Journal of Pharmaceutical and Biomedical Analysis 90 (2014) 58–63

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Monitoring of four dipyrone metabolites in communal wastewater by solid phase extraction liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry

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ARTICLE INFO

Article history:
Received 13 September 2013
Received in revised form
14 November 2013
Accepted 16 November 2013
Available online 24 November 2013

Keywords:
Metamizole
Biofilm
Analgesic
Degradation
Disinfection

ABSTRACT

Liquid chromatography quadrupole time-of-flight spectrometry (LC-Q-TOF-MS), equipped with electrospray ionization (ESI), was developed for the determination of the main metabolites of dipyrone – 4-aminoantipyrine (4-AA), 4-acetylaminoantipyrine (4-AAA), 4-formylaminoantipyrine (4-FAA) and 4-methylaminoantipyrine (4-MAA) in communal wastewater after reversed-phase solid phase extraction (SPE) in the low to several μg/l concentration range. Samples originated from conventional wastewater treatment plant (WWTP) using activated sewage sludge as well as from a pilot-scale WWTP operating in mixed mode (activated sewage sludge and cascade biofilms reactors with biofilms growing on fix beds and roots of greenhouse plants). Results of the present study confirmed the outcomes of our previous report according to which, 4-FAA was the most persistent metabolite, while 4-AAA and 4-MAA could be determined in the highest and lowest concentration, respectively. Moreover, the study of intraday variation of the concentration of these metabolites revealed that the concentration of 4-AA, 4-AAA and 4-FAA registered a 46%–75% increase in the samples collected at noon compared to those collected at 6 AM. Chlorination did not affect considerably the removal efficiency (about 15%) of these metabolites in samples collected for 3 months consecutively before and after disinfection. Both wastewater treatment techniques efficiently removed 4-AAA (between 80 and 96%). However, in the summer season, the removal efficiency of conventional WWTP using open-air aerated tanks is lower by 30%, (on average) than in the cold season. The concentration of the investigated metabolites showed increased concentrations in the winter season confirming the intake habits of the population from this popular analgesic and antipyretic drug.

1. Introduction

Dipyrone (metamizole sodium) is a pro-drug used as an antipyretic and analgesic which, after oral intake, is spontaneously hydrolyzed into its main metabolite, 4-methylaminoantipyrine (4-MAA). In the liver, the absorbed 4-MAA is metabolized to 4-aminoantipyrine (4-AA) via demethylation and further acetylated to acetylaminoantipyrine (4-AAA) by polymorphic N-acetyltransferase [1]. Another important metabolite, 4-formylaminoantipyrine (4-FAA), is generated by an, as yet, uncharacterized oxidation of the N-methyl group [2,3] (Fig. 1). The determination of such polar and medium polar compounds is usually carried out by liquid chromatography mass spectrometry (LC–MS) [4]. Due to the low environmental concentrations, a crucial step is the enrichment of the sample, which is traditionally performed using solid-phase extraction (SPE) [4]. Monitoring studies have demonstrated the discharge of the metabolites of dipyrone through municipal wastewater-treatment plants (WWTPs) [4,5]. Thus, occurrence of all dipyrone metabolites has already been reported in effluents as well as their dissipation in surface water [6,7].

The degradation of 4-AAA was shown to be favored under aerobic conditions [8,9]. The evaluation of the photochemical behavior of 4-AAA, 4-FAA and 4-MAA and under simulated solar irradiation revealed that 4-MAA was the most degradable dipyrone metabolite with half-life times ($t_{1/2}$) ranging from 0.12 to 0.58 h, depending on the irradiation conditions and salt composition of waters. To contrary, the $t_{1/2}$ of 4-FAA and 4-AAA were 24 and 28 h, respectively. Nevertheless, the application of an acute toxicity test (*Daphnia magna*) showed an increase in toxicity during the photolytic process [3].

Advanced oxidation processes (AOPs) consist of techniques (e.g., H_2O_2/UV, O_3/UV, γ-radiolysis, TiO_2 photocatalysis, photo-assisted Fenton and electro-Fenton nanostructured CeO_2/C gas diffusion

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http://dx.doi.org/10.1016/j.jpba.2013.11.019

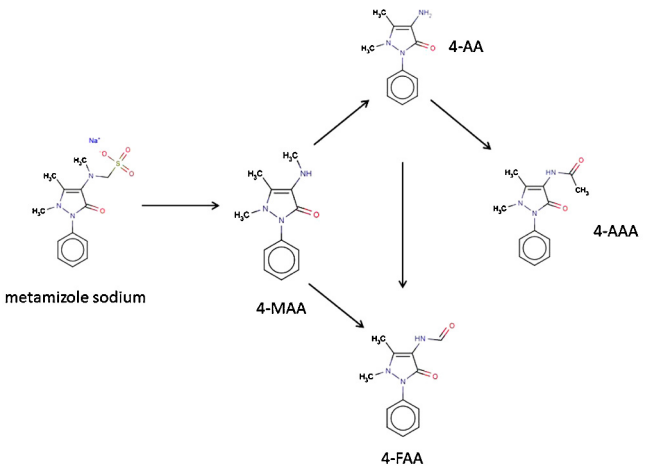

Fig. 1. Metabolization scheme of metamizole sodium in the human body.

electrode) producing powerful and non-selective hydroxyl radical (OH•) for direct degradation of organic pollutants present in drinking water and wastewater [10,11]. The AOP either completely mineralizes [12] or oxidizes several compounds to very low concentrations [13,14] and may generate environmentally friendly byproducts [15,16]. Thus, in a study on the degradation of 4-MAA by photo-Fenton treatment at low iron(II) concentration (2 mg/l), it was demonstrated that solution toxicity did not increase due to the degradation products formed [17].

