Pharmaceutical removal from different water matrixes by Fenton process at near-neutral pH: Doehlert design and transformation products identification by UHPLC-QTOF MS using a purpose-built database

E. Cuervo Lumbaque[a], R. M. Cardoso[a], A. Dallegrave[a], L. O. dos Santos[a], M. Ibáñez[b], F. Hernández[b], C. Sirtori[a*]

[a]Instituto de Química- Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Porto Alegre-RS, Brazil.

[b]Research Institute for Pesticides and Water, University Jaume I, Castellón 12071, Spain.

*Corresponding author

E-mail address: carla.sirtori@ufrgs.br

Abstract

This work evaluated the Fenton process in the removal of eight pharmaceuticals (gemfibrozil, nimesulide, furosemide, paracetamol, propranolol, dipyrone, fluoxetine, and diazepam), present at an initial concentration of 500 μg L^{-1} for each compound, from three different water matrixes (distilled water, simulated wastewater, and hospital wastewater). The Fenton process conditions (iron and hydrogen peroxide concentrations, and pH) were optimized for the distilled water matrix by Doehlert design associated with response surface methodology. These corresponded to an initial Fe^{2+} concentration of 12.5 mg L^{-1}, an initial hydrogen peroxide concentration of 533 mg L^{-1}, and pH 5.0. Mineralization rates and pharmaceutical degradation were monitored for all water matrixes and different experimental conditions employed. Unique iron addition, using low iron concentration (12.5 mg L^{-1}) and increased iron concentration (37.5 mg L^{-1}) were evaluated. These preliminary results motivated the study of the Fenton process employing successive iron additions and using an excess of hydrogen peroxide in the reaction medium. Multiple iron addition favored higher oxidation of the initial contaminants. Finally, the most persistent transformation products generated during the Fenton process were identified by liquid chromatography coupled with quadrupole-time of flight mass spectrometry (UHPLC-QTOF MS) using a purpose-built database that allowed monitoring of 97 transformation products, simultaneously, in one chromatographic analysis only. In this study, 12 transformation products (TPs) were tentatively identified employing this purpose-built database. Most TPs generated were classified as high toxicity (Cramer rules) and showed ready biodegradability (START biodegradability).

1. Introduction

Pharmaceuticals can be considered one of the most important groups of unregulated micropollutants, especially by the increase in the use of these substances, and by the enhanced limits of detection of these compounds due to the impressive advance in some analytical tools, such as chromatographic techniques coupled with mass spectrometry. These compounds may be candidates for future regulation, depending on the results of research into their effects on human health and aquatic biota, and on follow-up data regarding the frequency of their presence in the environment. Is known that pharmaceuticals do not need to persist in the environment to cause negative effects, since the high rates of transformation or removal that they are able to undergo can be offset by their continuous introduction into the environment (Barceló, 2003).

Recent studies have detected more than 80 compounds with pharmacological activity in different environmental aqueous matrixes at concentrations ranging from ng L^{-1} to μg L^{-1} (Ibáñez et al., 2017; Carraro et al., 2016; Marković et al., 2015; Lin et al., 2010; Mendoza et al., 2015; Santos et al., 2013; Gómez et al., 2007; Roberts and Thomaz, 2006; Lishman et al., 2006; Andreozzi et al., 2003; Sedlak et al., 2005; Lacey et al., 2008; Sui et al., 2010; Spongberg and Witter, 2008; Santos et al., 2005; Bueno et al., 2007; Kim et al., 2007; Gros et al., 2006; Ternes et al., 2001a; Ternes et al., 2001b; Farré et al., 2001; Metcalfe et al., 2004; Castiglioni et al., 2005; Bartelt-Hunt et al., 2009; Escher et al., 2011). Moreover, according to these studies, the highest levels of pharmaceuticals are found near sewage spillways (Heberer, 2002).

In this context, special attention should be given to hospital effluents, which are characterized by high concentrations of pharmaceuticals and metabolites, disinfectants, pigments/dyes, reagents, etc. (Gupta et al., 2009; Langford and Thomas, 2009). In

general, hospital effluents have an extra contribution of organic content that favors high COD, low BOD and, therefore, biological processes conventionally used are not suitable for the treatment of these hospital wastewaters (Elmolla and Chaudhuri, 2009; Rahim Pouran et al., 2015). According to Carraro et al. (2016) in only a few countries is hospital effluent classified as industrial wastewater, and because of its characteristics goes through some kind of pre-treatment before discharge into the sewage system.

In this sense, Advanced Oxidation Processes (AOPs) represent an option that has proven effective for the treatment of water contaminated with organic compounds. These processes are characterized by the generation of hydroxyl radicals, a highly oxidizing species, at atmospheric pressure and at room temperature. The high potential of AOPs for the treatment of water containing pharmaceuticals is widely recognized (Klavarioti et al., 2009; Giannakis et al., 2015). Among the different PAOs, photo-Fenton and Fenton processes have been shown to be good options for removal of pharmaceuticals from different effluents and environmental or water matrixes (Miralles-Cuevas et al., 2014a and 2014b; Rahim Pouran et al., 2015; Mirzaei et al., 2017; Li et al., 2012; Wilde et al., 2017; Perini et al., 2018).

The Fenton process combines H_2O_2 and Fe^{2+} as reactants (reactions 1 and 2) to produce hydroxyl radicals and other transient species that can oxidize a wide range of organic compounds. For the classical Fenton process, a more restricted pH control is necessary and for this reason this treatment process was traditionally performed at acid pH (2.5–3) (Gallard et al., 1998). However, now there are some studies demonstrating the possibility of working with near-neutral pHs. These studies emphasize the importance of working with almost neutral pH for practical applications of these treatment processes. Two interesting examples of manuscripts where this subject has

been properly highlighted were published by Vermilye and Volker (2009) and Clarizia et al. (2017).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-} \quad (1)$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + {}^{\bullet}HO_2 + H^{+} \quad (2)$$

Generally, in the Fenton process, organic compounds are oxidized by hydroxyl radicals and other reactive transient species, but are not completely mineralized, favoring generation of transformation products (TPs) (Ostra et al., 2007). The TPs formed can present numerous possible chemical structures because there is no previously defined favored path. The TPs are only identifiable through the use of advanced chromatographic techniques, mainly high-resolution mass spectrometry coupled to liquid chromatography (LC-HRMS) (Gago-Ferrero et al., 2016). There is no doubt that there are many studies in the literature that aim at identification of TPs during different AOP treatment processes (Gupta and Garg, 2018; Fatta-Kassinos et al., 2011; García-Galán et al., 2016; Jallouli et al., 2016). However, in these studies, only ideal conditions are considered: just one model compound was employed, at significantly higher initial concentrations than those found in natural or raw waters and wastewaters (at mg L^{-1}), and using distilled water as matrix. All these conditions are quite different from those found in real effluents where numerous pharmaceuticals, metabolites and TPs are present in low concentrations in complex aqueous matrixes.

In view of the above, this work aims, for the first time, to optimize the experimental conditions of a Fenton process using Doehlert design and, afterwards, to evaluate the influence of three different water matrixes – distilled water (DW), simulated wastewater (SW), and raw hospital wastewater (RHW) – to treat, simultaneously, eight pharmaceuticals at μg L^{-1} concentrations, by a Fenton process. As a final step, this study intends to identify the major TPs generated during the Fenton process by liquid chromatography coupled with quadrupole-time of flight mass

spectrometry (UHPLC-QTOF MS), employing a new purpose-built database that allowed tentative identification of 97 TPs in one chromatographic analysis.

2. Material and methods

2.1 Chemicals, solutions and water matrixes

All reagents used for chromatographic analyses were LC-MS grade. Acetonitrile (ACN) and methanol (MeOH) and formic acid (purity = 98%) were purchased from Merck (Darmstadt, Germany) and from Scharlau (Barcelona, Spain). Ammonium acetate LC-MS grade was purchase from Fluka, Sigma-Aldrich (Germany). All pharmaceuticals included in this work were purchased from different providers and were analytical grade (purity > 98.99%). Fenton experiments were performed using iron sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) purchased from Reagen (Rio de Janeiro, Brazil). Reagent-grade hydrogen peroxide (H_2O_2, 39% w/v) and sulfuric acid (H_2SO_4, 98%) used for pH adjustment were purchased from Synth (São Paulo, Brazil).

Eight pharmaceuticals selected were employed with individual initial average concentrations below 500 µg L^{-1}: gemfibrozil (GFZ), nimesulide (NMD), furosemide (FRS), paracetamol (PCT), propranolol (PPN), dipyrone (DIP), fluoxetine (FXT) and diazepam (DZP). Working solutions were prepared by appropriate dilution of the stock solutions that were prepared using analytical standards of the selected pharmaceuticals and MeOH/ACN, 1:2 (v/v). The choice of these pharmaceuticals was due to the fact that most of them are consumed continuously or in specific cases without medical prescription (analgesics). Moreover, the existence of national regulations in Brazil that control the acquisition and manipulation of many drugs led to the selection of the compounds mentioned above.

Experiments were carried out in three different water matrixes: DW, SW, and RHW. The composition of SW was from the method reported by the OECD (1999) to simulate the organic content of the real hospital wastewater employed in this work (Composition for 1L of SW: peptone 160 mg L^{-1}, beef extract 110 mg L^{-1}, urea 30 mg L^{-1}, $Mg_2SO_4 \cdot 7H_2O$ 2 mg L^{-1}, $CaCl_2 \cdot 2H_2O$ 4 mg L^{-1}). The RHW used in this study came from a public hospital located in the city of Porto Alegre (Brazil). Its main characteristics were: pH 7.85, chloride 35.7 mg L^{-1}, conductivity 691.3 μS cm^{-1}, BOD 130.3 mg L^{-1} O_2, DOC 72.9 mg L^{-1}, COD 291 mg L^{-1} O_2, phosphate 13.98 mg L^{-1}, total suspended solids 85.3 mg L^{-1}, and total solids 378 mg L^{-1}. RHW consisted of raw hospital wastewater, which was used as it came (without filtration) in degradation studies.

