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Dedicated to the memory of
Professor Candin Liteanu on his 100th anniversary

SIMULTANEOUS IDENTIFICATION OF FENTON DEGRADATION BY-PRODUCTS OF DICLOFENAC, IBUPROFEN AND KETOPROFEN IN AQUATIC MEDIA BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY

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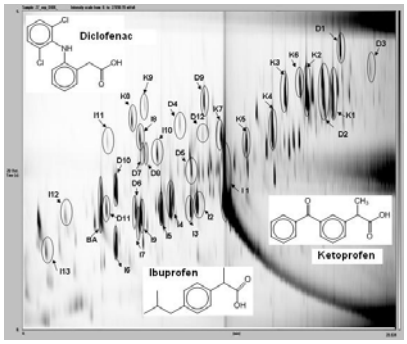
Diclofenac, ibuprofen and ketoprofen are anti-inflammatory drugs intensively used both in human and animal treatment. Due to their high stability these compounds are partially removed by wastewater treatment plants and from this reason the development of some alternative treatments such as advanced oxidative processes are necessary. The main problems in the optimization of an advanced oxidative process rise from the difficulties which appear in the identification of degradation by-products necessary for the establishment of degradation pathway. In this paper a developed method for the simultaneous identification of Fenton degradation by-products of the three above mentioned pharmaceuticals is presented. The obtained results show the comprehensive two-dimensional gas chromatography coupled with mass spectrometry as a proper method for the analysis of the complex mixture of compounds resulted from the Fenton degradation process. Moreover, some compounds never mentioned in the scientific literature were identified.



INTRODUCTION

Diclofenac, ibuprofen and ketoprofen are non-steroidal anti-inflammatory drugs (NSAIDs) with analgesic, antipyretic and platelet-inhibitory actions[1] which are used in large quantities for the human and animal treatment. Different studies[2-4] showed that some of these compounds have a toxic effect both for terrestrial vertebrate and aquatic wildlife being considered as the cause of the massive decline of vulture population in Pakistan, and on the Indian subcontinent.[5] Recently, the European Commission introduced diclofenac on the list of priority substances[6] and recommended to the member states their monitoring in different water bodies.

The NSAIDs input in the aquatic compartment is mainly through human waste by excretion under metabolized or unaltered parent compound forms as well as by their discharge during the manufacturing process.[7] Different studies[8-10] reported that NSAIDs are partially removed by the wastewater treatment plants and therefore the environmental input tends to considerably increase.

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In the last decade different advanced oxidation processes (AOPs) such as ozone oxidation,[11,12] O_3/UV, O_3/ultrasound (O_3/US), O_3/H_2O_2, O_3/UV/US, O_3/US/$FeSO_4$,[13] H_2O_2/UV,[11] heterocatalytic/UV,[14] Fe^{2+}/H_2O_2 (Fenton),[15-17] Fe^{2+}/H_2O_2/UV (photo-Fenton)[17-19] etc. have been applied to the degradation of NSAIDs.

Advanced oxidation processes (AOPs) are a group of treatment processes in the degradation of refractory compounds which use an oxidative species (O_3, •OH, chloride etc.) to mineralize the organic compounds into CO_2 and H_2O.[20] One of the most applied and promising method for the removal of organic compounds from aquatic samples is the Fenton oxidation process, because the reagents used in this method are cheap, easy to handle and environmentally friendly.[20]

The Fenton oxidation process uses the Fenton reagent, a mixture of H_2O_2 and Fe^{2+}, which generates •OH radicals according to the Eq. (1), for the oxidation of organic matter. These radicals are very active and react quickly with the organic compound generating an intermediate (Eq. 2). Then Fe^{2+} is regenerated through the reduction of Fe^{3+} by H_2O_2 (Eq. 3).[21]

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + \bullet OH + H_2O \quad (1)$$

$$\textit{Organic compounds} + \bullet OH \rightarrow \textit{intermediate} \quad (2)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO\bullet + H^+ \quad (3)$$

