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Photolysis and UV/H2O2 of diclofenac, sulfamethoxazole, carbamazepine, and trimethoprim: Identification of their major degradation products by ESI-LC-MS and assessment of the toxicity of reaction mixtures

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Abstract

The photolysis of diclofenac (DCF), sulfamethoxazole (SMX), carbamazepine (CBZ), and trimethoprim (TMP) was investigated using a low-pressure (LP) mercury ultraviolet (UV) lamp (254 nm) and a combination of UV with hydrogen peroxide (H_2O_2). For each experiment, 5 mg/L of each pharmaceutical was prepared in pure water and individually degraded by either UV alone or UV/H_2O_2. DCF and SMX were highly susceptible to UV treatment and completely degraded to below their LC-MS detection limit (1 µg/L) after only 8 min of UV irradiation. TMP and CBZ were more resistant to UV treatment, with only 58.2 and 25.2% degradation (after 1 h UV exposure). The combination of H_2O_2 addition (up to 0.2 g/L) with UV significantly improved the removal rate of TMP and CBZ up to 91.2 and 99.7% of the initial concentration, respectively. A number of novel transformation compounds were identified as UV or UV/H_2O_2 degradation products using LC-MS. The range and amount of these transformation compounds strongly depended on the applied treatment conditions. The toxicity of each pharmaceutical solution before and after treatment was also evaluated and all parent compounds were non-toxic at the tested concentration (i.e. 5 mg/L). DCF, in particular, but also CBZ and SMX, showed an increase in solution toxicity after treatment with UV only, indicating the presence of photolytic degradation products that are more toxic than the parent compounds. Treatment with UV/H_2O_2 reduced the toxicity of all solutions to below the detection limit of the assay.

Keywords: UV photolysis; UV/H_2O_2 advanced oxidation process; degradation product identification; BLT-Screen; Wastewater treatment

1. Introduction

The presence and fate of pharmaceutically active compounds (PhACs) in urban wastewater is of substantial environmental concern due to their widespread occurrence [1]. It has been repeatedly observed that conventional wastewater treatment is unable to sufficiently eliminate many PhACs [1-6]. Examples of persistent PhACs include diclofenac (DCF), sulfamethoxazole (SMX), carbamazepine (CBZ), and trimethoprim (TMP), which are anti-inflammatory, antibiotic, anticonvulsant, and antibiotic drugs, respectively. These compounds are resistant to conventional water treatment processes, as shown by their global detection in wastewater treatment plant effluents, surface water and groundwater [1, 3, 7-9]. The presence of PhACs in surface and drinking waters elicits concerns about both their ecological impact on aquatic organisms and their potential to adversely affect public health [10]. Aquatic toxicity, the development and maintenance of antibiotic resistance and endocrine disruption have all been associated with particular PhAC pollutants [2, 11-14]. For example, the chronic exposure of bacteria to trace concentrations of antibiotic pharmaceuticals such as TMP and SMX in the aquatic environment can result in induction of antibacterial resistant genes [15]. DCF and CBZ were observed to have adverse impacts on rainbow trout (*Oncorhynchus mykiss*) and zebrafish, respectively [16-18]. However, research into chronic health impacts of long term PhACs exposure to humans and the environment has not yet reached its maturity [19].

Advanced oxidation processes (AOPs) have been shown to be a promising technology to degrade PhACs present in wastewater prior to discharge into the aquatic environment. A number of AOPs have been investigated such as ozonation [20, 21], Fenton and photo-Fenton oxidation [22], photolysis and UV/hydrogen peroxide (H_2O_2) [23], and heterogeneous photo-catalysis [24]. The formation of pharmaceutical degradation products by AOPs has also been identified as a challenge, as degradation products may be more toxic than the parent

