerging contaminants (Rivera-Utrilla et al., 2013). Because routinely applied methods of WW treatment (consisting of two stages: physicochemical and biological) are usually ineffective in this case, novel, many potentially more appropriate methods were suggested as the additional step of pharmaceutical substances removal (so-called tertiary treatment). The idea of a very wide group of these methods – advanced oxidation processes (AOPs) – is based on the creation of reactive oxygen species (ROS), such as hydroxyl radical or superoxide anion radical, which are usually able to completely decompose the contaminants, leading to the total mineralization of sample. Amongst AOPs major role play methods utilizing catalytic processes. Depending on the number of applied phases, they are divided into two groups: homogeneous (Fenton reaction, and its modifications, e.g. photo-Fenton, hydrogen peroxide combined with UV–Vis radiation) and heterogeneous photocatalysis. Effectiveness of the second one proceeds from interaction of radiation

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with surface of the catalyst particles. Since materials used as catalysts are semiconductors, absorption of photon of sufficient energy results in creation of electron-hole pair, followed by emergence of ROS, as well as direct degradation of contaminant molecule (Trawiński and Skibiński, 2017). However fitness of numerous substances for the photocatalytic removal of contaminants was investigated, still mostly used is TiO_2, especially composition of 25% rutile and 75% anatase (Hurum et al., 2003). Popularity of this semiconductor arises from its satisfactory physico-chemical properties and relatively low toxicity (Friedmann et al., 2010; Kanakaraju et al., 2014). Nonetheless various modifications of TiO_2 structure were introduced in order to enhance its photoreactivity and enable its application on an industrial scale (for instance doping, coupling with nanomaterials, immobilization). In spite of TiO_2 could be in general considered as the most effective photocatalyst, in some particular cases other semiconductors (e.g. zinc, tungsten, molybdenum, bismuth or vanadium compounds) may be more useful (Di Paola et al., 2012; He et al., 2016).

Plenty of approved pharmaceuticals are photolabile substances (over 250, according to European Pharmacopoeia), thus studies of their interaction with radiation of solar spectrum allow to investigate a significant part of drugs environmental fate, as well as development of suitable methods of their removal from wastes or environmental samples. On the other hand, in the case of photostable molecules (absorbing only < 290 nm radiation), application of AOPs could be an excellent way to solve the problem of ineffective WW treatment (Trawiński and Skibiński, 2017). It should be also noted, that assessment of elimination kinetic solely, without elucidation of intermediates or transformation products (TPs), may be insufficient, because in some cases formed TPs are more toxic than the parent compound (for example possibly genotoxic products of naproxen irradiation) (Isidori et al., 2005).

Asenapine ((3aRS,12bRS)-5-chloro-2-methyl-2,3,3a,12b-tetrahydro-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole) is an atypical antipsychotic drug, belonging to the dibenzoxepin class, used in treatment of schizophrenia and bipolar disorder. The molecule possesses affinity to dopaminergic (D_1 – D_4), serotoninergic (5-HT_{2A}, 5-HT_{2C}, 5-HT_6 and 5-HT_7), α_1 and α_2 adrenergic, and H_1 histaminergic receptors and was approved by both FDA and EMA in 2010 (Boer et al., 2012; Miller et al., 2013). Susceptibility of asenapine to UV–Vis irradiation was investigated by its exposition to sunlight for 24 h, and no degradation was observed (Chhalotiya et al., 2012). Since the psychotropic pharmaceuticals are frequently reported as the problematic pollutants, evaluation of their fate, as well as looking for effective methods of their elimination is crucial. For instance presence of mianserin, a compound possessing similar chemical structure to asenapine, in the environmental samples was proved by numerous studies, and its toxicity towards the model organisms was in some cases relatively high (Giebułtowicz and Nałęcz-Jawecki, 2014; Villain et al., 2016; Xiang et al., 2018). Taking into account that asenapine belongs to the group of frequently prescribed antipsychotic medications (Farina et al., 2014), its presence in the environment should be expected. Moreover, despite the structural similarities between mianserin and asenapine, these compounds posses significantly different mode of action – therefore also differences concerning their toxicity could be anticipated. According to the available literature asenapine possesses low or moderate toxic properties at several endpoints, such as endocrine disruption, genotoxicity, carcinogenicity or developmental toxicity (Amerio et al., 2015; Singh et al., 2018), nonetheless toxicity of the asenapine TPs was not estimated until now.