Unfortunately, parallel reactions can consume •OH radicals, as described in Eqs. (4) and (5), leading to a reduction of the Fenton reagent efficacy over time.[22]

$$\bullet OH + Fe^{2+} \rightarrow Fe^{3+} + OH^- \quad (4)$$

$$\bullet OH + H_2O_2 \rightarrow H_2O + HOO\bullet \quad (5)$$

The application of Fenton and photo-Fenton oxidation processes on the removal of NSAIDs from aquatic media has been reported in literature.[15,16,18,19,23,24] Usually, these studies have been focused on the investigation of degradation rate of some NSAIDs under different conditions. Few studies provide some degradation pathways[17,18] but, most of them are focused only to one compound providing information about its degradation mechanism and its degradation by-products. According to our knowledge, studies related to a simultaneous identification of the degradation by-products of a NSAID mixture were not presented in literature. This lack appears due to the technical limits of the analysis methods used for the identification of the degradation by-products which are able to analyze a reduced number of compounds. The identification of an increased number of degradation by-products can be realized by using multidimensional chromatographic methods.[25-28]

Comprehensive two-dimensional gas chromatography (GC×GC) appears nowadays as the main analytical tool for the study of complex mixtures of volatile and semi-volatile organic compounds.[25] This technique uses for separation two columns with different polarities coupled together by an interface called modulator.[26] In this way, two mechanisms of separation, one based on the volatility and another one on polarity, are combined,[27] having as results the improvement of the chromatographic resolution due to the increase of peak capacity, the decrease of detection limits by cryofocusing and the ability to present structures of chromatograms in a chemically ordered manner according to the volatility and polarity of the compounds.[28]

The aim of this study is to apply the comprehensive two-dimensional gas chromatography coupled with quadrupole mass spectrometry (GC×GC-qMS) for the simultaneous identification of a large number of degradation by-products of selected NSAIDs (diclofenac, ibuprofen and ketoprofen) under Fenton oxidation process and for a better understanding of their degradation pathway.

RESULTS AND DISCUSSION

The results of performed experiment showed that the GC×GC-qMS method gives the possibility to identify the most of the main degradation by-products which are formed during the Fenton oxidation of the selected NSAIDs. Thus, 12 degradation compounds for the case of diclofenac, 13 for ibuprofen, and 9 for ketoprofen have been identified (Fig. 1).

Among the diclofenac by-products, four of them namely Benzene, 1,3-dichloro-2-isocyanato- (D6); 2,6-Dichloronitrobenzene (D7); 2,6-Dichlorophenyl isocyanide (D8) and N-(2,6-Dichlorophenyl)-formamide (D9), were identified for the first time, these compounds never being mentioned in literature referring to the diclofenac Fenton degradation pathway. Nevertheless there are still other compounds which cannot be identified due to the instrument performance limits.

Regarding the separation of the degradation by-products, it can be observed that these compounds are well distributed on the whole chromatogram. In the lower part are situated the hydroxyl and acidic compounds while at the upper part the carbonyl compounds.

Meaning of the abbreviations, the names and the structural formulas of the identified degradation compounds are presented in Tables 1-3.