compounds. Ultraviolet (UV) light irradiation is a widely used process for disinfection of drinking water [25], and is a promising process for wastewater purification. The photolytic reaction mechanism is especially complicated as it may involve a number of different reaction pathways forming many unknown degradation products. Some photolytic degradation products of UV treatment may be more toxic than, or at least as toxic as, their parent compounds [26]. Many common pharmaceutical compounds are susceptible to degradation by UV at typical disinfection doses [27, 28]. Experiments have shown that DCF and SMX are well degraded during UV photolysis and UV/H_2O_2 treatment [29]. On the other hand, low rates of removal of CBZ have been observed during photo-degradation [30, 31]. Whilst its degradation rates are low, the major photolytic degradation products formed, acridine and acridone, are known to be more toxic than their parent compound, possessing considerable mutagenic and carcinogenic characteristics [26, 32-34]. The combination of UV with H_2O_2 significantly improves the removal of resistant pharmaceuticals compared to UV alone. The hydroxyl radical ($^{\bullet}OH$) is produced during UV/H_2O_2 as a result of UV photolysis of H_2O_2. $^{\bullet}OH$, which is strong oxidant, can attack a wide range of organic compounds causing degradation through a series of steps including hydroxylation and dehydrogenation [23].

Photo-degradation reactions can occur directly and indirectly [35, 36]. In direct photo-degradation, a compound absorbing radiation can become unstable and consequently decay, whereas the indirect pathway involves naturally occurring compounds that produce strong reactive species (such as singlet oxygen, and/or hydroxyl radicals) that then react with organic compounds. The photolytic degradation products formed may have molecular weights lower than their parent compounds [30, 37, 38]. Conversely the detected photolytic degradation products could be observed with slightly higher molecular weights than their parent compounds, as a result of radical additions rather than direct photolysis [30, 37].

92 Additionally, photolytic degradation products with significantly larger molecular weights,
93 such as the formation of dimers, can be produced during irradiation through
94 photopolymerization [39, 40]. In general, the degradation products formed tend to display
95 different reactivity within the system to those of their parent compounds. However, research
96 conducted into the identification of these degradation products is limited. Consequently,
97 further investigation is required to fully elucidate the UV photolysis and UV/H_2O_2
98 degradation of PhACs.

99 The overarching purpose of our work was to comprehensively investigate the degradation of
100 four pharmaceuticals, namely, diclofenac (DCF), sulfamethoxazole (SMX), carbamazepine
101 (CBZ), and trimethoprim (TMP), by UV photolysis and UV/H_2O_2 advanced oxidation
102 process. The scope of this current paper was to analyse the range of degradation products
103 formed and identify previously unreported degradation products. Additionally, to confirm the
104 structure, where possible, of compounds previously reported using m/z ratio and mass
105 fragmentation. This also involved investigating the rate of degradation of the four (parent)
106 pharmaceuticals and the evolution of major degradation products. In addition, the toxicity of
107 the treated solutions was tested in a typical bacteria toxicity assay in order to evaluate the
108 relative toxicity of the mixture of degradation products compared to their parent compounds.

109 **2. Materials and Methods**

110 **2.1. Materials and chemicals**

111 Four pharmaceuticals were selected for investigation in this study: diclofenac (DCF),
112 sulfamethoxazole (SMX), carbamazepine (CBZ), and trimethoprim (TMP) (Fig. 1). The
113 pharmaceuticals were of analytical grade and purchased from Sigma-Aldrich (Castle Hill,
114 Australia). All other chemicals (including H_2O_2) and solvents used in this study were of
115 analytical or high performance liquid chromatography (HPLC) grade and purchased from

VWR International (Sydney, Australia). All experiments were carried out using Milli-Q quality water.