2.2 Fenton process

Fenton experiments were performed in a borosilicate-glass vessel (1L) equipped with magnetic stirring. During the reaction, the system was protected from light. For this, the reaction was carried out in a black box with total insulation and, in addition, the system was covered with black polymeric material. This material did not come into contact with the treated solution and only guaranteed extra protection from radiation. The optimization of Fenton reaction conditions was carried out using Doehlert designs.

In all experiments, pH was adjusted using H_2SO_4, and H_2O_2 (39% w/v) was added only at the beginning of the experiments. For Fe(II) two different approaches were evaluated: a single dose of Fe^{2+} at the beginning of the treatment process, and other successive iron additions at different times during the treatment. The selected pharmaceuticals were added (as described in Section 2.1), to each water matrix, before the pH adjustment and iron and H_2O_2 additions, in that order.

2.3 Analytical Determinations during Fenton Process

Mineralization was followed by measuring the dissolved organic carbon (DOC) by Analytik Jena AG multi N/C 2100 S. Total iron concentration was monitored using colorimetric determination with 1,10-phenanthroline, following ISO 6332 (1988), using a UV-vis Cary 50 spectrophotometer. Hydrogen peroxide was analyzed by a spectrophotometric method using ammonium metavanadate, based on the formation of a red-orange peroxovanadium cation during the reaction of H_2O_2 with metavanadate (Nogueira et al., 2005). Monitoring of the degradation of the selected pharmaceuticals was performed by the reduction of the chromatographic peak area (A/A_0) in UHPLC-QTOF MS at different treatment times.

2.4 UHPLC-QTOF MS instrumentation

Pharmaceutical TPs generated during the Fenton process were monitored, for the first time, at Federal University of Rio Grande do Sul (UFRGS-Brazil) by an UHPLC system (Shimadzu Nexera X2) connected to a QTOF mass spectrometer (Bruker Daltonics, Impact II). The UHPLC was equipped with a reverse-phase Luna®Omega C18 analytical column (2.1 mm × 50 mm × 1.6 μm). When ionization in positive mode was selected, the mobile phase was a mixture of MeOH acidified with 0.1% formic acid (A) and H_2O acidified with 0.1% formic acid (B) at a flow rate of 0.28 mL/min. In this case, the gradient progressed from 10% A (initial conditions) to 90% A in 10 min, and then maintained for 2 min. The QTOF mass spectrometer was operated in positive ionization mode under the following conditions: capillary 4000 V, nebulizer 40 psi, drying gas 9 L/min, gas temperature 200 °C. In turn, when the analysis was conducted in negative ionization mode, the mobile phase employed was composed by a mixture of

methanol acidified with 0.01% formic acid (A) and water acidified with 0.01% formic acid (B) at a flow rate of 0.28 mL/min. The same gradient as applied in positive mode was used. The QTOF mass spectrometer was operated in negative ionization mode under the following conditions: capillary 2500 V, nebulizer 40 psi, drying gas 9 L/min, gas temperature 190 °C. In all analyses, the injection volume was 10 µL. The samples injected were previously filtered through a 0.22 µm PVDF filter. The QTOF MS system was operated in broadband collision-induced dissociation (bbCID) acquisition mode that provided MS and MS/MS spectra at the same time. All MS information was recorded over the *m/z* range of 50−1000 with a scan rate of 2 Hz. The bbCID mode allowed for work with two different collision energies: one with a Low Collision Energy (LE) of 10 eV, and a second that applies a High Collision Energy (HE) of 20 eV to obtain MS/MS spectra. Data from TP analysis were processed with DataAnalysis 4.2 software. Elemental composition and double-bond equivalent (RDB) were selected. In most cases, possible elemental compositions for ions with a deviation of ±5 ppm of error were assigned.

In a second moment, due to the higher complexity of the hospital wastewater and difficulties associated with the identification of compounds, the TPs generated during the Fenton process in RHW were also analyzed in University Jaume I (UJI-Spain) in order to complement the present study. A Waters Acquity UHPLC system (Waters, Milford, MA, USA) coupled to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters Micromass, Manchester, UK), using an orthogonal Z-spray-ESI interface was used, operating in both positive and negative ionization modes. The chromatographic separation was performed using an Acquity UHPLC BEH C18 analytical column (2.1 mm × 100 mm × 1.7 µm) from Waters. The mobile phase employed was composed by a mixture of methanol acidified with 0.01%

formic acid (A) and water acidified with 0.01% formic acid (B), at a flow rate of 300 µL/min. The initial percentage of A was 10%, which was linearly increased to 90% in 14 min, followed by a 2 min isocratic period, and then returned to initial conditions over 2 min. The QTOF MS was operated in both ionization modes under the following conditions: capillary voltages of 700 (ESI+) and 2000 V (ESI-), cone voltage of 20 V, desolvation temperature set to 600 °C, the source temperature to 130 °C and the column temperature to 40 °C. In all analyses, the injection volume was 25 µL. The samples injected were previously filtered through a 0.22 µm filter.

For MS^E experiments, two acquisition functions with different collision energies were selected. The low energy function (LE), selecting a collision energy of 4 eV, and the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV were used, in order to obtain a greater range of fragment ions. The LE and HE functions settings were both for a scan time of 0.4 s. Mass data was acquired with MassLynx v 4.1 (Waters) and all data were processed by ChromaLynx application manager (within MassLynx v 4.1). In all cases, possible elemental compositions for ions with a deviation of 2 mDa were assigned.

2.5 Predicted biodegradability and the toxicological risk of TPs

Toxtree software (version 2.6.13) was used to evaluate the predicted biodegradability (START biodegradability) and the toxicological risk (Cramer rules) of the TPs tentatively identified.

3. Results and Discussion

3.1 Doehlert design

In this study, the Doehlert design was applied to optimize experimental Fenton conditions, such as Fe^{2+} and H_2O_2 concentrations and pH, using DW matrix. Initial average concentration for each selected pharmaceutical was below 500 μg L^{-1} and the initial solution was prepared according was described in Section 2.1.

Multivariate models, such as the Doehlert model, are second-order experimental designs, which are more complete than univariate models, since they allow the study of several variables simultaneously (Ferreira et al., 2004). This model reduces the number of experiments to be performed and it is used in conjunction with multivariate statistic techniques such as response surface methodology (RSM). This approach is very useful to optimize reaction conditions, and is based on the fit of a polynomial equation to the experimental data, to describe the behavior of a data set aiming at statistical previsions (Bezerra et al., 2008). The levels evaluated for each variable are presented in Table 1.

Table 1. Variables and levels tested in Doehlert design and their respective responses as mineralization (%) after 60 minutes for DW matrix.

Fe^{2+} levels (mg L^{-1})						
-1	**-0.5**		**0**	**0.5**		**1**
5.0	7.5		10.0	12.5		15.0
H_2O_2 levels (mg L^{-1})						
-0.866	**-0.577**	**-0.289**	**0**	**0.289**	**0.577**	**0.866**
100	208	316	425	533	641	750
pH levels						
-0.817		**0**		**0.817**		
2.8		3.9		5.0		

Test	**Fe^{2+}** (mg L^{-1})	**H_2O_2** (mg L^{-1})	**pH**	**Response (mineralization % after 60 min)**
1	15.0	425.0	3.9	23.7
2	12.5	750.0	3.9	22.0
3	12.5	533.5	5.0	27.3
4	5.0	425.0	3.9	19.3
5	7.5	100.0	3.9	4.3
6	7.5	316.5	2.8	7.5
7	12.5	100.0	3.9	5.6
8	12.5	316.5	2.8	13.5

9	7.5	750.0	3.9	7.5
10	10.0	641.5	2.8	20.8
11	7.5	533.5	5.0	9.3
12	10.0	208.5	5.0	9.0
13	10.0	425.0	3.9	20.2
14	10.0	425.0	3.9	24.4
15	10.0	425.0	3.9	23.4
16	10.0	425.0	3.9	21.0

Sixteen experiments were required by the Doehlert design. The mineralization percentage achieved in the Fenton reaction after 60 min was used as experimental response. The optimum conditions for the Fenton process were achieved with 12.5 mg L^{-1} of Fe^{2+}, 533 mg L^{-1} of H_2O_2, and pH 5 (test number 3). The obtained data were used in RSM and the visualization of the predicted model was obtained in the graphical representation of the relationship between pH and H_2O_2 concentration (see Equation 1 and Figure 1). This illustrates the profile in the optimization of the two variables where the maximum point is located within the experimental region, a relationship deemed to be important since both the concentration of hydrogen peroxide and pH are main factors for the Fenton process. The quality of the adjusted model was assessed by Analysis of Variance (ANOVA), allowing for the evaluation of the significance of the regression. This verification was carried out using electronic spreadsheets (Teófilo et al., 2006) to show whether the mathematical model was well adjusted to the experimental data. All results obtained from the Doehlert design are presented in supplementary information (Tables S.1.1, S.1.2, Figures S.1.1, S.1.2 and S.1.3). In this case, the present model explained more than 88% of the experimental data. There was a good strong match between the model's predicted sensibility efficiency values and the experimental values. For this reason, the optimized experimental conditions (test number 3) were selected to evaluate Fenton reaction performance to degrade pharmaceuticals in the three water matrixes studied.

$$R(i,j) = (11.22) + (7.383521916)*(x(i))+(0.788176186)*(y(j)) + (-9.469458407)*(x(i)^2) + (-0.014800799)*(y(j)^2) + (-6.48633116)*((x(i))*(y(j))) \quad \text{(Equation 1)}$$

