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Application of Metabolite Profiling Tools and Time-of-Flight Mass Spectrometry in the Identification of Transformation Products of Iopromide and Iopamidol during Advanced Oxidation

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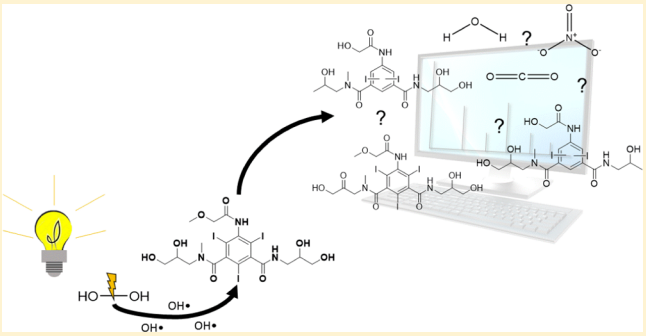
Supporting Information

ABSTRACT: The efficiency of wastewater treatment systems in removing pharmaceuticals is often assessed on the basis of the decrease in the concentration of the parent compound. However, what is perceived as "removal" during treatment may not necessarily mean mineralization of the pharmaceutical compound but simply conversion into different transformation products (TPs). Using liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer (LC-QToF-MS), we demonstrated conversion of iopromide in wastewater to at least 14 TPs after an advanced oxidation process (AOP) using UV (fluence = 1500 mJ/cm^2) and H_2O_2 (10 mg/L). Due to the complexity of the wastewater matrix, the initial experiments were performed using a high concentration (10 mg/L) of iopromide in order to facilitate the identification of TPs. Despite the high concentration of iopromide used, cursory inspection of UV and mass spectra only revealed four TPs in the chromatograms of the post-AOP samples. However, the use of METLIN database and statistics-based profiling tools commonly used in metabolomics proved effective in discriminating between background signals and TPs derived from iopromide. High-resolution mass data allowed one to predict molecular formulas of putative TPs with errors below 5 ppm relative to the observed m/z. Tandem mass spectrometry (MS/MS) data and isotope pattern comparisons provided necessary information that allowed one to elucidate the structure of iopromide TPs. The presence of the proposed iopromide TPs was determined in unspiked wastewater from a municipal wastewater treatment plant, but no iopromide and TPs were detected. Using analogous structural modifications and oxidation that results from the AOP treatment of iopromide, the potential TPs of iopamidol (a structurally similar compound to iopromide) were predicted. The same mass fragmentation pattern observed in iopromide TPs was applied to the predicted iopamidol TPs. LC-QToF-MS revealed the presence of two iopamidol TPs in unspiked AOP-treated wastewater.



INTRODUCTION

The presence of pharmaceuticals and personal care products (PPCPs) in many water bodies has been a growing concern. Data from many parts of the world show the high detection frequency of PPCPs in lakes, rivers, and marine environments that receive municipal wastewater discharges.[1−3] Due to the continuous input from anthropogenic activities, PPCPs have been considered contaminants of emerging concern (CEC).[4−7] CECs comprise a broad range of chemicals including pesticides, industrial and commercial compounds, PPCPs, and steroid hormones that are introduced into the environment in a plethora of ways, such as from wastewater treatment plants (WWTPs) and hospital effluents.[8−10] Some PPCPs can cause adverse effects at environmentally relevant concentrations on many organisms across different trophic levels. For example, synthetic estrogens have caused the collapse of fathead minnow population after exposure to 5 ng/L of ethinylestradiol. In addition, while the toxicity of a single pharmaceutical at the expected environmental concentrations may not be observed, synergistic effects may occur in the presence of a mixture of several PPCPs.[9,11−13] Notably, the occurrence of low levels of antibiotics in the environment has been linked to the increased emergence of resistant bacteria and antibiotic resistance genes, which are now also considered as CECs.[14]

A risk assessment study conducted by Santos et al.[9] determined the hazard quotient of PPCPs in hospital effluents to fish, daphnids, and algae, based on the worst-case scenario using the highest measured environmental concentrations of each compound and its predicted no-effect concentration.