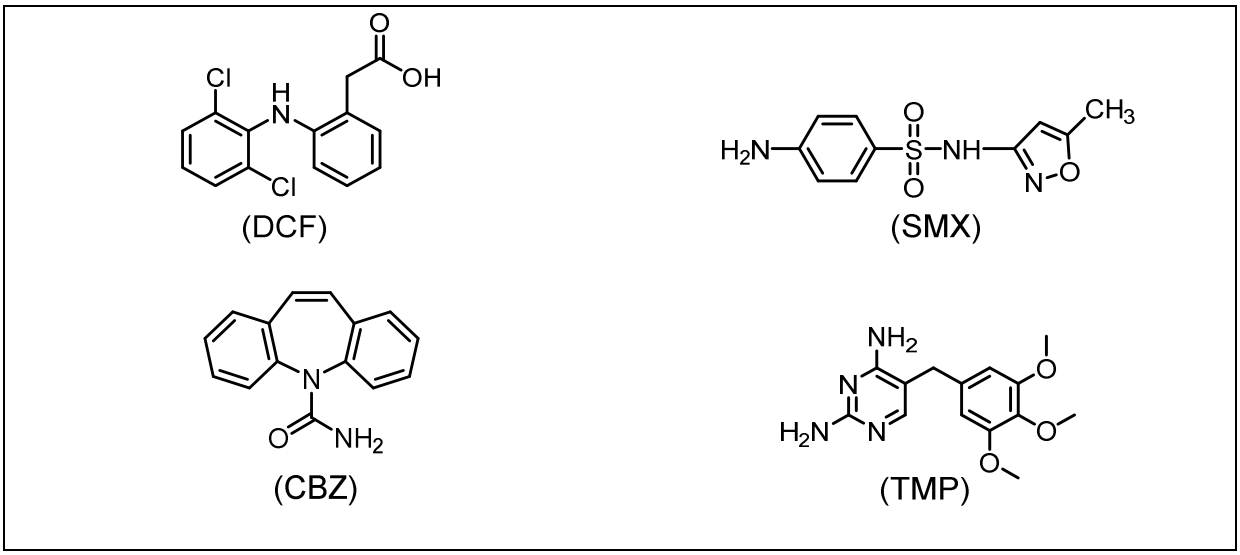


Figure 1: Molecular structures of the four selected pharmaceuticals. DCF = diclofenac; SMX = sulfamethoxazole; CBZ = carbamazepine; TMP = trimethoprim.

2.2. UV system

Batch experiments of UV irradiation and UV/H_2O_2 were carried out in a 0.4 L laboratory-scale UV oxidation system supplied by Ace Glass (Vineland, NJ, USA) that consisted of a reactor and a low pressure mercury lamp (LP-UV). The low-pressure mercury lamp had a length of 27 cm, a total UV energy output of 83 W and UV intensity of 1.04 W/cm^2. It emitted UV light at a principal wavelength of 254 nm. The UV light was located in the middle of the reactor, and the solution surrounded the light during the experiments to provide uniform exposure. A magnetic stirrer was continuously used to provide good mixing of the reaction solutions during the photolytic reaction (Fig. 2). Further description of the UV system is available elsewhere [41].

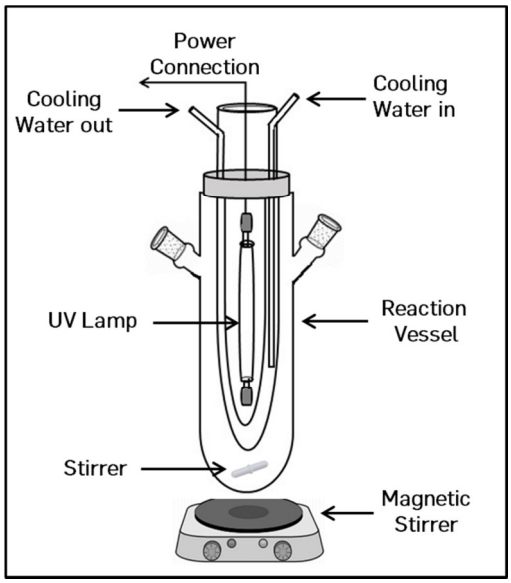


Figure 2: Schematic diagram of UV system used in this study.

The system was connected to a chiller to maintain the temperature at 20ºC. The solution temperature before and immediately after UV exposure deviated from the set point by 1 to 5ºC depending on the exposure time. Therefore, the solution temperature can be taken as 23 ± 2ºC regardless of the exposure time. It should be noted that the exposure of the solution to the UV light and the amount of energy absorbed by the compounds is specific to the configuration of the system. The kinetics of degradation and transformation kinetics are thus also configuration and system dependent.

2.3 Experimental protocol

Stock solutions containing 5 g/L of DCF, SMX, CBZ, and TMP were prepared using pure methanol. These were stored in amber bottles and kept in a freezer at -18ºC prior to use. For LC-MS analysis, calibration standards at concentrations of 1, 10, 50, 100, and 200 µg/L for each pharmaceutical were freshly prepared from the stocks.