Direct UV irradiation and O_3 are considered as powerful disinfection agents, but chlorinating agents (e.g., molecular chlorine, sodium hypochlorite, chloroamine and chlorine dioxide) are the most widespread. Molecular chlorine is more reactive in oxidation reactions and in reactions with double bonds, while sodium hypochlorite has a higher activity in electrophilic aromatic substitutions [18].

Whereas in the human body the major metabolic routes are known for dipyrone, the products of the biodegradation brought about by microorganisms are little known. In a recent study, Pieper et al. [19] demonstrated that 4-MAA was readily degraded microbially to different metabolites using a continuous flow reactor with natural biofilms derived from river water, while methyl groups and amines of 4-MAA were metabolized to 4-FAA and 4-AA.

The aim of this study was to investigate the intraday and seasonal changes of dipyrone metabolites in communal wastewater collected from WWTPs applying different wastewater treatment technologies (activated sewage sludge with or without disinfection vs. cascade biofilm reactor system) by further developing of a suitable reversed-phase solid phase extraction liquid chromatography quadrupole time-of-flight mass spectrometric (RP-SPE-LC-ESI-Q-TOF-MS) method as a follow-up of our pilot study done in this field [20].

2. Experimental

2.1. Chemicals

Three dipyrone metabolites, namely 4-MAA, 4-AAA and 4-FAA were synthesized at the Institute of Biochemistry and Medical Chemistry, Medical School, University of Pécs according to literature methods [20]. Analytical grade 4-AA (Sigma Aldrich, Budapest, Hungary) was recrystallized from toluene. For the SPE, HPLC gradient methanol (MeOH) and formic acid were purchased from LGC Standards GmbH (Wesel, Germany).

2.2. Sample collection

In order to study the intraday variation of dipyrone metabolites in the influent wastewater, corresponding samples were collected for 24 h with a sampling frequency of 6 h starting at 6:00 AM from the North-Pest Wastewater Treatment Plant (WWTP) of the Budapest Sewage Works Ltd., in Hungary, using conventional activated sludge technology. Moreover, during July 2011 and April 2012, municipal wastewater samples were collected from the North-Pest WWTP. Simultaneously, communal wastewater samples were collected from a pilot-scale wastewater treatment plant in the village of Telki, found in the suburban area of Budapest where a cascade biofilm reactor system is applied. Samples were collected concomitantly at the effluent and the influent sides in amber-colored glass bottles. Generally, sampling was performed monthly.

The effect of chlorination on the concentration of dipyrone metabolites was studied by taking biologically treated chlorinated wastewater samples monthly between July and September 2011 from the South-Pest WWTP applying activated sludge technology.

In total, 43 samples collected in triplicate were analyzed.

2.3. Sample preparation

Sample preparation of the collected wastewater samples was done as reported in our previous work [20]. Briefly, 250–500 ml of sewage water samples was filtered through a GF/A ⌀ 125 mm glass microfiber paper of pore size 1.6 μm supplied by Whatman (Maidstone, UK). The filtrates were loaded onto pre-conditioned StrataX 33 μ polymeric 200 mg/3 ml reversed-phase cartridges (Phenomenex, Torrance, CA, USA) SPE columns placed on a 12-port Visiprep DL Vacuum Manifold (Supelco, Bellefonte, PA, USA) by using disposable Supelco PTFE liners. After this step, the cartridges were washed with 6 ml of 5% v/v MeOH (pH = 8 with acetic acid). Samples were eluted with 6 ml of MeOH containing 0.1% v/v formic acid. The methanolic eluates were stored after the SPE at 4 °C.

2.4. Preparation of calibration solutions

From the synthesized and purified standards, individual stock solutions in concentration of 3 g/l for each metabolite were prepared in Falcon® PP centrifuge tubes by dissolution of the solid substances in deionized water (18 MΩ cm). Working standards were freshly diluted from this solution after two dilution steps. The concentration range of the calibration solutions was between 1.5 μg/l and 300 μg/l.

2.5. Instrumentation and sample analysis

Liquid chromatographic separation was carried out on a Synergy-Hydro RP column (150 × 2 mm; 4 μm, Phenomenex, Torrance, CA, USA) and an AQ C18 SecurityGuard guard cartridge (4 × 2 mm; 4 μm, Phenomenex, Torrance, CA, USA) both thermostated at 40 °C. The LC system consisted of a Waters Acquity Binary Solvent Manager, Waters Acquity Sample Manager equipped with a 10 μl loop, and a Waters Acquity Column Manager purchased from Waters Corp (Milford, MA, USA). MassLynx (Waters Corp., Milford, MA, USA) was used for the control of the equipment and for data evaluation.

A multi-step gradient method was applied by using an aqueous solution of 5% v/v acetonitrile (containing 0.05% v/v formic acid as well) as eluent A and 100% v/v MeOH as eluent B at a flow rate of 0.4 ml/min. The initial composition of the mobile phase was 95% v/v eluent A followed by a linear gradient to 90% v/v eluent B from 0.5 to 1.5 min, after which the mobile phase composition was maintained at 90% v/v eluent B for other 1.5 min. At the end