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Among the PPCPs detected are the X-ray contrast agents iopromide and iopamidol, which were classified to have a high hazard quotient on algae and fish. Excreted almost unchanged (90%), iopromide is one of the most frequently detected PPCPs in hospital effluents and has been detected at concentrations ranging from ng/L to μg/L.[9,15−17] Nonionic contrast media have been shown to induce oxidative stress and apoptosis in human neutrophils.[18] Because the concentrations of iopromide in the environment are typically low (except in hospital effluents), TPs are less likely to cause toxic effects on organisms; however, the concern is more on the contribution of iodinated compounds on the formation of disinfection byproducts. Iodinated disinfection byproducts that are generated upon treatment of iodinated contrast media-containing water have been shown to cause mammalian cell toxicity.[19] In addition, the production of a toxic mixture of disinfection byproducts after iopamidol underwent chlorination has also been reported.[20,21]

Iopromide and other iodinated X-ray contrast media (e.g., iopamidol) have been shown to undergo biotransformation in activated sludge and drinking water treatment processes.[16,22,23] The TPs generated, however, are not effectively removed from the drinking water supply.[16] Therefore, there is a need to find more effective treatment processes to remove iopromide from effluents of WWTPs. Recently, the effectiveness of the advanced oxidation process (AOP) based on UV/H_2O_2 followed by biodegradation, resulting in the mineralization of carbamazepine which is otherwise a recalcitrant pharmaceutical, has been demonstrated.[24] Additional studies have also reported that the UV/H_2O_2-based AOP results in the high removal rate of other PPCPs in wastewater.[25−28] However, many of these studies reporting "removal" of PPCPs measured only the concentrations of the parent compounds before and after the AOP and did not determine the TPs formed. As highlighted in the recent viewpoint article by Stadler et al., disappearance of PPCPs during treatment may simply be a transformation process producing compounds that may still have environmental effects; therefore, analysis of TPs is warranted.[29]

The goal of this study is to examine the fate of iopromide during the UV/H_2O_2 AOP and identify the resulting major TPs. In addition, on the basis of the identified TPs of iopromide, the TPs of a structurally related compound (iopamidol) in real wastewater were deduced after the AOP treatment. It is important to identify recalcitrant TPs, especially those that retain biological activity and could potentially be more toxic than their parent compounds.[30] Liquid chromatography (LC) coupled to mass spectrometry (MS) has been the method of choice in the targeted and untargeted analysis of contaminants in water. Identification of unknown TPs at low concentrations in a complex sample matrix is very challenging. The advent of MS instruments capable of high accurate mass measurements, such as a quadrupole time-of-flight mass spectrometer (QToF-MS), combined with high-throughput data acquisition and powerful software to aid in statistical analysis could alleviate the seemingly daunting task of unknown identification of TPs. The high mass resolving power of the QToF-MS allows discrimination of masses down to the ppb error range. The identified masses can be utilized to calculate probable empirical formulas corresponding to the observed mass. Databases such as METLIN (http://masspec.scripps.edu/) contain more than 60 000 structures and about 12 000 metabolites with high-resolution tandem mass spectrometry (MS/MS) data that can be exported and compared to serve as reference for the high-resolution m/z of metabolites and their respective fragments.[31] To handle the large amount of data produced, QToF-MS instruments are equipped with high-throughput data management systems that are capable of identifying molecular species and their related adducts. The data system has the ability to calculate the fold changes in the signal intensities of specific m/z. Fold changes are the differences in the ion counts of specific m/z between different samples (e.g., collected at different time intervals) and facilitate the identification of species that are down- or up-regulated in the experimental time series. The QToF-MS instruments also contain a collision cell that generates fragmentation data, which can facilitate structural elucidation of unknown compounds. With these capabilities, LC-QToF-MS instruments have been extensively used to determine biomarkers of diseases and profiling metabolites in complex biological matrices.[32−34] This study demonstrates how LC-QToF-MS facilitated the identification of novel iopromide TPs formed during the AOP. To demonstrate that this approach can be extended to other PPCPs, the concentration of endogenous iopamidol (unspiked) in wastewater was measured before and after the AOP; its TPs were identified using the same approach used for iopromide. The use of LC-QToF-MS combined with powerful data analyses prove extremely useful in identifying environmentally relevant TPs and contaminants that are otherwise difficult to detect in complex matrices.

MATERIALS AND METHODS

Iopromide (99%) was purchased from U.S. Pharmacopeia (Rockville, MD). Deuterated carbamazepine (D-10 CBZ) was obtained from CDN Isotopes (Quebec, CA). Iopamidol (95%) was obtained from Sigma-Aldrich (St. Louis, MO). Tuning solution (G1969-85000) was from Agilent Technologies (Santa Clara, CA). A Barnstead NANOpure Diamond purification system (Waltham, MA) was used to obtain 18.2 MΩ water throughout all the experiments. LC/MS grade methanol (Optima LC/MS) was used for LC separations. Methanol and formic acid (88%) were purchased from Fisher Scientific (Pittsburgh, PA).