The aim of this study was to investigate photolytic and photocatalytic transformation of asenapine with the use of xenon lamp mimicking full solar spectrum, H_2O_2 and TiO_2 as the catalysts, elucidation of TPs structures, identification of reaction pathways, and comparison of the kinetic parameters. Toxicity, biodegradability and bioaccumulation factors of detected products was then calculated and compared by PCA.

2. Experimental

2.1. Materials

Asenapine maleate, formic acid for LC-MS, water for LC-MS and titanium (IV) oxide, nanopowder 21 nm particle size (Aeroxide® 25) were purchased from Sigma-Aldrich Co. (St. Louis, USA). Hypergrade acetonitrile for LC-MS and propan-2-ol (gradient grade) were purchased from Merck (Darmstadt, Germany). Hydrogen peroxide 30% pure p.a. was purchased from Chempur, (Piekary Śląskie, Poland).

2.2. Sample preparation

Stock solution of asenapine maleate was prepared in acetonitrile at concentration 1.41 mg/mL which corresponded to 1 mg/mL concentration of asenapine as a base, and was refrigerated at 7 °C. Working solutions were prepared by diluting the stock solutions in water and H_2O_2 to obtain 10 μg mL^{-1} concentration of asenapine and 0.03% (w/w) of H_2O_2. Concentration of acetonitrile did not exceed 1%, as it was recommended in the OECD Guidelines for the Testing of Chemicals (OECD, 2008).

Calibration of the quantitative method for the determination of asenapine was performed in the range 0.1–14.0 mg L^{-1}. Calibration solutions were prepared by diluting stock solution in water.

In order to investigate the adsorption process of asenapine, TiO_2 was added to aqueous working solutions at four loadings: 100, 200, 500 and 1000 mg L^{-1}, and then vigorously stirred in darkness for 30 min. Concentration of asenapine was determined at the beginning and at the end of the experiment. For photocatalytic experiments loading of 200 mg L^{-1} was chosen. In this case the adsorption kinetics was investigated in the range of time: 0–30 min.

2.3. Photolytic and photocatalytic experiments

For all experiments solutions and suspension were transferred into 3.5 mL quartz caped cells (l = 1 cm) mounted horizontally in Atlas Suntest CPS + photostability chamber (Linsengericht, Germany), and irradiated simultaneously. The irradiance was set to 250 W m^2 which corresponded to energy dose of 900 kJ m^{-2} h^{-1}. The chamber was equipped with a xenon lamp and D65 filter simulating full solar spectrum. The temperature in the chamber was controlled and kept below 35 °C. The TiO_2 sample was vigorously stirred (500 rpm) with the use of microstirrer (MINI Stirrer, Cimarel: Telemodul, Thermo Electron LED GmbH, Germany) and PTFE covered bar (l = 6 mm) during whole experiment. Dark control samples were also performed by exposing analyzed solutions and suspension in quartz cells wrapped in aluminum foil for the same period of time. In the case of direct photolysis and H_2O_2 photocatalytic experiment, 50 μL aliquots were collected every 30 min up to 2 h, and afterwards every 60 min up to 7 h. In the case of TiO_2 photocatalysis, 100 μL aliquots were collected, and then centrifuged at 15,000 rpm for 5 min. Applied time schedule was identical to H_2O_2 and direct photolysis, however the experiment was terminated after 6 h. Then UHPLC-DAD-ESI(+)-Q-TOF analysis was performed.

2.4. Analytical procedure

UHPLC-MS/MS analysis was performed with the use of Agilent Accurate-Mass Q-TOF LC/MS G6520B system with dual electrospray (DESI) source and Infinity UHPLC system (Agilent Technologies, Santa Clara, USA) and Hibar RP-18e (2.1 × 50 mm, dp = 2 μm) HR column (Merck, Darmstadt, Germany). A mixture of acetonitrile (A) and water containing 5% of acetonitrile (B) with addition of 0.1% solution of formic acid in both media was used as a mobile phase. The gradient elution was carried out at constant flow 0.3 mL min^{-1} from 5% A (95% B) to 50% 0–9 min. 2 min post time was performed to return to initial conditions. The injection volume was 2 μL and the column temperature