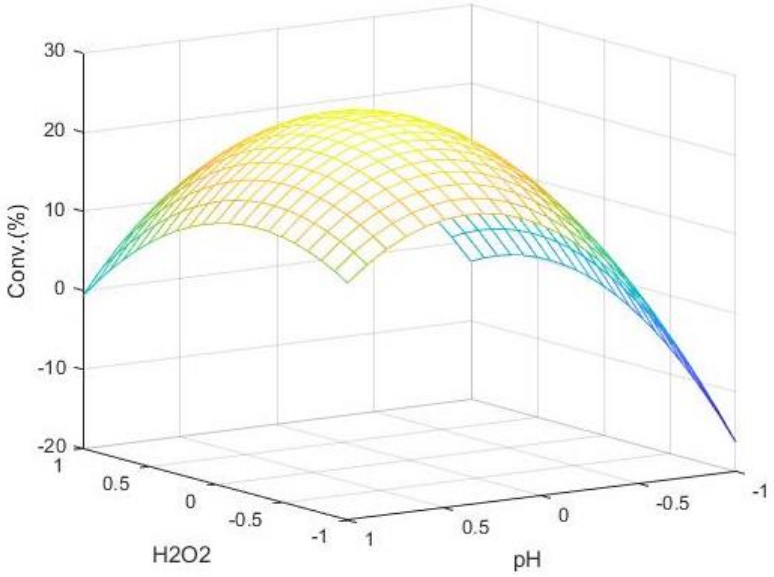


Figure 1. Response surface for H_2O_2 and pH interaction.

3.2. Single iron addition

The Fenton process was carried out at pH 5.0, using a single Fe^{2+} addition that corresponded to 12.5 mg L^{-1} and an initial H_2O_2 concentration of 533.5 mg L^{-1} (see Figure 2) in the three matrixes studied. The results showed that some pharmaceuticals were eliminated after 1 min of treatment. In this case, DIP and FRS showed the highest degradation rate in DW and SW. These data are in agreement with studies by Klamerth et al., (2013), where FRS exhibits rapid degradation in the first minutes of the treatment process. On the other hand, NMD, FXT, DZP and GFZ were more persistent in all matrixes evaluated.

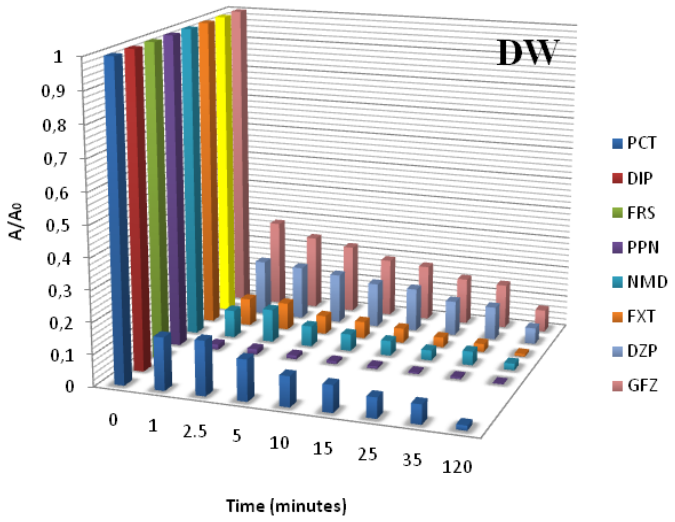
Experiments performed in DW showed a reduction of 27% in the initial DOC after 120 min. In turn, SW did not present significant mineralization until the final

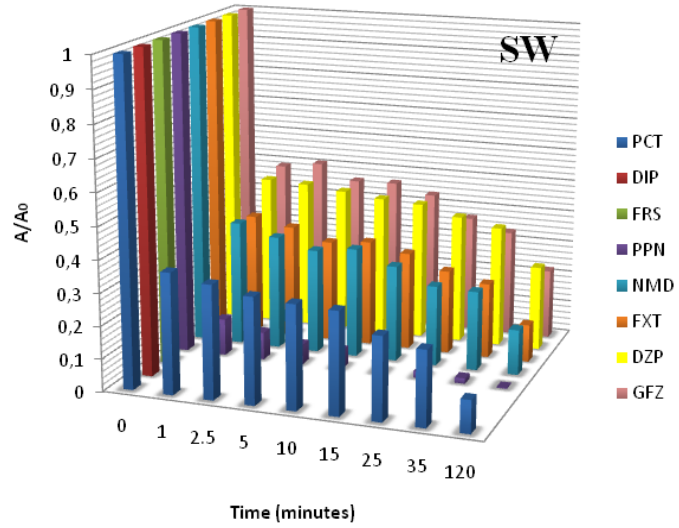
treatment time period monitored. At this stage, for SW and RHW, 5% and 3% of the initial DOC was reached, respectively. Besides, at this point, for both these water matrixes – SW and RHW – initial H_2O_2 consumed was 258 mg L^{-1} and 270 mg L^{-1}, respectively. In all cases, 533 mg L^{-1} of H_2O_2 was used to provide an excess and to avoid limiting the reaction. Iron(II) remained practically constant during the Fenton reaction in all water matrixes studied when a single dose of iron was used (see Figure 3). Our results for H_2O_2 and iron(II) are in agreement with data reported by Carra et al. (2013) for dark Fenton and where Fenton was evaluated and compared to solar photo-Fenton processes.

For RHW, it is important consider that the presence of some pharmaceuticals in this real wastewater was to be expected. Thus, in order to evaluate whether the model pharmaceuticals were already present in this matrix, another study was performed and is the subject of other manuscript that now is in preparation. These preliminary analyses indicated that paracetamol, dipyrone and diazepam are the only pharmaceuticals that were presented in the RHW matrix. However, since the degradation of these and other drugs selected for study was monitored by the reduction of the peak area in relation to the initial area, the efficiency of the treatment process took into account the potential additional loading of these three analytes.

In this context, it is important to highlight that in this study it was decided to carry out the direct injection of initial and degraded samples from all water matrixes evaluated; that is, no extraction and/or pre-concentration method was performed in the present study. This option aimed to provide minimum manipulation of the samples, and thus reduce the possibility of losses of the initial pharmaceuticals and, principally, their TPs. Additionally, according Togolla et al. (2014), Solid Phase Extraction (SPE) techniques are therefore used, but require large quantities of samples, and in our case, as

the experiments were performed in batch and with an initial volume of 1L, these conditions did not favor the application of SPE techniques.





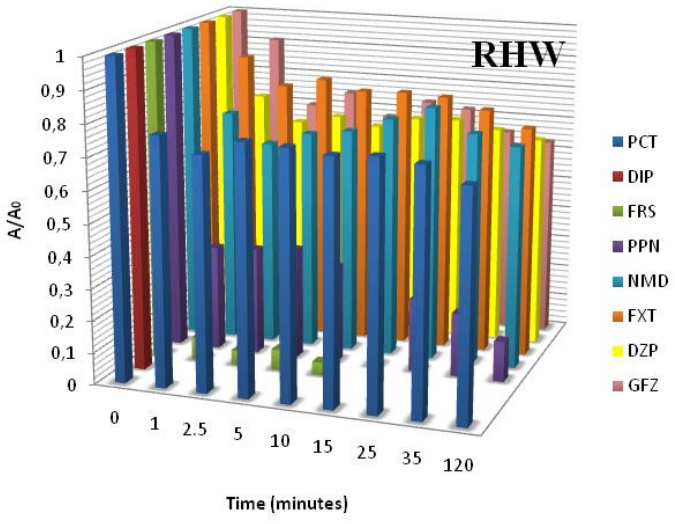


Figure 2. Pharmaceuticals degradation during the Fenton process using single iron addition (Fe^{2+} 12.5 mg L^{-1}; H_2O_2 533 mg L^{-1} and pH 5) for all water matrixes evaluated.

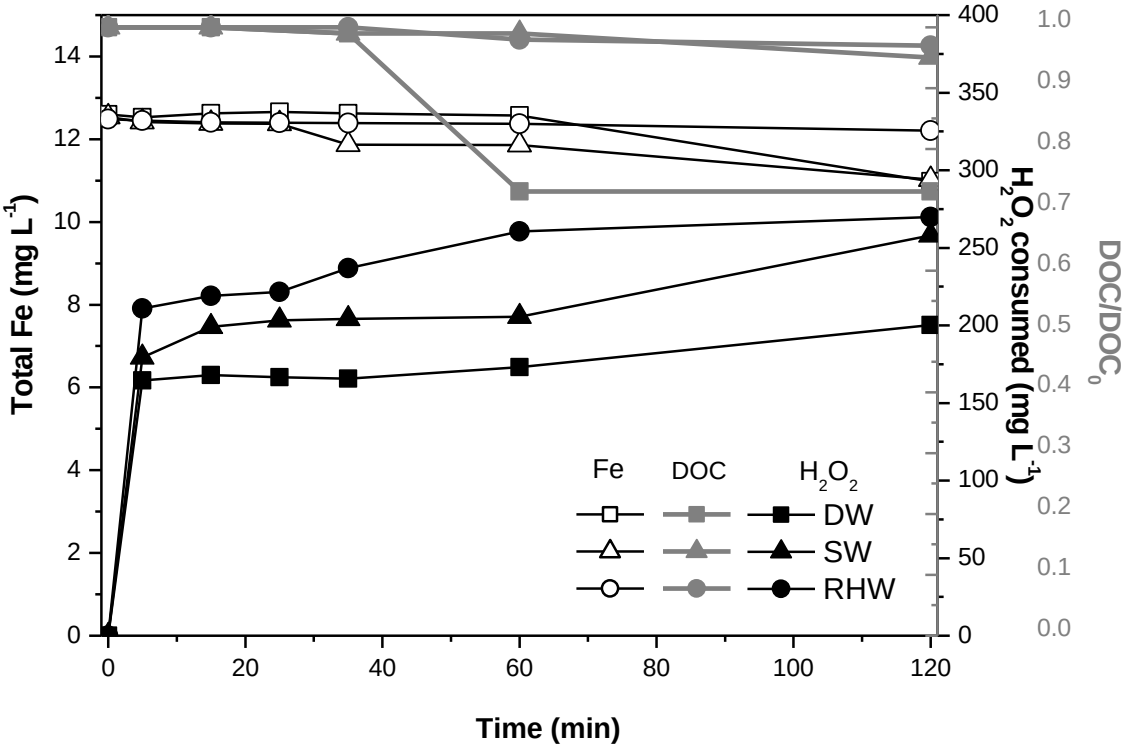


Figure 3. Total iron, H_2O_2 consumption and DOC/DOC_0 ratio determined during Fenton process for three different water matrixes evaluated using single iron addition (Fe^{2+} 12.5 mg L^{-1}; H_2O_2 533 mg L^{-1} and pH 5).