Sample Collection and Shipment. The AOP experiments were carried out at the University of Colorado (Boulder, CO) using secondary WWTP effluents spiked with iopromide (to a final concentration of 10 mg/L) in order to allow detection of novel TPs and monitor their formation during the AOP and removal during biodegradation. All effluents were collected from the Boulder WWTP. The spiked WWTP effluent was fed into a UV/H_2O_2 bench scale reactor followed by biofiltration as described in an earlier publication by Keen et al.[24] Iopromide concentrations up to 10 mg/L were found not to be inhibitory towards microbial cultures in previous studies conducted by Khunjar et al.[35] Samples were taken before the AOP (pre-AOP), after the AOP (post-AOP), and after 72 h of biofiltration. Unspiked wastewater served as the blank. All samples were filtered through a 0.45 μm nylon filter to remove particulate matter and minimize bacterial degradation and then kept frozen and shipped overnight to the University at Buffalo (Buffalo, NY), where they were kept frozen for no longer than 1 week before analysis.

Subsequent experiments using the same AOP conditions and biofiltration setup were performed to determine the presence of endogenous levels of iopromide and a structurally similar compound, iopamidol, in the wastewater before and after the AOP treatment. By analogy, putative TPs in spiked iopromide

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Table 1. Identified AOP Transformation Products of Iopromide with the Proposed Molecular Formula, Characteristic MS/MS Fragmentation, and the % Removal after Biodegradation

chemical formula	theoretical m/z	actual m/z	average error,[a] ppm	retention time, min	biological removal after 72 h	distinguishing features
$C_{18}H_{22}I_3N_3O_8$	789.8620	789.8610	2.1 ± 0.3	4.03	49% removed	loss of 103.07
$C_{18}H_{22}I_3N_3O_8$	789.8620	789.8610	2.1 ± 0.3	4.57	49% removed	loss of 89.05
$C_{17}H_{22}I_3N_3O_8$	777.8620	777.8622	0.3 ± 0.1	3.37	not removed	loss of 105.09, 91.06, HI + H_2O, and HI + 91.06; presence of 2 peaks indicating diastereomers
$C_{17}H_{20}I_3N_3O_8$	775.8464	775.8464	1.1 ± 0.5	3.37	not removed	loss of 103.07 and HI + 103.07; presence of 2 peaks indicating diastereomers
$C_{17}H_{20}I_3N_3O_8$	775.8464	775.8464	1.1 ± 0.5	3.59	53% removed	loss of 89.06 and HI + 89.06
$C_{15}H_{18}I_3N_3O_6$	717.8409	717.8405	0.7 ± 0.3	4.51	not removed	loss of HI + 91.06, 91.06, and H_2O
$C_{18}H_{26}I_2N_3O_9$	681.9759	681.9759	0.7 ± 0.3	3.45	27% removed	loss of 105.09, 91.06, and H_2O; presence of 2 peaks indicating diastereomers
$C_{17}H_{23}I_2N_3O_8$	651.9653	651.9652	1.1 ± 0.1	3.94	19% removed	loss of HI + 91.06, 91.06, and H_2O; presence of 2 peaks indicating diastereomers
$C_{16}H_{21}I_2N_3O_8$	637.9497	637.9519	2.9 ± 0.2	3.37	20% removed	loss of HI + 91.06, HI + H_2O, 91.06, and HI; presence of 2 peaks indicating diastereomers
$C_{16}H_{19}I_2N_3O_8$	635.9340	635.9341	2.0 ± 0.5	3.75	40% removed	loss of HI, HI + 75.04, 91.06, and 75.03; presence of 2 peaks indicating diastereomers
$C_{16}H_{19}I_2N_3O_8$	635.9340	635.9336	2.0 ± 0.5	4.62	98% removed	loss of HI, HI + 75.04, 91.06, and 75.03
$C_{15}H_{19}I_2N_3O_7$	607.9391	607.9382	2.2 ± 0.9	4.48	25% removed	loss of HI, 91.06, HI + 35.05, and 35.05
$C_{15}H_{19}I_2N_3O_6$	591.9442	591.9444	1.4 ± 0.7	4.91	15% removed	loss of HI + 91.06, HI, and 91.06
$C_{18}H_{24}I_1N_3O_9$	554.0636	554.0636	2.1 ± 0.8	4.51	29% removed	loss of 105.09 and loss of H_2O

[a] $n = 4$.

and their MS/MS fragmentation patterns were used to guide in the identification of the AOP TPs of endogenous iopamidol in unspiked wastewater. The iopamidol concentration in wastewater was determined using standard addition.