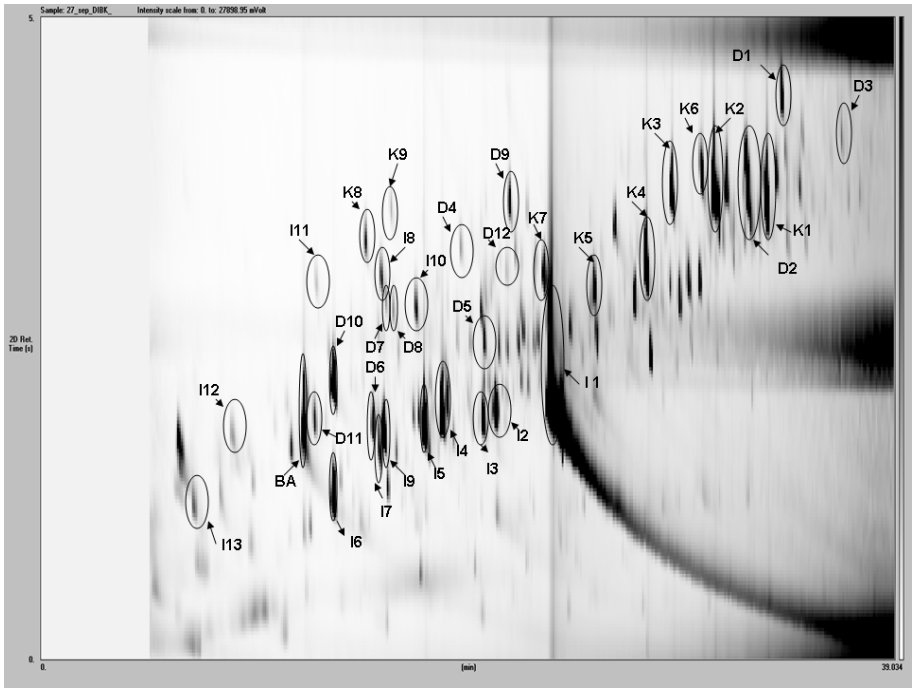
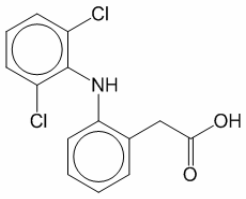
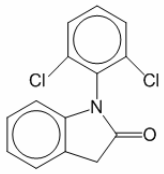
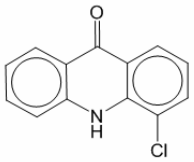
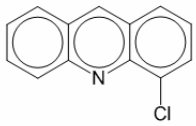
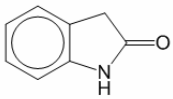
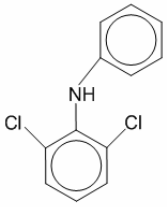
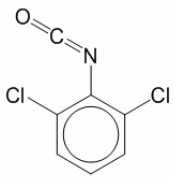
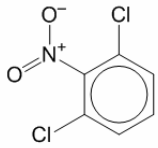
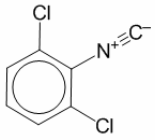
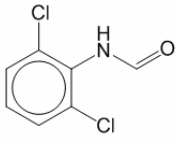
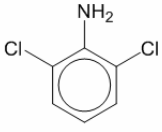
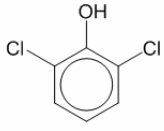
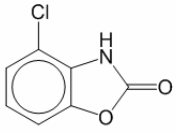


Fig. 1 – GC×GC chromatogram of the identified Fenton degradation by-products of diclofenac, ibuprofen and ketoprofen.

Table 1

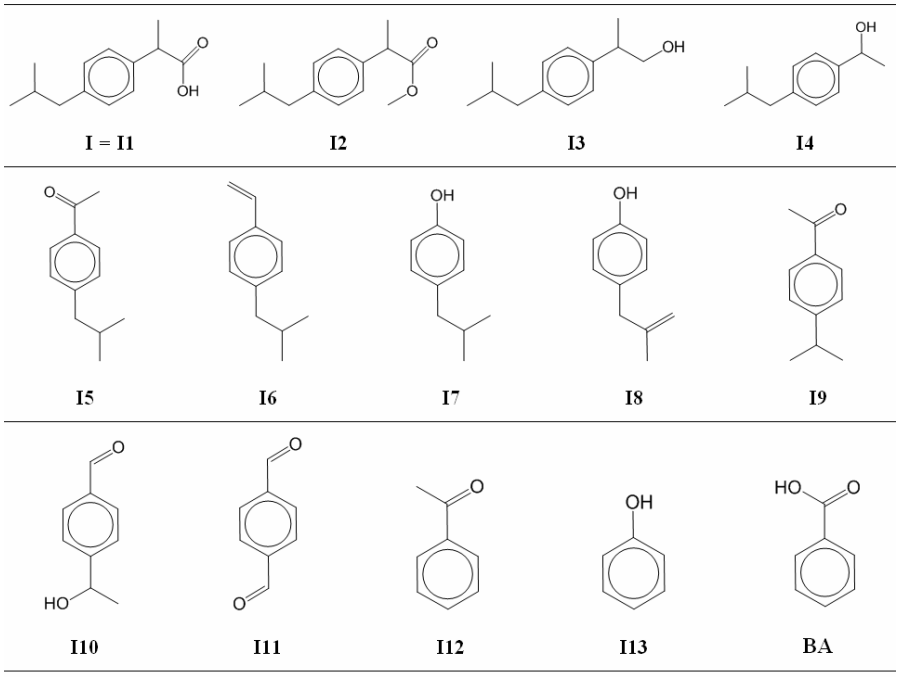
Diclofenac (D) and Fenton degradation by-products of diclofenac (D1-D12).
Structural formula and chemical name from NIST mass spectral library