The discrete efficiency of the Fenton process in RHW and, especially in SW matrixes, could be justified by the presence of organic matter and different ionic species (Sirtori et al., 2010). Also, according to Bang et al. (2016), the presence of natural organic matter has a negative effect on the removal of some compounds. In their study, the authors demonstrated that the removal was inversely proportional to the TOC concentration in the synthetic water used in their study. Thus, the presence of natural organic matter caused a significant increase in energy consumption, due to the strong absorption properties and the high reactivity with OH radicals in the treatment process evaluated by the authors. In our results, the effect of the water matrix composition on the pharmaceutical degradation during the Fenton process was quite prominent.

In this context, according to Zhang et al. (2016) and García-Muñoz et al. (2017), pharmaceuticals are much more reactive than the other organic content present in complex wastewaters (real or simulated) during different advanced treatment processes. Moreover, Lanzafame et al. (2017) demonstrated that different ions commonly found in wastewater, such as chloride or carbonate among others, can act as scavengers of the hydroxyl radicals and lead to the formation of species such as $Cl_2^{\bullet -}$ and $CO_3^{\bullet -}$ (significantly less reactive than the hydroxyl radical). This would lead to lower degradation of pharmaceuticals in SW. In addition, the presence of phosphate could lead to decreasing iron solubility, due to the increase in inter-ionic forces in the solution, decreasing the efficiency of the treatment process. In this case, RHW contains 13.98 mg L^{-1} of phosphates, unlike the SW matrix which does not contain phosphate.

Thus, in order to increase the efficiency of the process, the single addition assay was repeated using an initial concentration of iron at 37.5 mg L^{-1}, maintaining the initial H_2O_2 at 533 mg L^{-1} (since it had already been added in excess and was only partially consumed after 120 min of treatment) and pH 5. Only DW matrix was evaluated with a higher initial concentration of iron (37.5 mg L-1) and the results are shown in Figure 4. In this case it is observed that the increase in iron(II) concentration from 12.5 to 37.5 mg L^{-1} as initial single addition favored the increase of the mineralization by 12%. On the other hand, when multiple iron(II) additions (with a total final concentration of 37.5 mg L^{-1} of Fe (II)) was tested, 39% of the initial organic content was mineralized. These results indicated that higher concentrations of iron(II) in multiple additions favor the Fenton process.

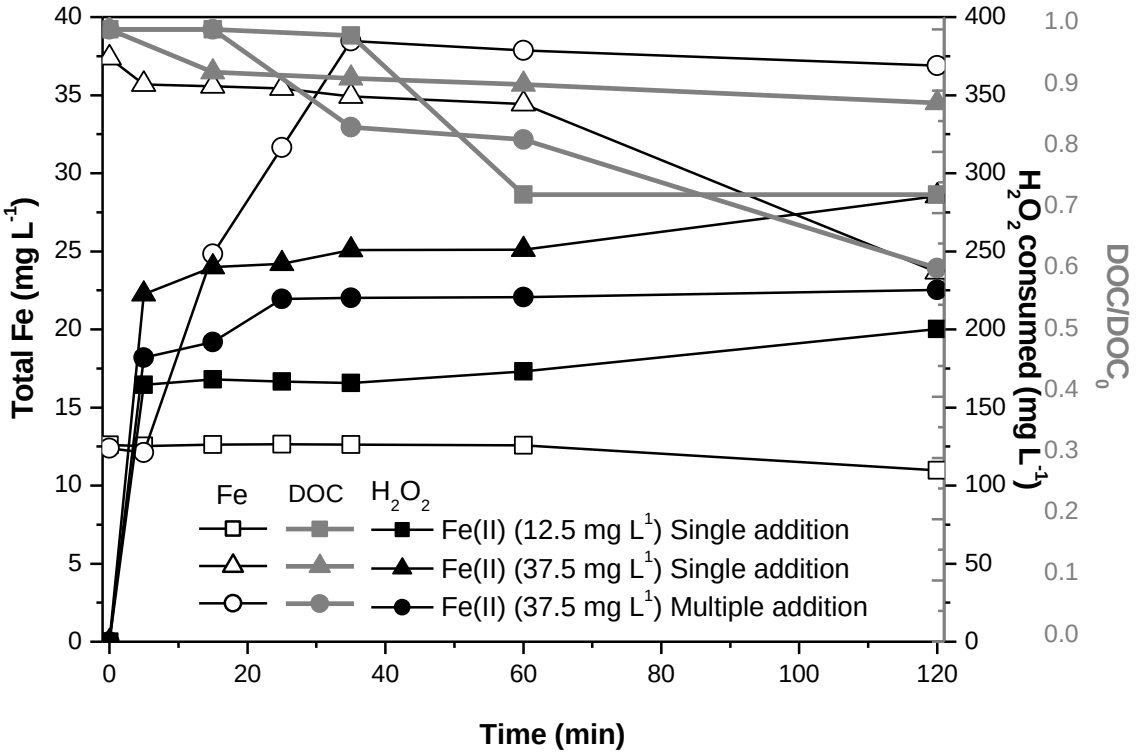


Figure 4. Total iron, H_2O_2 consumption and DOC/DOC_0 ratio determined during Fenton process for DW matrix using single addition (Fe^{2+} 12.5 mg L^{-1}) and single addition (Fe^{2+} 37.5 mg L^{-1}) and multiple iron addition (Fe^{2+} 37.5 mg L^{-1}). All experiments were performed using pH 5 and initial H_2O_2 concentration of 533 mg L^{-1}.

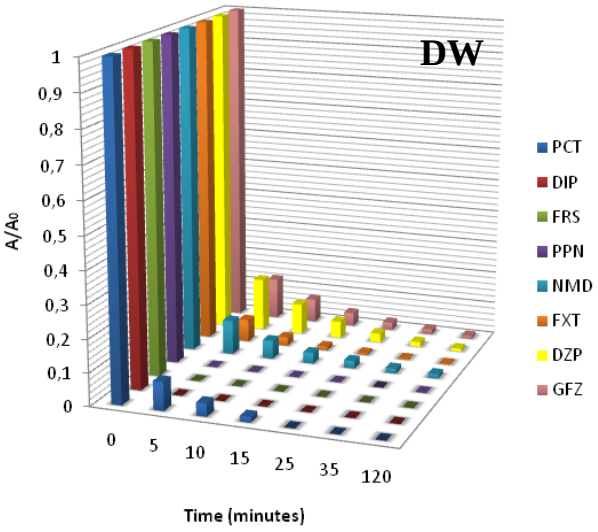
3.3 Multiple iron additions

In an attempt to improve pharmaceutical degradation by the Fenton process, multiple iron(II) additions were made with the same initial H_2O_2 concentration of 533 mg L^{-1}, based on studies of sequential iron dosage as a strategy for the successful removal of environmental pollutants at neutral pH (Carra et al., 2013). Different iron dosages were performed (0 min: 12.5 mgL^{-1}; 5 min: 6.25 mgL^{-1}; 10 min: 6.25 mgL^{-1}; 15 min: 6.25 mgL^{-1}; 25 min: 6.25 mgL^{-1}) for a total iron dose of 37.5 mg L^{-1}. This strategy was evaluated because, as is known, the periodic addition of iron(II) to the water maintains a stable catalyst concentration (ferrous ions), reflected in superior efficiency of the treatment process.

DIP, FRS and PPN showed the highest degradation rate in the three matrixes (Figure 5), and they exhibited rapid degradation in the initial treatment time range. On

the other hand, PCT, NMD, FXT, DZP and GFZ were more persistent in RHW. It seems that increasing the concentration of iron available in the system by successive iron additions favors the formation of hydroxyl radicals, as long as there is an excess of hydrogen peroxide in the reaction medium. Consequently, a greater degree of oxidation of the contaminants was achieved.

Experiments performed in DW matrix showed a DOC reduction of 40% after 120 min. In turn, SW and RHW presented a moderated mineralization (around 15%) until the final treatment time period monitored (see Figure 6). At the final treatment time, H_2O_2 consumption was 225.2, 328.4 and 223.8 mg L^{-1} for DW, SW and RHW, respectively. Iron concentration was reduced by 10% only at 120 min for RHW (final concentration was 33.4 mg L^{-1}).