Identification of Transformation Products Using LC-QToF-MS. Separation was carried out using an Agilent 1260 LC system (Santa Clara, CA) with degasser, binary pump, autosampler, and isopump. A reversed phase column, Thermo Beta Basic C-18 (Waltham, MA), with dimensions 200 mm × 2.1 mm and 5 μm particle size, was used. Column temperature was kept at 25 °C. A 20 μL sample was injected, and isocratic elution using 90% A (water with 0.3% formic acid) and 10% B (methanol) at a rate of 0.2 mL/min was performed. An Agilent 6530 Accurate Mass Q-ToF-MS with jet stream technology was used under positive electrospray ionization (+ESI) in the full scan mode. Samples were initially analyzed under both +ESI and −ESI mode, but only data from +ESI gave enough information that allowed detection of potential TPs that fit the criteria defined during the analysis. Therefore, subsequent data analyses focused only on the results obtained from +ESI runs. The likelihood that a few TPs were undetected using +ESI is very low, but possible, because many compounds can undergo wrong-way round ionization, allowing simultaneous detection of multiple classes of compounds without the need to switch the polarity.[36] The approach of using wrong-way round ionization in LC/MS/MS has been used in the analysis of PPCPs in environmental samples.[37,38]

Before use, the mass spectrometer was calibrated using the manufacturer prescribed tuning solution. Purine and hexakis-(1*H*,1*H*,3*H*-tetrafluoropropoxy)phosphazine were used as reference masses. The mass spectrometer had a 6 L/min gas flow (N_2) at 300 °C, nebulizer pressure of 30 psig, and sheath gas flow of 11 L/min at 300 °C. The fragmentor voltage was set at 175 V while the skimmer voltage was set at 65 V; no fragmentation of iopromide molecular ion was observed at this voltage. High resolution mass spectra were collected from 50−1700 m/z at a rate of 1 spectra/s. Samples were analyzed in quadruplicate.

Data Analysis. Profinder Professional software (Agilent Technologies) was used to identify features, composed of the $[M + H]^+$, $[M + Na]^+$, and the $[M + K]^+$ for this specific study. Mass Profiler Professional (MPP) was used to compare samples of different treatment conditions. This was done in order to identify features (ions) that are statistically different across samples and have a minimum 2-fold change in ion counts. One-way ANOVA at a 95% confidence level was performed followed by the Tukey HSD (honestly significant difference) posthoc test to compare means and a Benjamini-Hochberg FDR (false discovery rate) multiple testing corrections. To identify novel TPs produced during the AOP, post-AOP samples were compared against the pre-AOP samples and the blank.

Features that met the criteria of the tests were compared to the METLIN database. Features that were not previously identified or reported were assigned formulas that are within 10 ppm error using MPP ID Browser (Agilent Technologies). Carbon, hydrogen, nitrogen, oxygen, and iodine were included in the list of elements that are present. The presence of Na and K adducts with the appropriate exact mass served as an additional way of verifying an observed ion. Extracted ion chromatograms were generated using the suspected m/z values that corresponded to potential TPs in order to verify if the peaks of putative TPs can indeed be observed in the chromatograms. Further MS/MS characterization of iopromide TPs was limited to the compounds that met the following criteria: predicted chemical formulas are within less than 5 ppm error relative to the observed m/z values, chromatographic peaks are observed within the retention time window of potential TPs as observed in earlier LC-UV analysis, and elemental compositions are consistent with the expected reactions of iopromide with hydroxyl radicals.

RESULTS AND DISCUSSIONS

Mass Spectral Fragmentation Patterns of Iopromide and Its Transformation Products. Using LC-UV analysis as a starting point, we observed four potential TPs of iopromide in the post-AOP samples (Supporting Information, Figure S1).