 **D**	 **D1**	 **D2**	 **D3**
 **D4**	 **D5**	 **D6**	 **D7**
 **D8**	 **D9**	 **D10**	 **D11**	 **D12**

(D) Diclofenac; (D1) 3H-Indol-2-one, 1-(2,6-dichlorophenyl)-; (D2) 10(H)Acridin-9-one, 4-chloro-; (D3) Acridine, 9-chloro-; (D4) 2H-Indol-2-one, 1,3-dihydro-; (D5) Benzenamine, 2,6-dichloro-N-phenyl-; (D6) Benzene, 1,3-dichloro-2-isocyanato-; (D7) 2,6-Dichloronitrobenzene; (D8) 2,6-Dichlorophenyl isocyanide; (D9) N-(2,6-Dichlorophenyl)formamide; (D10) Benzenamine, 2,6-dichloro-; (D11) Phenol, 2,6-dichloro-; (D12) 4-Chloro-1,3-benzoxazol-2(3H)-one.

Table 2

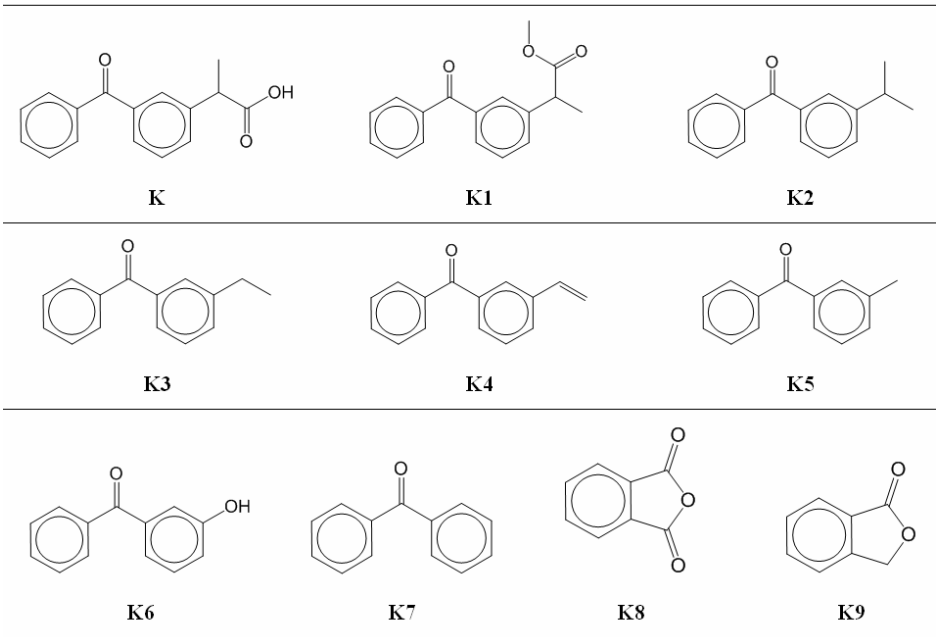
Ibuprofen (I) and Fenton degradation by-products of ibuprofen (I1-I13, BA).
Structural formula and chemical name from NIST mass spectral library



(I=I1) Ibuprofen; (I2) Ibuprofen methyl derivative; (I3) Benzeneethanol, β-methyl-4-(2-methylpropyl)-; (I4) Benzene, 1-(1-hydroxyethyl)-4-isobutyl-; (I5) 4'-(2-Methylpropyl)acetophenone; (I6) Benzene, 1-ethenyl-4-(2-methylpropyl)-; (I7) Phenol, 4-(2-methylpropyl)-; (I8) Phenol, p-(2-methylallyl)-; (I9) Ethanone, 1-[4-(1-methylethyl)phenyl]-; (I10) 4-(1-Hydroxyethyl)benzaldehyde; (I11) 1,4-Benzenedicarboxaldehyde; (I12) Acetophenone; (I13) Phenol; (BA) Benzoic acid.