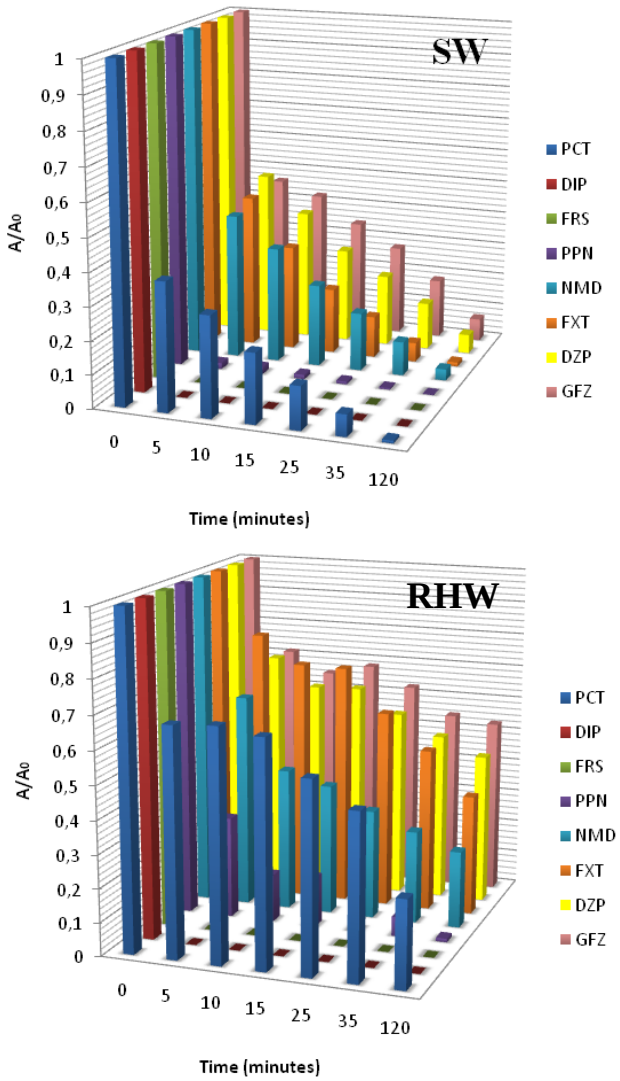


Figure 5. Pharmaceuticals degradation during the Fenton process using multiple iron additions (Fe^{2+} 37.5mg L^{-1}; H_2O_2 533 mg L^{-1} and pH 5) for all water matrices evaluated.

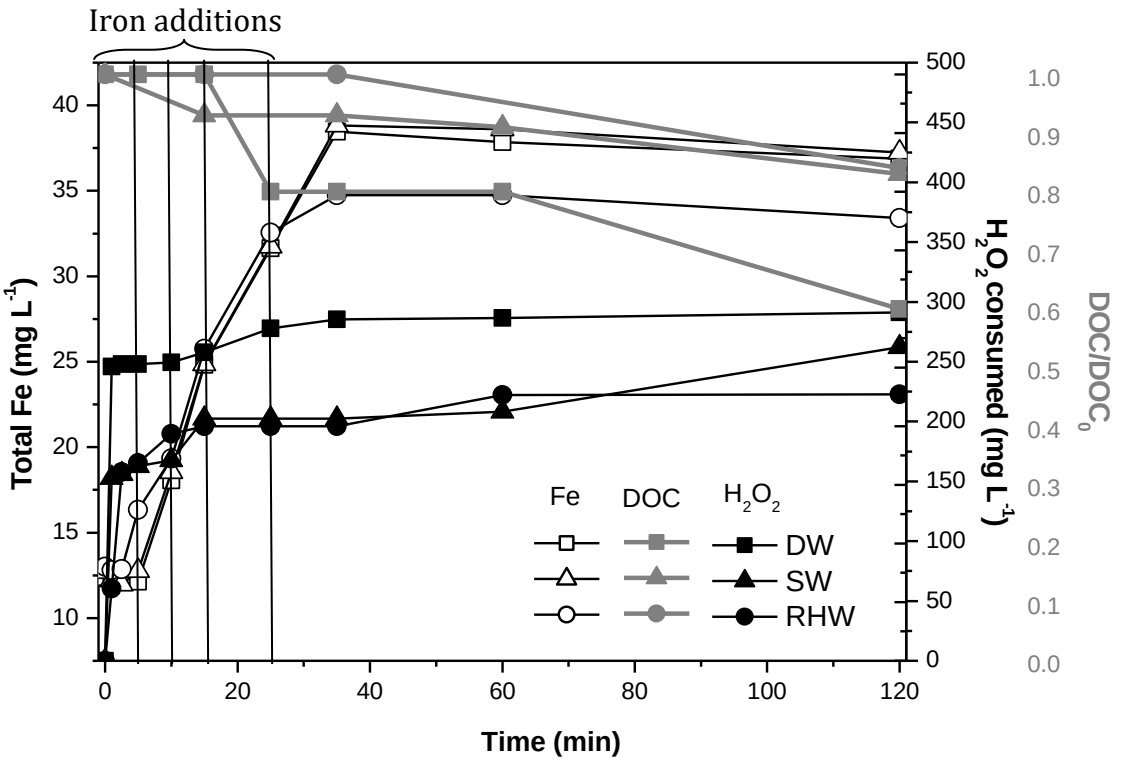


Figure 6. Total iron, H_2O_2 consumption and DOC determined during Fenton process for three different water matrixes evaluated using multiple iron additions (Fe^{2+} 37.5mg L^{-1}; H_2O_2 533 mg L^{-1} and pH 5).

3.4 Transformation Products

In order to detect the presence of possible TPs formed, samples from each experiment were analyzed by a UHPLC-QTOF MS instrument operating in positive and negative ionization modes, as previously described in Section 2.4. A purpose-built database containing a total of 97 TPs reported in the literature for the eight pharmaceuticals under study was elaborated. The purpose-built database included the elemental composition of the TPs as well as information about the product ions reported for these species, if available (for more details, see Table S.2.1 - Supporting Information).

It is important to note that, in general, in studies where transformation products are proposed, as mentioned in the introduction, only one drug is normally used at high initial concentrations (in the order of tens of mg L^{-1}). These individual studies performed with individual pharmaceuticals are extremely useful because they bring

valuable data that were used as a data source for the purpose-built database. Thus, once the purpose-built database was elaborated, the data processing was adapted from other previous studies that used this tool to qualitatively identify the presence of different compounds (such as pharmaceuticals, pesticides, metabolites, among others, with or without analytical standard available) (Hernández et al., 2014; Díaz et al., 2013). Thus, as far as is known, the elaboration and use of a purpose-built database for tentative identification of different TPs generated by AOPs is a new strategy that allows the degradation of pharmaceuticals simultaneously and in concentrations closer to those found in hospital effluents (Mendoza et al., 2015). For this reason, it can be considered that the present work, by bringing this additional aspect of the use of the purpose-built database, from an analytical point of view, provides a new application for an automated method for the search for TPs.

Table 2 summarizes the analytical information relative to the TPs identified in all water matrixes studied, such as elemental composition, theoretical and experimental accurate masses of the ions, their respective errors in ppm and double bond equivalents (DBE) provided by the software. The low mass errors observed (below 5ppm in most cases), allowed for the correct assignation of the elemental compositions.

Table 2. TPs identified during the Fenton process treatment by UHPLC-QTOF MS using the proposed-built database.

Compound	Elemental composition $[M+H]^+$/ $[M-H]^-$	Ion Mass (m/z)		Error (ppm)	DBE*	Ionization mode	Matrices occurrence
		Experimental	Calculated				
TP3 PPN	$C_6H_{16}NO_2$	134.1175	134.1176	0.4	0.5	P	DW
	$C_6H_{14}NO$	116.1069	116.1070	-0.3	0.5		
	$C_6H_{12}N$	98.0974	98.0964	-9.5	1.5		
	C_3H_8NO	74.0603	74.0600	-1.5	0.5		
	$C_4H_{10}N$	72.0807	72.0808	0.6	0.5		
	C_3H_6N	56.0492	56.0495	4.7	1.5		

TP6 PPN	$C_{16}H_{22}NO_4$	292.1545	292.1543	-0.7	6.5	P	DW, SW, RHW
	$C_{10}H_7O_2$	159.0422	159.0441	11.7	7.5		
	C_9H_7O	131.0490	72.0808	-4.1	0.5		
	$C_6H_{14}NO$	116.1067	116.1070	2.8	0.5		
	C_8H_7	103.0536	103.0542	5.8	5.5		
	$C_6H_{12}N$	98.0968	98.0964	-4.3	1.5		
	$C_4H_{10}N$	72.0806	72.0808	1.9	0.5		
	C_3H_6N	56.0496	56.0495	-2.9	1.5		
TP7 PPN	$C_{14}H_{20}NO_4$	266.1394	266.1387	-1.7	5.5	P	DW, SW, RHW
	$C_6H_{12}N$	98.0969	98.0964	-4.7	1.5		
	C_3H_8NO	74.0610	74.0600	-13.1	0.5		
	$C_4H_{10}N$	72.0813	72.0808	-6.7	0.5		
TP19 PPN or TP5 PPN	$C_{14}H_{20}NO_5$	282.1340	282.1336	-1.4	5.5	P	DW, SW, RHW
	$C_6H_{14}NO$	116.107	116.107	0.3	0.5		
	$C_6H_{12}N$	98.0969	98.0964	-4.6	1.5		
	$C_4H_{10}N$	72.0811	72.0808	-4.0	0.5		
	C_5H_6N	56.0495	56.0495	-0.6	1.5		
TP26 PPN	$C_{16}H_{22}NO_5$	308.1501	308.1492	-2.8	6.5	P	DW, SW, RHW
	$C_9H_7O_2$*	147.0456*	147.0446*	6.8*	6.5*		
	$C_6H_{14}NO$	116.1072	116.1070	-1.5	0.5		
	$C_4H_{10}N$	72.0811	72.0808	-4.1	0.5		
	C_3H_6N	56.0491	56.0495	5.9	1.5		
TP1 FXT	$C_{17}H_{19}F_3NO_2$	326.1368	326.1362	-1.4	7.5	P	DW, RHW
	$C_{10}H_{14}NO$	164.1067	164.1070	1.5	4.5		
	C_9H_9O	133.0657	133.0648	-6.9	5.5		
	C_3H_7O	59.0493	59.0491	-2.9	0.5		
TP1 NMD	$C_{13}H_{11}N_2O_6S$	323.0333	323.0343	3.2	9.5	N	DW, RHW
	$C_{12}H_9N_2O_4$	245.0563	245.0568	1.9	9.5		
	$C_{10}H_4N_3O_2S$	229.9993	230.0030	16.0	10.5		
TP6 PCT	$C_8H_8NO_5$	198.0404	198.0408	1.8	5.5	N	DW
	$C_8H_5O_5$	181.0136	181.0142	3.5	6.5		
	$C_7H_8NO_3$	154.0526	154.0510	-10.5	4.5		
	$C_6H_6NO_3$	140.0355	140.0353	-1.4	4.5		
TP1 GFZ	$C_{15}H_{21}O_4$	265.1481	265.1445	-13.3	5.5	N	DW, RHW

TP3 GFZ	$C_8H_9O_2$	137.0606	137.0608	1.7	4.5	N	DW
TP7 GFZ	$C_{15}H_{19}O_4$	263.1277	263.1289	4.6	6.5	N	DW
	C_8H_9O	121.0670	121.0659	-9.2	4.5		
TP8 GFZ	$C_9H_9O_3$	165.0580	165.0557	-14.1	5.5	N	DW

* For more information see Figure S 2.2 (supporting information).