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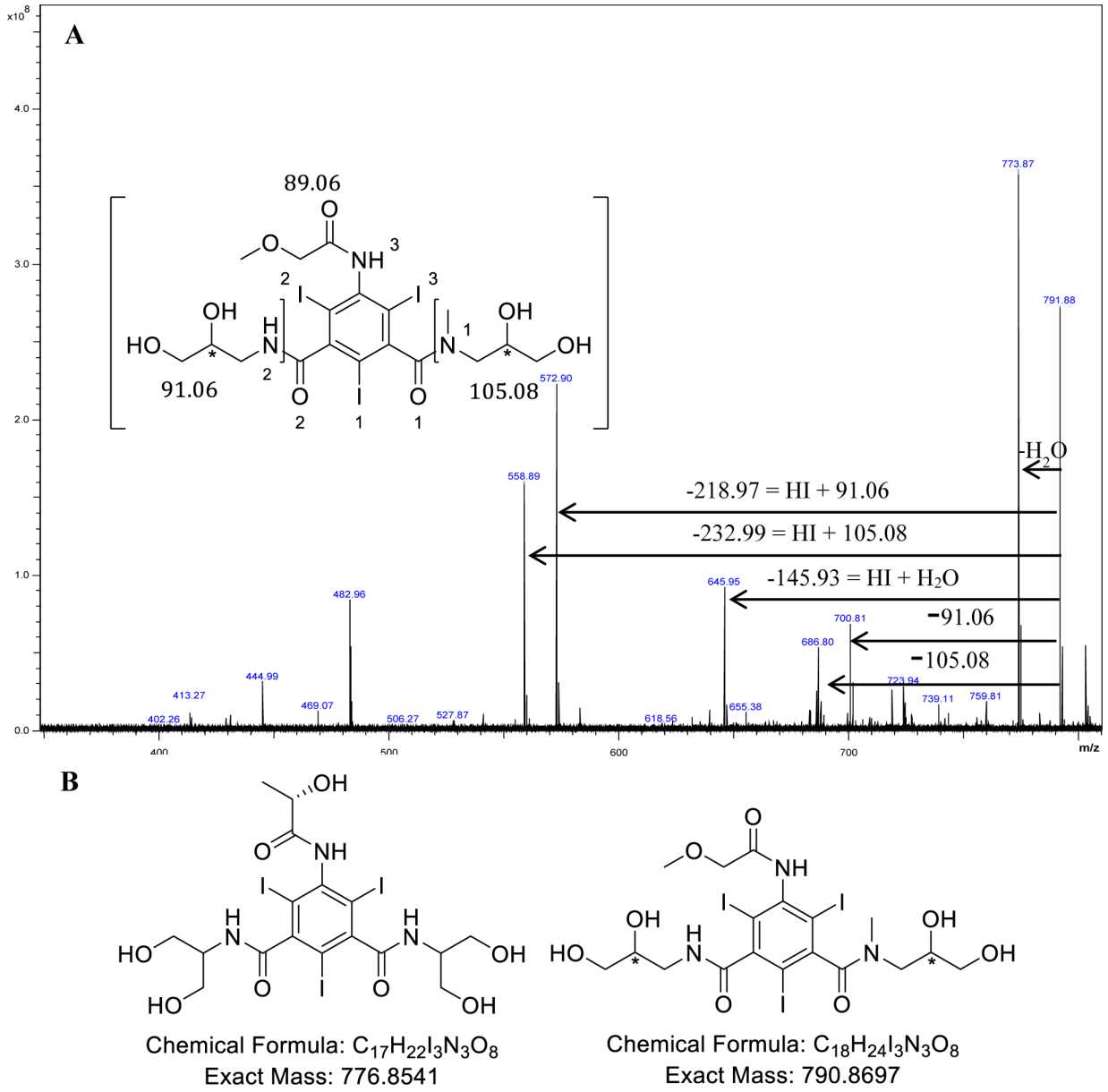

Figure 1. (A) Fragmentation of iopromide shows losses due to cleavage of the amide bonds, loss of HI, and loss of water. (B) Structure of iopamidol (left) and iopromide (right). Note that iopamidol does not have the two chiral carbons (indicated by asterisks) that are present in the alkyl amide side chains of iopromide.

Spectroscopic methods such as UV absorbance measurements have been shown to complement MS methods in the identification of TPs and metabolites of pharmaceuticals containing chromophores.[23,39] By knowing the m/z values that correspond to the observed UV peaks, further MS/MS experiments can be performed to elucidate their chemical structures. However, due to the high signal-to-noise levels in full scan MS, identification of the TPs proved challenging despite the high concentration of iopromide used to spike the sample. The combination of poorly resolved chromatographic peaks of iopromide and its TPs, and the saturation of the mass spectra, makes identification of TPs using low resolution MS a formidable task. While detection of new UV peaks is undeniably a good starting point, the sensitivity and selectivity of using LC-UV is limited in complex matrices such as in wastewater effluents.

To overcome the challenges of identifying unknown TPs, we employed LC-QToF-MS to obtain high-resolution mass data from samples before and after the AOP. Using METLIN and the MS/MS spectra, newly formed compounds related to iopromide were putatively identified. The ability of the statistical and profiling software to make automated comparisons of different samples, identify isotope patterns based on the number of C, H, N, and O, and detect the formation of adducts facilitated data mining by only focusing on compounds that significantly changed before and after the AOP. Molecular formulas for the identified TPs of iopromide were assigned.