Table 3

Ketoprofen (K) and Fenton degradation by-products of Ketoprofen (K1-K9).
Structural formula and chemical name from NIST mass spectral library



(K) Ketoprofen; (K1) Ketoprofen methyl ester; (K2) Methanone, [3-(1-methylethyl)phenyl]phenyl-; (K3) Methanone, (3-ethylphenyl)phenyl-; (K4) Methanone, (3-ethenylphenyl)phenyl-; (K5) Methanone, (3-methylphenyl)phenyl-; (K6) m-Hydroxybenzophenone; (K7) Benzophenone; (K8) Phthalic anhydride; (K9) 1(3H)-Isobenzofuranone.

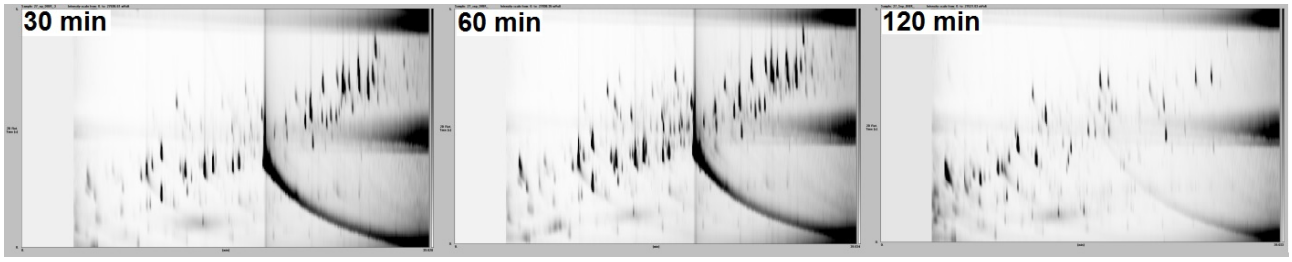


Fig. 2 – The evolution of Fenton degradation process of selected NSAIDs.

Concerning the degradation mechanism, the results showed that each compound has its own degradation mechanism and no reaction between the different oxidation by-products occurs. It is also observed that the oxidation process starts with the oxidation of aliphatic chain bonded to the aromatic rings. Only in the case of diclofenac, the degradation mechanism can follow two pathways, (a) and (b). In the pathway (a), diclofenac can be dehydrated due to the acidic pH of solution resulting (2,6-diclorophenyl)-indolin-2-one (*i.e.*, ring closure reaction),[15,29] while in the pathway (b) can take place a dechlorination which subsequently underwent cyclization forming a six-membered ring, such as 10(H)acridin-9-one, 4-chloro- or acridine, 4-chloro-.[30] Further the degradation mechanism of diclofenac continues with the cleavage of the C–N bonds leading to the formation of various aromatic compounds, each with one benzene ring (Table 1).

Regarding the Fenton oxidative process efficiency over the selected NSAIDs, the performed experiments showed that the Fenton process is slow and the time necessary for their complete degradation is long. Fig. 2 presents the GCxGC chromatograms obtained after 30, 60 and 120 minutes of Fenton degradation.

It can be observed that, as the oxidative degradation process takes place, the bottom left corner of the chromatogram is populate due to lower retention of the compounds in both dimensions of the GC separation. This means that the molecular structures of the formed products become simpler and their polarity is modified by the losing of the organic functions which may confer them some polar characteristics. As opposed the short degradation times lead to an agglomeration of the top right corner of the chromatogram due to the formation of the first NAIDs degradation by-products, compounds with high molecular structure and polarity.

EXPERIMENTAL

1. Chemicals

Diclofenac sodium, ibuprofen and ketoprofen, each of 99% purity, were purchased from Fluka-Sigma-Aldrich. A working solution of 1 mg/L of each compound has been prepared in methanol. Ethyl acetate (99.5%) and methanol (99.9%) were purchased from Carlo Erba. Sulphuric acid solution (0.5 mol/L) AVS TITRINORM was purchased from Chemicals VWR BDH Prolabo (Paris, France). H_2O_2 (30%), iron (II) sulfate heptahydrate (99%) and sodium chloride (99%) were purchased from Sigma-Aldrich. Helium (99.9999%) was purchased from Messer (France). Milli-Q water (18.2 Ω conductivity) obtained by a Millipore ultrapure water system was used for the preparation of iron (II) solution and for the degradation experiments.