As it has been previously indicated, only those by-products that had been previously reported in the literature were sought (Santiago-Morales et al., 2013; Zhao et al., 2017; Sun et al., 2016; Moctezuma et al., 2012; Najjar et al., 2014; Chen et al., 2017). Likewise, in this study, the data processing to identify some TPs was adapted with some modifications from Ibáñez et al. (2017) and Llorca et al. (2016). An automatic screening for peak detection was performed, and a mass error of the suspected TPs (± 5ppm) was used. As shown in Table 2, 12 TPs were found in the matrixes studied.

As Table 2 shows, four TPs were identified in the three types of water tested, three more TPs were identified in DW and RHW only, and four TPs were found in DW only. The fact that more TPs were present in DW might be explained by the formation of a greater number of OH radicals, which in turn provided a greater degradation and mineralization of the pharmaceuticals. Consequently, a greater number of TPs were detected in that matrix. In addition, the composition of the other matrixes could favor ion suppression effects that would have an effect on the lower number of TPs observed for SW and RHW.

According to our results, a total of five TPs were found for PPN (see Figure S.2.1). Of these, three (TP3 PPN, TP6 PPN and TP7 PPN) could be associated with photocatalytic by-products using Ce-doped TiO_2 as catalyst, as reported by Santiago-Morales et al. (2013). The fourth of the TPs (*m/z* 282.1336 Da, $C_{14}H_{20}NO_5$) could not be fully elucidated because two isomeric compounds were plausible (TP5 PPN or TP19

PPN). Both of them present similar fragmentation, with few specific characteristic fragments. Unfortunately, specific fragments of the suspected TPs were not observed, making it to unfeasible to identify which of the two TPs was involved.

The last PPN TP corresponded to *m/z* 308.1492 Da, with an elemental composition $C_{16}H_{22}NO_5$. It might correspond to TP26 PPN or TP27 PPN. In this case, the complementary QTOF analysis performed at UJI allowed for the identification of the characteristic signal fragment (*m/z* 147.0446, $C_9H_7O_2$) of TP26 PPN. Accurate mass spectra obtained for TP26 PPN at high collision energy are shown in Figure S 2.2 (Supporting Information).

Regarding FXT, only one TP was observed. TP1 FXT exhibited an *m/z* of 326.1368 Da, corresponding to the elemental composition $C_{17}H_{19}F_3NO_2$. This TP had

been previously identified by Zhao et al. (2017) as an intermediate product generated after ozonation processes at different pHs. In that study, the authors also indicated that generation of this hydroxylated TP was enhanced with increasing pH, by increasing the concentration of hydroxyl radicals in the alkaline medium of the treatment process.

In addition to the TPs discussed above, and identified in positive mode, six more TPs were identified in negative ionization mode: one for NMD and PCT and four for GFZ. TP1 NMD showed an accurate mass of 323.0338 Da, corresponding to an elemental composition of $C_{13}H_{11}N_2O_6S$ (expressed as deprotonated molecule). This hydroxylated metabolite was one of the major compounds observed in human plasma by Sun et al. (2016), after administration of NMD. Thus, it seems that this metabolite can also be considered a transformation product from the Fenton degradation.

In the case of paracetamol, the identified TP was TP6 PCT (*m/z* 198.0404 Da, $C_8H_8NO_5$). This TP was previously identified as a transformation product generated by

photocatalytic degradation (UV–TiO_2) (Moctezuma et al., 2012) and ozonation, or a UV–H_2O_2 TP of PCT in aqueous medium (Najjar et al., 2014).

For the GFZ, four identified TPs (TP1 GFZ, TP3 GFZ, TP7 GFZ and TP8 GFZ) were by-products previously found by Cheng et al. (2017) in the degradation of gemfibrozil by photocatalysis mediated by sunlight-driven TiO_2 (carbon dots photocatalyst). These TPs showed reduced fragmentation, even when employing high collision-energy HE, as can be seen in Figure S.2.1.

It is possible that other TPs could have been produced during treatment with the Fenton process in all matrixes evaluated. However, they were not detected definitively due to their low persistence throughout the process, or to their formation in very small concentrations. It must be pointed out that analyses were made by direct injection of the samples, without any type of pre-concentration, in order to minimize potential losses of TPs of different polarities associated with the sample treatment.

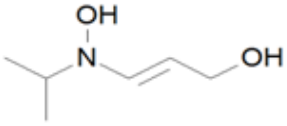
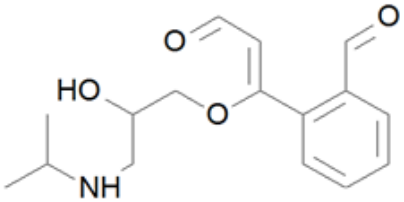
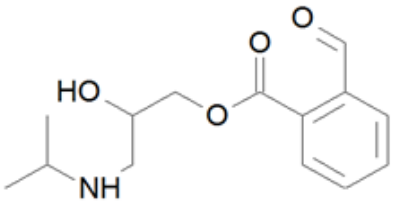
Finally, tentatively identified TPs were evaluated by Toxtree software (version 2.6.13), which determined, based on predictions of the chemical structure of the molecule to be analyzed, the biodegradability (START biodegradability) and the toxicological risk (Cramer rules) that each TP offers. This strategy was previously employed by Urbano et al. (2017) for toxicity and biodegradability determination of some TPs of sulfaquinoxaline generated by an ozonation process. The results obtained by the Toxtree software for TPs tentatively identified in the present study can be seen in Table 3.

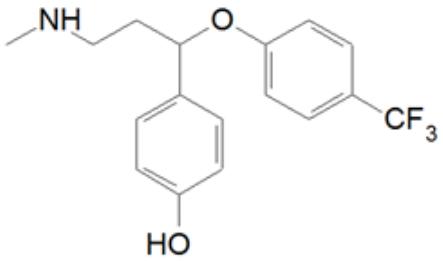
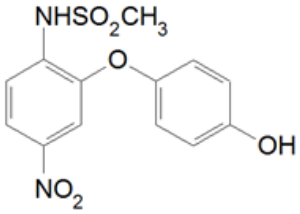
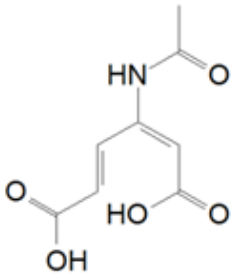
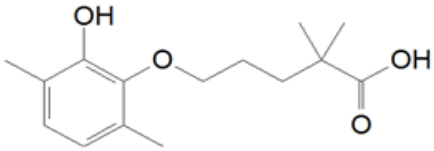
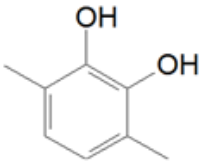
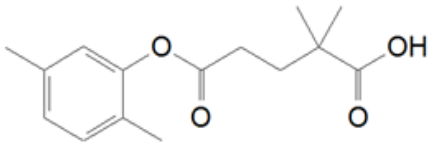
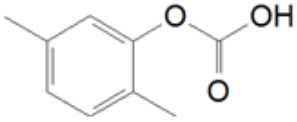
The transformation products TP6 PPN, TP1 FXT, TP1 NMD, TP6 PCT, and TP3 GFZ were classified as highly toxic according to Cramer's rules. These TPs are substances that have significant toxicity, or still have reactive functional groups. In turn, TP1 GFZ has been classified as of intermediate toxicity; that is, it is clearly less

innocuous than those of Class I, but does not provide either a positive indication of toxicity, or a lack of those characteristics in Class III. Finally, TP3 PPN, TP7 PPN, TP7 GFZ and TP8 GFZ were classified as Class I. Compounds classified as Class I are substances with those structures and related data suggesting a low order of oral toxicity. If combined with low human exposure, they should not present a particular priority for research (Cramer et al., 1978).

Evaluating the biodegradability of the different TPs it was observed that TP3 PPN, TP6 PPN, TP1 FXT and TP1 NMD were classified as persistent substances. The other TPs (TP7 PPN, TP6 PCT, TP1 GFZ, TP3 GFZ, TP7 GFZ and TP8 GFZ) which were present in greater amounts, in turn, were considered to be readily biodegradable. This capability of the Fenton process, and of AOPs in general, to favor the formation of more biodegradable substances is an important aspect already reported in previous studies (Sirtori et al., 2010; Zapata et al., 2010).

Table 3. Toxicological risk (Cramer rules) and START biodegrability determined by the TPs generated during Fenton process.