A combination of accurate mass data and metabolic profiling tools revealed 14 TPs of iopromide (Table 1) formed during AOP. The experimentally determined $[M + H]^+$ of all TPs have mass errors less than 2.9 ppm, which is below the acceptable 5 ppm tolerance. All TPs were observed to form Na and K adducts with accurate mass meeting the criteria set for the

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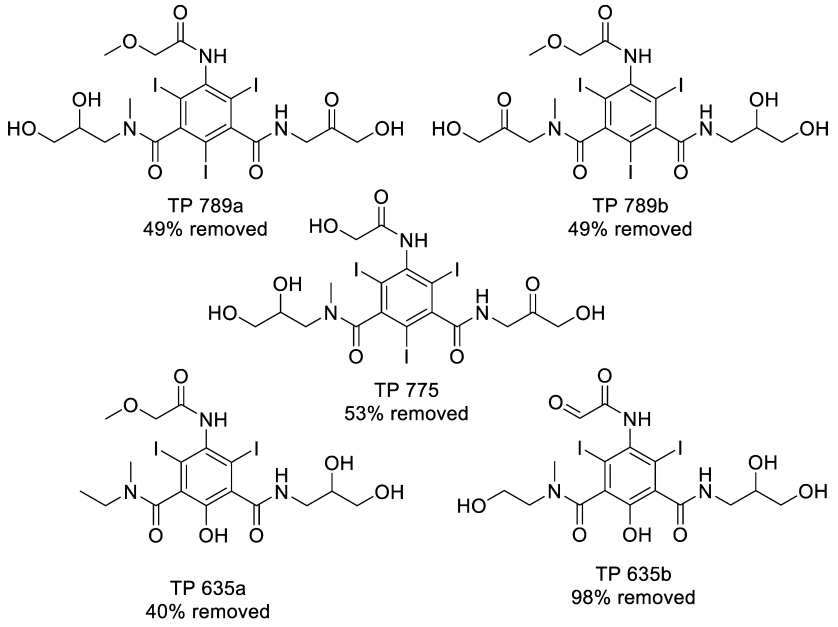

Figure 2. Structures of TPs that are removed at >40% after 72 h of biodegradation. The positions of iodine in TP 635a and TP 635b are assigned arbitrarily because the exact location of iodine removed during the AOP is not possible to be determined by MS alone. Chirality of 2 carbons were maintained in TP 777, TP 681, and TP 651, and two resolved chromatographic peaks were observed in LC/MS/MS.

probable TPs; the presence of these adducts served as additional confirmation that an observed $[M + H]^+$ ion is not a mere artifact. Note that the adducts should have the same retention time as the molecular ion; during profiling, MPP compares the retention time of molecular ions and their respective adducts and reports the different species as one. The chemical structures of the TPs (Supporting Information, Table S1) were proposed on the basis of the mass fragmentation pattern of the precursor ion in comparison to the fragmentation behavior of the parent iopromide. The fragmentation study was done using three different collision cell energies. It has been observed that at higher collision energies the intensity of the fragments with higher m/z values decreases. Similar fragments were observed at different collision energies with varying intensities. Probable TPs that do not show this behavior were not considered further in the analysis.

Iopromide MS fragmentation is characterized by loss of water (m/z 18.0), loss of HI (m/z 127.9), and cleavage of the three amide bonds (Figure 1A). The loss of 105.08, 91.06, and 89.06 correspond to the cleavage of the amide bonds containing N1, N2, and N3, respectively. Observing a mass difference of 105.08, for example, implies that the alkyl amide side chain containing N1 was retained during the AOP. Modifications in the observed mass losses can be attributed to the changes in the parent iopromide molecule resulting from the AOP. For example, TP 789 shows a mass loss of 103.07. This fragment is analogous to the loss of 105.09 observed in iopromide (Figure 1A), less two hydrogens due to the oxidation of a primary or secondary alcohol into an aldehyde or a ketone (Figure 2), respectively. This MS fragmentation of iopromide is key to predicting the location of the chemical modification that occurred during the AOP. For instance, the MS/MS of TP 717 (structure shown in the Supporting Information, Table S1) shows the loss of 218.96 and 91.06. By referring back to the fragmentation pattern of iopromide in Figure 1, we can easily see that the loss of 91.06 in the TP is due to the loss of the alkyl amide side chain containing N2. The loss of 218.96 can be attributed to HI (127.9) plus the m/z 91.06 fragment. For TP 681, we observed the loss of both 105.08 and 91.06 that is characteristic of iopromide. Both TP 717 and 681 also show loss of water. The hydroxyl substitution of iodine 1 is more likely to occur because it is more susceptible to nucleophilic attack as a result of the presence of the electron withdrawing oxygen 1 and 2 on the carbonyl carbons directly attached to the phenyl ring. None of the proposed structures involved the loss of nitrogen, and the nominal masses observed were consistent with the nitrogen rule.