2. Instruments and methods

For the analysis of the Fenton degradation by-products of NSAIDs, a Thermo Trace GC×GC gas chromatograph equipped with a dual CO_2 cryogenic modulator and coupled to a quadrupole mass spectrometer (qMS) model ThermoISQ (Courtaboeuf, France) was used. Helium of high purity at a constant flow rate of 1 mL min^{-1} was used as carrier gas for GC×GC analysis. The mass spectrometer frequency of acquisition was 50 Hz, and the total ion current (TIC) MS signal was used for data collection setting a mass range from 50 to 300 *m*/*z*. The ionization was performed by Electron Impact Ionization using a voltage of −70 eV. The ion source temperature was 230°C and that for transfer line 270°C. The inlet temperature was set at 270ºC and the injection was made in splitless mode.

For the separation, conventional phase orthogonal set columns which consist in a non polar column in the first dimension and a medium polarity column in the second dimension were used. A Factor Four VF-1 ms column (100% dimethylpolysiloxane), 15 m × 0.25 mm ID, 1.0 µm film thickness (Varian) was used in the first dimension and a DB-17 column (50% phenyl/50% dimethylpolysiloxane), 1.5 m × 0.10 mm ID, 0.10 µm film thickness (Agilent) was used for the second dimension. The separation of compounds was performed with a gradient temperature program, starting by a slowly heating of 5ºC/min from 70ºC to 265ºC, with 1 minute final hold time. The modulation period was 5 seconds and the initial off-set was 1.2 second.

The data acquisition was performed using the X-Calibur software and the GC×GC representation was realized by the Chrom-Card software. The identification of the degradation by-products was done by comparison the obtained mass

spectra with those from NIST (classical) mass spectral library. Only the compounds for which the similarity between the obtained mass spectra and the mass spectra from NIST library exceed 60 % have been taken into account.

3. Fenton degradation

For the Fenton degradation experiment, 10 mL of a Milli Q water sample acidified at pH 2 with 100 µL H_2SO_4 0.5 mol/L containing 200 ng of each studied anti-inflammatory drug (diclofenac sodium, ibuprofen and ketoprofen) was placed into a 50 mL brown glass bottle. Immediately, 500 µL of Fe^{2+} solution (1 mg/mL Fe^{2+}) and 200 µL of H_2O_2 (30%) were added into the brown bottle which was kept under magnetic stirring and room temperature for different time intervals in order to perform the degradation process. Because the catalyst (Fe^{2+}) and the oxidant (H_2O_2) are consumed very fast, these were refreshed at each 30 minutes in the same amounts as it was described previously. To study the Fenton oxidative process efficiency over the selected drugs, different experiments at 30, 60 and 120 minutes were performed. After each Fenton oxidative experiment, the degradation by-products of NAIDs were extracted with organic solvent and analyzed by GC×GC-qMS.

4. Extraction procedure

The sample resulted after each Fenton experiment was transferred into a separation funnel where 1.5 g of NaCl was added to create a salt out effect. After a manually shaking to dissolve the salt, 2 mL of ethyl acetate were added into the separation funnel followed by 5 minutes of vigorous shaking extraction. After the phase separation, the organic one was collected into a 5 mL glass vial and evaporated at 200 µL under nitrogen. One microliter of the resulted extract was manually injected into GC×GC using a micro-syringe of 10 µL.

CONCLUSIONS

After the Fenton oxidation process of the selected anti-inflammatory drugs were identified 12 degradation by-products of diclofenac, 13 of ibuprofen and 9 of ketoprofen. Among the diclofenac by-products, four of them namely Benzene, 1,3-dichloro-2-isocyanato- (D6); 2,6-Dichloronitrobenzene (D7); 2,6-Dichlorophenyl isocyanide (D8) and N-(2,6-Dichlorophenyl)-formamide (D9), were identified for the first time, these compounds never being mentioned in literature referring to the diclofenac Fenton degradation pathway.

The obtained results by the developed Fenton experiments prove that GC×GC technique could be a convenient alternative to monitor the advanced oxidative processes of organic compound mixture due to their ability to analyze in a single run compounds with different physico-chemical properties. Coupling this technique with the mass spectrometry gives the possibility to identify a huge number of degradation by-products and to provide enough data to estimate a realistic degradation pathway of the organic compounds under advanced oxidation processes.

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