TP	Chemical structure	Toxicological risk	START biodegrability
TP3 PPN		Low (class I)	Persistent chemical (class 2)
TP6 PPN		High (class III)	Persistent chemical (class 2)
TP7 PPN		Low (class I)	Easily biodegradable chemical (class 1)

TP1 FXT		High (class III)	Persistent chemical (class 2)
TP1 NMD		High (class III)	Persistent chemical (class 2)
TP6 PCT		High (class III)	Easily biodegradable chemical (class 1)
TP1 GFZ		Intermediate (class II)	Easily biodegradable chemical (class 1)
TP3 GFZ		High (class III)	Easily biodegradable chemical (class 1)
TP7 GFZ		Low (class I)	Easily biodegradable chemical (class 1)
TP8 GFZ		Low (class I)	Easily biodegradable chemical (class 1)

Conclusions

In this work, the optimum experimental conditions to perform the Fenton process on three different waster matrixes containing eight pharmaceuticals were

achieved using a Doehlert model. For the DW matrix, the treatment process using increased and multiple Iron(II) additions studied showed an efficient mineralization rate and degradation of the selected pharmaceuticals. A less favorable behavior was observed for the other matrixes under study (SW and RHW). The purpose-built database containing up to 97 TPs was elaborated for analysis of the samples by UHPLC-QTOF MS. This allowed identification of 12 TPs generated during the Fenton process, making use of the accurate masses and fragmentation behavior. Most TPs identified were hydroxylation products of the pharmaceuticals initially present, and these compounds were classified as high toxicity (Cramer rules). Finally, the biodegradability assessment (START biodegradability) indicated that most TPs generated by the Fenton process are readily biodegradable.

Acknowledgments

The authors wish to thank the Brazilian Ministry of Science, Technology, Innovation and Communications-CNPq (Processo: 403051/2016-9) and Mrs. Eng. Tainá Flores da Rosa and HCPA for the RHW. Carla Sirtori thanks the CNPq for her Research grant (Processo: 303474/2015-7) and thanks the CAPES Foundation for her Postdoctoral Research grant (Processo: 88881.119908/2016-01). Elisabeth Cuervo Lumbaque thanks the CAPES Foundation for her PhD. Research grant. The authors from UJI acknowledge the financial support from Generalitat Valenciana (Research Group of Excellence, PrometeoII/2014/023).

References

Andreozzi R., Raffaele M., Nicklas P., 2003. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere. 50, 1319-1330.

APHA – Standart Methods for the examination of water and wastewater, 19th edition, 3500D, 1995.

Bang, H., Slokar, Y.M., Ferrero, G., Kruithof, J.C., Kennedy, M.D. 2016. Removal of taste and odor causing compounds by UV/H_2O_2 treatment: effect of the organic and inorganic water matrix. Desalin. Water Treat. 57, 1-10.

Barceló, D., 2003. Emerging pollutants in water analysis. TrAC Trends Analyt. Chem. 22, xiv-xvi.

Bartelt-Hunt S.L., Snow D.D., Damon T., Shockley J., Hoagland K., 2009. The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. Environ. Pollut. 157, 786-791.

Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta76, 965-977.

Bueno M.J.M., Agüera A., Gómez M.J., Hernando M.D., García J.F., Fernández-Alba A.R., 2007. Application of Liquid Chromatography/Quadrupole-Linear Ion Trap Mass Spectrometry and Time-of-Flight Mass Spectrometry to the determination of pharmaceuticals and related contaminants in wastewater. Anal. Chem. 79, 9372-9384.

Carra, I., Casas López, J.L., Santos-Juanes, L., Malato, S., Sánchez Pérez, J.A., 2013. Iron dosage as a strategy to operate the photo-Fenton process at initial neutral pH. Chem. Eng. J. 224, 67–74.

Carraro, E., Bonetta, S., Bertino, C., Lorenzi, E., Bonetta, S., Gilli, G., 2016. Hospital effluents management: Chemical, physical, microbiological risks and legislation in different countries. J. Environ. Manage. 168, 185-199.

Castiglioni S., Bagnati R., Calamari D., Fanelli R., Zuccato E., 2005. A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters. J. Chromatogr. A. 1092, 206-215.

Chen, P., Wang, F., Chen, Z.-F., Zhang, Q., Su, Y., Shen, L., Yao, K., Liu, Y., Cai, Z., Lv, W., Liu, G., 2017. Study on the photocatalytic mechanism and detoxicity of gemfibrozil by a sunlight-driven TiO_2/carbon dots photocatalyst: The significant roles of reactive oxygen species. Appl. Catal. B Environ. 204, 250–275.

Clarizia, L., Russo, D., Di Somma, I., Marotta, R., Andreozzi, R., 2017. Homogeneous photo-Fenton processes at near neutral pH: A review. Appl. Catal. B Environ. 209, 358–371

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard – a decision tree approach. Fd. Cosmet. Toxicol. 16, 255-276.

Diaz, R., Ibáñez, M., Sancho, J.V., Hernández, F., 2013. Qualitative validation of a liquid chromatography–quadrupole-time of flight mass spectrometry screening method for organic pollutants in waters. J. Chromatogr. A. 1276, 47-57

El Najjar, N.H., Touffet, A., Deborde, M., Journel, R., Vel Leitner, N.K., 2014. Kinetics of paracetamol oxidation by ozone and hydroxyl radicals, formation of transformation products and toxicity. Sep. Purif. Technol.136, 137-143.

Elmolla, E.S., Chaudhuri, M., 2009. Degradation of the antibiotics amoxicillin, ampicillin and cloxacillin in aqueous solution by the photo-Fenton process. J. Hazard. Mater. 172(2–3), 1476-1481.

Escher, B. I., Baumgartner, R., Koller, M., Treyer, K., Lienert, J., McArdell, C.S., 2011. Environmental toxicology and risk assessment of pharmaceuticals from hospital wastewater. Water Res. 45(1), 75-92.

Farré M., Ferrer I., Ginebreda A., Figueras M., Olivella L., Tirepu L., Vilanova M., Barceló D., 2001. Determination of drugs in surface water and wastewater samples by liquid chromatography-mass spectrometry: methods and preliminary results including toxicity studies with *Vibrio fischeri*. J. Chromatogr. A. 938, 187-197.

Fatta-Kassinos, D., Vasquez, M.I., Kümmerer, K., 2011. Transformation products of pharmaceuticals in surface waters and wastewater formed during photolysis and advanced oxidation processes – Degradation, elucidation of byproducts and assessment of their biological potency. Chemosphere, 85 (5), 693-709.

Ferreira, S.L.C., dos Santos, W.N.L., Quintella, C.M., Neto, B.B., Bosque-Sendra, J.M., 2004. Doehlert matrix: a chemometric tool for analytical chemistry-review. Talanta 63, 1061–1067.

Gago-Ferrero, P., Schymanski, E.L., Hollender, J., Thomaidis, N.S., 2016. Chapter 13: Non target Analysis of Environmental Samples Based on Liquid Chromatography Coupled to High Resolution Mass Spectrometry (LC-HRMS), Comprehensive Analytical Chemistry.71, 381-403.

Gallard H., De Laat J., Legube B., 1998. Influence du pH sur la vitsse d´oxydation de composes organiques par FeII/H_2O_2. Mechanismes reactionnels et modelization. New J. Chem. 263-268.

García-Galán, M.J., Anfruns, A., Gonzalez-Olmos, R., Rodríguez-Mozaz, S., Comas, J., 2016. UV/H_2O_2 degradation of the antidepressants venlafaxine and O-

desmethylvenlafaxine: Elucidation of their transformation pathway and environmental fate. J. Hazard Mater. 311, 70-80.

García-Muñoz, P., Pliego, G., Zazo, J.A., Munoz, M., de Pedro, Z.H., Bahamonde, A., Casas, J.A. 2017. Treatment of hospital wastewater through the CWPO-photoassisted process catalyzed by ilmenite. J. Environ. Chem. Eng., *In Press*, Available online 24 August 2017.

Giannakis, S., Gamarra Vives, F. A., Grandjean, D., Magnet, A., De Alencastro, L. F., Pulgarin, C., 2015. Effect of advanced oxidation processes on the micropollutants and the effluent organic matter contained in municipal wastewater previously treated by three different secondary methods. Water Res. 84, 295-306.

Gómez M.J., Bueno M.J.M., Lacorte S., Fernández-Alba A.R., Agüera A., 2007. Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. Chemosphere. 66, 993-1002.

Gros M., Petrovic M., Barceló D., 2006. Development of a multi-residue analytical methodology based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters. Talanta. 70, 678-690.

Gupta, A., Garg, A., 2018. Degradation of ciprofloxacin using Fenton's oxidation: Effect of operating parameters, identification of oxidized by-products and toxicity assessment. Chemosphere, 193, 1181-1188.

Gupta, P., Mathur, N., Bhatnagar, P., Nagar, P., Srivastava, S., 2009. Genotoxicity evaluation of hospital wastewaters. Ecotoxicol Environ Saf. 72(7), 1925-1932.

Heberer T., 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol. Lett. 131, 5–17.

Hernández, F., Ibáñez, M., Bade, R., Bijlsma, L., Sancho, J.V., 2014. Investigation of pharmaceuticals and illicit drugs in waters by liquid chromatography-high-resolution mass spectrometry. TrAC Trends Anal. Chem. 63, 140-157.

Ibáñez, M., Borova, V.,Boix, C., Aalizadeh, R., Bade, R., Thomaidis, N.S., Hernández, F., 2017. UHPLC-QTOF MS screening of pharmaceuticals and their metabolites in treated wastewater samples from Athens. J. Hazard Mater. 323, 26-35.

ISO 6332, Water quality - Determination of iron - Spectrometric method using 1,10-phenanthroline, 1988.

Jallouli, N., Elghniji, K., Hentati, O., Ribeiro, A.R., Silva, A.M.T., Ksibi, M., 2016. UV and solar photo-degradation of naproxen: TiO_2 catalyst effect, reaction kinetics, products identification and toxicity assessment. J. Hazard Mater. 304, 329–336.