Iopromide is a diastereomer (Figure 1), and therefore, it shows two distinct chromatographic peaks (Supporting Information, Figure S1). The diastereomeric nature of iopromide provides another means of elucidating the structures of its TPs. When the chirality in the molecule is retained in TPs, the characteristic 2-peak profile in the extracted ion chromatogram is observed. However, when one of the chiral centers is lost during the AOP then only one chromatographic peak is observed. In the case of multiple structures such as for TPs 681 and 651, two peaks are observed in the extracted ion chromatograms suggesting that (1) the chains containing the chiral carbons were retained and (2) oxidation occurred on the chain that contains the ether moiety. On the other hand, the extracted ion chromatogram of TP 591 supports the proposed structure where one of the chiral carbons was oxidized into a ketone group.

Inspection of the proposed TP structures indicates that substitution of the iodo group by a hydroxyl group, oxidation of an alcohol group, and homolytic cleavage of the peripheral alkyl groups connected to N3 were the common transformation reactions of iopromide during the AOP. Cleavage of the carbon−oxygen ether bond into an alcohol group was also observed. All TPs with m/z greater than 700 have intact tri-iodinated ring structures, with the transformations limited to the peripheral chains. The changes in the signal intensities of the putative TPs were monitored over time during biodegradation to determine their relative biodegradability

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(Supporting Information, Figure S2). However, we did not attempt to identify the biodegradation products of the iopromide TPs at this time. The structures of TPs that were more than 40% removed during biodegradation are shown in Figure 2.

Data Handling: Profiling and Statistical Analysis. Using Profinder, more than 2000 different molecular species were identified to be present after the AOP. MPP statistically compared features that are found between the blank, pre-AOP samples, and post-AOP samples. Using one-way ANOVA at a 95% confidence level, the list was reduced to less than 500 statistically significant compounds from an original list of more than 2000; a diagram (Figure S3) is provided in the Supporting Information to provide a brief description of the process. We defined the level of stringency that will be applied when it comes to comparing features between different samples.

In this work, 4 replicates were run to show that a peak is not a mere artifact. Features were considered real if they are found in at least 3 of the 4 runs and have minimum ion abundance at peak maximum of 3000 ion counts. A posthoc Tukey HSD test is applied to enable the comparison of means from different batch sizes.[40] False positives were eliminated by employing the Benjamini-Hochberg FDR test for multiple testing corrections.[41,42] Multiple testing corrections were performed to adjust *p*-values derived from a series of statistical tests; this was made necessary by the fact that, by doing a statistical test, the experimenter increases the probability of getting false positive results. Compared to the Bonferroni test, the Benjamini-Hochberg test is less stringent and provides a good balance between eliminating false positives and false negatives. These statistical analyses are commonly utilized in profiling studies to identify statistically significant differences between experimental groups.[43,44]

Criteria for Identifying Probable TPs Using an Online Database and Formula Generator. Using the mass filtering scheme described earlier, data were further processed by comparing the remaining features against the METLIN database and assigning empirical formulas to features that did not get any hits from the accurate mass database. On the basis of the chemical formula of iopromide, the elements were limited to carbon, hydrogen, nitrogen, oxygen, and iodine. A list of 10 chemical formulas with errors less than 10 ppm was proposed by MPP for each statistically significant feature. Possible TPs were limited to those that meet the following criteria: (1) the observed molecular mass should be within less than 5 ppm error compared to the theoretical mass corresponding to the proposed empirical formula, and (2) the TPs must elute ±5 min from the retention time of iopromide. The latter criterion was based on the observed UV peaks that appeared in the chromatograms of the post-AOP sample spiked with iopromide.

While the software is a powerful tool in data mining, it is critical to have knowledge of the reaction mechanisms in order to have reasonable prediction of probable TPs. Since the AOP produces hydroxyl radicals that attack the parent compound, as either an electrophile or a nucleophile, addition of a hydroxyl group, bond cleavage, and oxidation of the alcohol groups to aldehydes, ketones, or carboxylic acids are possible. A single molecule of iopromide only contains 18 carbon atoms, 3 iodine atoms, and 3 nitrogen atoms. TPs of iopromide can have more oxygen and hydrogen atoms but not more than the number of carbon, iodine, and nitrogen atoms than the original molecule. Using the guidelines above, the list of 500 features was further narrowed down to 20 compounds.

A set of guidelines must be followed in deciding whether an observed feature is a potential TP based on the chemical process being investigated. The 5 ppm error is an accepted limit for QToF-MS instruments when used to acquire accurate mass measurements for predicting empirical formulas.[45] The chromatographic retention time window is arbitrary and depends on the property of the analyte. Since the AOP introduces hydroxyl radicals, TPs are expected to be more hydrophilic than parent iopromide. However, the possibility of less polar TPs cannot be discarded since oxidation of an alcohol group can lead to formation of aldehyde and ketone groups that are less hydrophilic. Work by Pérez et al.[23] and Shulz et al.[17]showed the formation of aldehyde and ketone groups on iopromide during biodegradation in activated sludge and sewage treatment. While very little data can be extracted from the LC-UV chromatograms, this information is still very useful in visually establishing the retention time windows when potential TPs can be expected to elute.