For each UV experiment, 5 mg/L of each target compound were prepared in Milli-Q water and 0.3 L was transferred into the UV reactor. Although, these pharmaceuticals are frequently

146 detected in wastewater at concentrations of up to several µg/L, a relatively high concentration
147 (i.e. 5 mg/L) was used to facilitate detection and identification of potential degradation
148 products. The initial concentration of the selected pharmaceuticals is not expected to play any
149 major role in the photolytic reaction pathways. The UV light was switched on until the
150 desired exposure time was achieved. After the reaction took place 1 mL of the sample was
151 transferred to a LC-MS vial to be analysed. The remaining sample was stored in an amber
152 glass bottle for solid phase extraction (SPE). For each UV/H_2O_2 experiment, the desired H_2O_2
153 concentration was directly added to the solution prior switching the UV light on.

154 The sensitivity of the analysis for the identification of the degradation products was increased
155 by pre-concentrating with SPE cartridges. Sep-Pak cartridges (C18 6 cc Vac; 500 mg sorbent
156 per cartridge; 55-105 µm particle size) were purchased from Waters (Rydalmere, NSW,
157 Australia). The cartridges were first pre-conditioned with a 7 mL dichloromethane and
158 methanol mixture (1:1 v/v), 7 mL of methanol, followed by 7 mL of Milli-Q water. Then, the
159 samples were loaded onto the cartridges at a flow rate of 1–5 mL/min. The extracted
160 degradation products were eluted from the cartridge using 5 mL of methanol at a flow rate of
161 1–5 mL/min.

162 **2.4. Analytical methods**

163 A liquid chromatography – electrospray ionisation - mass spectrometer (LC-ESI-MS)
164 (Shimadzu single quadrupole LC-MS 2020) was used to identify parent compounds and
165 degradation products with a variety of masses and polarities. To optimise the LC-MS
166 parameters a number of preliminary experiments were conducted. The detected degradation
167 product peaks were classified as large (L), medium (M), or small (S) based on the
168 chromatographic peak abundance. Those with a signal-to-noise (S/N) ratio of < 10 were
169 eliminated from further analysis.

Compounds were separated using a Kinetex® PFP 100A column (100 mm × 3 mm, 12.6 μm) and a binary gradient made of (A) 0.1% formic acid in Milli-Q water, and (B) acetonitrile at a flow rate of 500 μL/min. The injection volume was 20 μL. The gradient used for SMX, CBZ, and TMP was (%B): 0.01 min (5%), 5 min (10%), 20 min (45%), 23 min (90%), 28.1 min (90%), 29 min (10%), 33 min (10%), and 35.01 min controller stop. The column temperature was maintained at 31°C. For DCF the gradient was (%B): 0.01 min (10%), 5 min (20%), 15 min (30%), 23 min (30%), 28.1 min (45%), 33 min (45%), 40 min (90%), 41.01 min (10%), 43.01 min (10%), and 45.01 min controller stop. Mass spectrometric data were collected in both positive and negative ion mode, from m/z 150 to 550. The detected degradation products were then confirmed using selected scan mode. All of the detector parameters were held constant during analysis. These include interface temperature (350ºC), nebulizing gas flow (1.5 L/min), dry gas flow (3 L/min), DL temperature (250ºC) and heat block temperature (200ºC).