Kern, D.I., Schwaickhardt, R.d.O., Mohr, G., Lobo, E.A., Kist, L. T., Machado, Ê.L., 2013. Toxicity and genotoxicity of hospital laundry wastewaters treated with photocatalytic ozonation. Sci Total Environ. 443, 566-572.

Kim S.D., Cho J., Kim I.S., Vanderford B.J., Snyder S.A., 2007. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking and waste waters. Water Res. 41, 1013-1021.

Klamerth, N., Malato, S., Agüera, A., Fernández-Alba, A., 2013. Photo-Fenton and modified photo-Fenton at neutral pH for the treatment of emerging contaminants in wastewater treatment plant effluents: A comparison.Water Res.47, 833–840.

Klavarioti, M., Mantzavinos, D., Kassinos, D., 2009. Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. Environ. Int. 35, 402–417.

Lacey C., McMahon G., Bones J., Barron L., Morrissey A., Tobin J.M, 2008. An LC–MS method for the determination of pharmaceutical compounds in wastewater treatment plant influent and effluent samples. Talanta. 75, 1089-1097.

Langford, K. H., Thomas, K.V., 2009. Determination of pharmaceutical compounds in hospital effluents and their contribution to wastewater treatment works. Environ. Int. 35(5), 766-770.

Lanzafame, G.M., Sarakha, M., Fabbri, D., Vione, D., 2017. Degradation of Methyl 2-Aminobenzoate (Methyl Anthranilate) by H_2O_2/UV: Effect of Inorganic Anions and Derived Radicals. Molecules 22, 619.

Li, W., Nanaboina, V., Zhou, Q., Korshin, G.V., 2012. Effects of Fenton treatment on the properties of effluent organic matter and their relationships with the degradation of pharmaceuticals and personal care products. Water Res. 46, 403-412.

Lin, A. Y.-C., Wang, X.-H., Lin, C.-F., 2010. Impact of wastewaters and hospital effluents on the occurrence of controlled substances in surface waters. Chemosphere. 81(5), 562-570.

Lishman L., Smyth S.A., Sarafin K., Kleywegt S., Toito J., Peart T., Lee B., Servos M., Beland M., Seto P., 2006. Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. Sci Total Environ. 367, 544-558.

Marković, M., Jović, M., Stanković, D., Kovačević, V., Roglić, G., Gojgić-Cvijović, G., Manojlović, D., 2015. Application of non-thermal plasma reactor and Fenton reaction for degradation of ibuprofen. Sci Total Environ. 505, 1148-1155.

Mendoza, A., Aceña, J., Pérez, S., López de Alda, M., Barceló, D., Gil, A., Valcárcel, Y., 2015. Pharmaceuticals and iodinated contrast media in a hospital

wastewater: A case study to analyse their presence and characterise their environmental risk and hazard. Environ Res. 140, 225-241.

Metcalfe C.D., Miao X.S., W., H.,R.,L., 2004. Pharmaceuticals in the Canadian environment in Pharmaceuticals in the environment- Sources, fate, effects and risks. Kümmerer K. (ed). Berlin, Heidelberg, New York: Springer, pp. 67-90.

Miralles-Cuevas, S., Audino, F., Oller, I., Sánchez-Moreno, R., Sánchez Pérez, J. A., Malato, S., 2014a. Pharmaceuticals removal from natural water by nanofiltration combined with advanced tertiary treatments (solar photo-Fenton, photo-Fenton-like Fe(III)–EDDS complex and ozonation). Sep. Purif. Technol. 122, 515-522.

Miralles-Cuevas, S., Oller, I., Pérez, J. A. S., & Malato, S., 2014b. Removal of pharmaceuticals from MWTP effluent by nanofiltration and solar photo-Fenton using two different iron complexes at neutral pH. Water Res. 64, 23-31.

Mirzaei, A., Chen, Z., Haghighat, F., Yerushalmi. L., 2017. Removal of pharmaceuticals from water by homo/heterogonous Fenton-type processes: A review. Chemosphere, 174, 665-688.

Moctezuma, E., Leyva, E., Aguilar, C.A., Luna, R.A., Montalvo, C., 2012. Photocatalytic degradation of paracetamol: Intermediates and total reaction mechanism. J. Hazard Mater. 243, 130-138.

Nogueira, R., Oliveira, M., Paterlini, W., 2005. Simple and fast spectrophotometric determination of H_2O_2 in photo-Fenton reactions using metavanadate. Talanta 66, 86–91.

OECD Guidelinesfor Testing of Chemicals, Simulation Test-Aerobic Sewage Treatment 303ª, 1999.

Ostra, M., Ubide, C., Zuriarrain, J., 2007. Interference modelling, experimental design and pre-concentration steps in validation of the Fenton's reagent for pesticides determination. Anal. Chim. Acta 584, 228–235.

Perini, J.A.L., Tonetti, A.L., Vidal, C., Montagner, C.C., Nogueira. R.F.P., 2018. Simultaneous degradation of ciprofloxacin, amoxicillin, sulfathiazole and sulfamethazine, and disinfection of hospital effluent after biological treatment via photo-Fenton process under ultraviolet germicidal irradiation. Appl. Catal. B Environ. 224, 761–771

Rahim Pouran, S., Abdul Aziz, A.R., Wan Daud, W.M.A., 2015. Review on the main advances in photo-Fenton oxidation system for recalcitrant wastewaters. J. Ind. Eng. Chem. 21, 53-69.

Roberts P.H., Thomas K.V., 2006. The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci Total Environ. 356, 143-153.

Santiago-Morales, J., Agüera, A., Gómez, M del M., Fernández-Alba, A.R., Giménez, J., Esplugas, S., Rosal, R., 2013. Transformation products and reaction kinetics in simulated solar light photocatalytic degradation of propranolol using Ce-doped TiO_2. Appl. Catal. B Environ.129, 13–29.

Santos J.L., Aparicio I., Alonso E., Callejón M., 2005. Simultaneous determination of pharmaceutically active compounds in wastewater simples by solid extraction and highperformance liquid chromatography with diode array and fluorescence detectors. Anal. Chim. Acta. 550, 116-122.

Santos, L.H.M.L.M., Gros, M., Rodriguez-Mozaz, S., Delerue-Matos, C., Pena, A., Barceló, D., Montenegro, M.C.B.S.M., 2013. Contribution of hospital effluents

to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. Sci Total Environ. 461–462, 302-316.

Sedlak D.L., Pinkston K., Huang C.H., 2005. Occurrence survey of pharmaceutically active compounds. Denver, CO: Awwa Research Foundation.

Sirtori, C., Zapata, A., Gernjak, W., Malato, S., Lopez, A., Agüera, A., 2010. Solar photo-Fenton degradation of nalidixic acid in waters and wastewaters of different composition. Analytical assessment by LC TOF-MS. Water Res. 45, 1736-1744.

Spongberg A.L., Witter J.D., 2008. Pharmaceutical compounds in the wastewater process stream in Northwest Ohio. Sci Total Environ., 397, 148-157.

Sui Q., Huang J., Deng S., Yu G., Fan Q., 2010. Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. Water Res. 44, 417-426.

Sun, X. Xue, K-L., Jiao, X-Y., Chen, Q., Xu, L., Zheng, H., Ding, Y-F., 2016. Simultaneous determination of nimesulide and its four possible metabolites in human plasma by LC–MS/MS and its application in a study of pharmacokinetics. J. Chromatogr. B. 1027, 139-148.

Teófilo, R.F., Ferreira, M.M.C., 2006. Chemometrics II: Spreadsheets for experimental desing calculations, a tutorial. Quim. Nova 29, 338-350.

Ternes T.A., Bonerz M., Schmidt T., 2001a. Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography-electrospray tandem mass spectrometry. J. Chromatogr. A. 938, 175-185.

Ternes T.A., 2001b. Analytical methods for the determination of pharmaceuticals in aqueous environmental samples. TrAC Trends Analyt. Chem. 20, 419-434.

Togola, A., Baran, N., Coureau, C., 2014. Advantages of online SPE coupled with UPLC/MS/MS for determining the fate of pesticides and pharmaceutical compounds. Anal. Bioanal. Chem. 406 (4), 1181-1191.

Urbano, V.R., Maniero, M.G., Perez-Moya, M., Guimaraes, J.R., 2017. Influence of pH and ozone dose on sulfaquinoxaline ozonation. J. Environ. Manage. 195, 224-231.

Vermilye A.W., Voelker, B., 2009. Photo-Fenton Reaction at Near Neutral pH. Environ. Sci. Technol. 43, 6927–6933.

Wilde, M.L., Schneider, M., Kümmerer, K., 2017. Fenton process on single and mixture components of phenothiazine pharmaceuticals: Assessment of intermediaries, fate, and preliminary ecotoxicity. Sci Total Environ. 583, 36-52.

Zapata, A., Oller, I., Sirtori, C., Rodríguez, A., Sánchez-Pérez, J.A., López, A., Mezcua, M., Malato, S., 2010. Decontamination of industrial wastewater containing pesticides by combining large-scale homogeneous solar photocatalysis and biological treatment. Chem. Eng. J. 160, 447–456.

Zhang, R. Yang, Y., Huang, C-H., Zhao, L., Sun, P., 2016. Kinetics and modeling of sulfonamide antibiotic degradation in wastewater and human urine by UV/H_2O_2 and UV/PDS. Water Res. 103, 283-292.

Zhao, Y., Yu, G., Chen, S., Zhang, S., Wang, B., Huang, J., Deng, S., Wang, Y., 2017. Ozonation of antidepressant fluoxetine and its metabolite product norfluoxetine: Kinetics, intermediates and toxicity.Chem. Eng. J. 316, 951–963.