Iopamidol: Application in Real Environmental Samples. To demonstrate the application of LC-QToF-MS, coupled with the statistical tools, in the identification of TPs at environmentally relevant conditions, unspiked wastewater samples were collected and analyzed for the presence of iopromide and its putative TPs. However, iopromide was not detected in any of the wastewater samples collected. The absence of iopromide in the wastewater samples is most likely due to the reduced use of iopromide in the US, which may have been replaced by iopamidol. Therefore, the same samples were analyzed for iopamidol, and an analogous MS fragmentation pattern in iopromide was applied to iopamidol. It is reasonable to expect that structurally similar compounds, such as iopromide and iopamidol (Figure 1B), will oxidize in the same manner during the AOP and produce corresponding TPs.

The concentration of iopamidol in the wastewater effluent was measured to be 33 mg/L before the AOP. After the AOP, the concentration of iopamidol was reduced by 88%, indicating that transformation or removal occurred. TPs for iopamidol were predicted using the knowledge gained from the AOP of iopromide like alcohol oxidation, hydroxyl substitution of iodine, or cleavage at the ether bond or which bonds are more labile and which groups are observed to oxidize more. When the same process was employed of profiling and performing MS/MS experiments for the identification of iopromide TPs, two TPs were identified for iopamidol (Figure 3). By oxidation of one alcohol group, iopamidol TP 775 is produced while substitution of one of the iodines by a hydroxyl groups gives TP 667. Furthermore, the proposed structure of TP 775 was supported by the observed MS/MS fragmentation corresponding to a loss of 72.02 and 91.06. For TP 667, fragments 74.03 and 91.06 were observed, indicating hydroxyl substitution of one of the iodine atoms in the ring. After 72 h of biodegradation, TP 775 and TP 667 were reduced by 47% and 27%, respectively, indicating that the AOP transformed iopamidol to more biodegradable compounds.

The analytical approach based on high accurate mass measurements offered by LC-QToF-MS and the powerful statistical analysis and database information afforded by MPP and METLIN proved successful in the identification of TPs of two pharmaceuticals during the treatment of wastewater by an AOP. The combination of the knowledge of the MS fragmentation pattern, the nitrogen rule, and predicted

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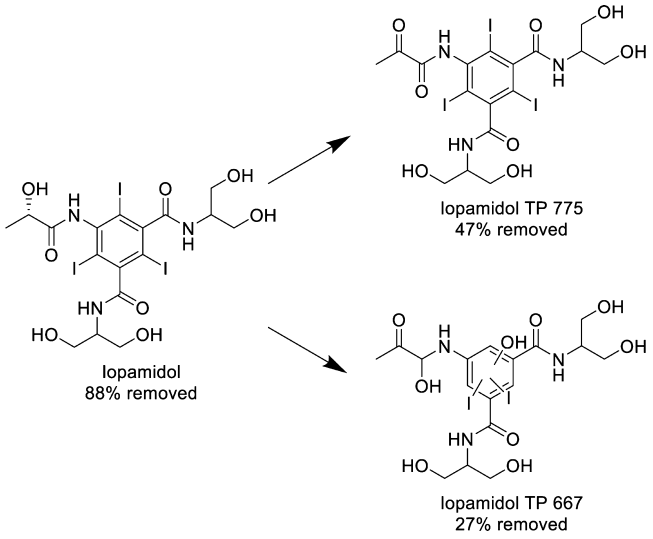

Figure 3. Two detectable TPs of iopamidol are found in wastewater after the AOP.

empirical formulas resulted in the prediction of reasonable structures for iopromide and iopamidol TPs. The potential applications of this approach in environmental analysis are wide ranging, especially in the identification of unknown TPs and metabolites during chemical and biological treatment processes. The TPs formed during the AOP were more biodegradable than their parent compounds, which is consistent with our earlier studies demonstrating the increased biodegradability of PPCPs when subjected to the AOP prior to biodegradation.

■ ASSOCIATED CONTENT

Supporting Information

Table S1, proposed transformation products of iopromide formed during UV/H_2O_2 treatment; Figure S1, HPLC/UV chromatograms of wastewater before and after the advanced oxidation process; Figure S2, identified TPs of iopromide showing changes in abundance after 72 hours of biodegradation; Figure S3, diagram showing the number of TPs (called features) identified at each level and after several criteria are applied using profiling and statistics. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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