2.5. Toxicity assessment

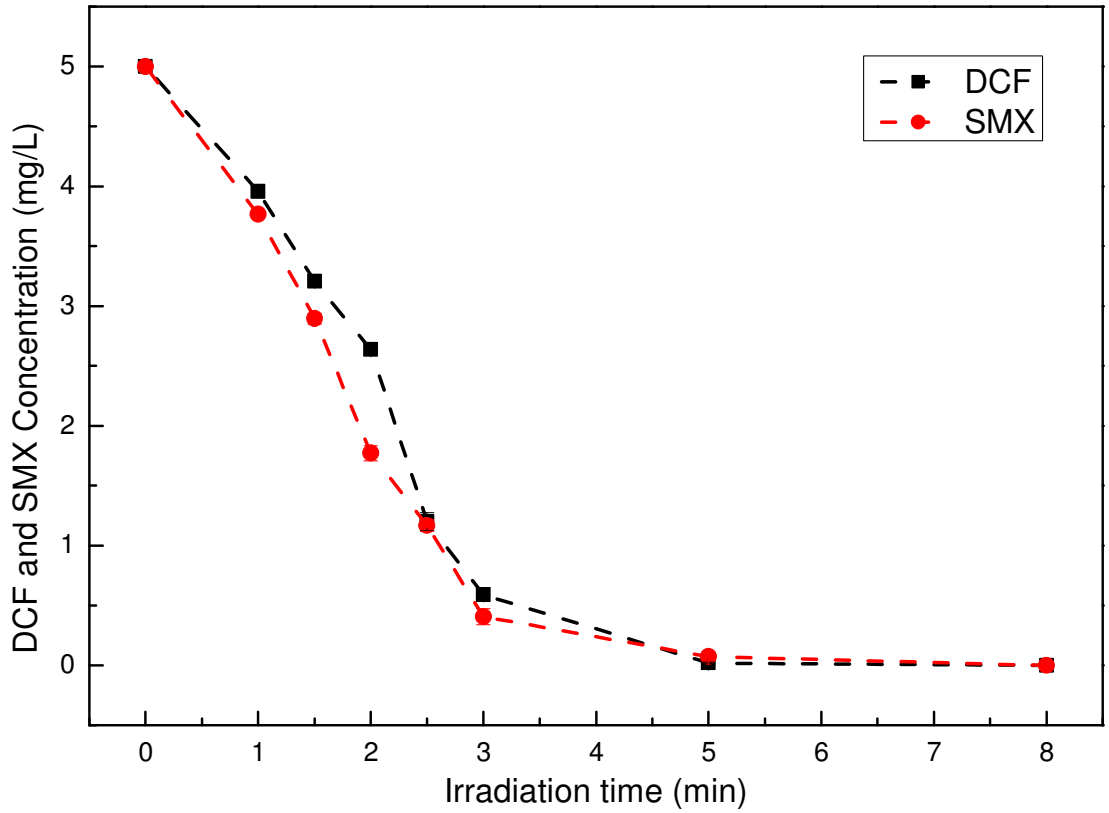
The toxicity of sample solutions before and after the treatment was evaluated using the bacterial luminescence toxicity screen (BLT-Screen) [42] .The samples were treated using UV only or UV/H_2O_2 for the desired UV exposure time and H_2O_2 amount. Then, the treated and untreated solutions were extracted using SPE cartridges (as described above) and eluted using 3 mL methanol, for an SPE enrichment factor of 100. A detailed description of the BLT-Screen method is available elsewhere [42], but in brief, the samples were serially diluted in a phosphate buffered saline medium and an aliquot of the naturally luminescent bacteria, *Photobacterium leiognathi*, was added to each dilution. Following 30 min of exposure, the luminescence in each dilution was measured on a Fluostar Omega plate reader (BMG Labtech, Germany) and the inhibition of luminescence was calculated relative to a negative control. The concentration of the sample required to inhibit bacterial luminescence

by 20% (IC_{20}) was then calculated for each sample by linear regression of the % inhibition *vs.* relative enrichment factor [42] within the linear range of the concentration-effect curve (from 0 to 40% inhibition). All results are presented as a relative toxicity unit (rTU), the reciprocal of the IC_{20}.

3. Result and discussion

3.1. Effect of UV and UV/H_2O_2

Experiments to determine the reactivity of 5 mg/L solutions of DCF, SMX, CBZ and TMP toward low pressure mercury UV light were performed over different UV exposure times. DCF and SMX were effectively degraded by UV exposure, with >90% reduction after 3 min and complete removal (to below their LC-MS detection limit of 1 µg/L) within 8 min (Fig. 3). Therefore, it can be concluded that DCF and SMX were readily photodegradable by UV photolysis and that a combination of UV with H_2O_2 was not required for more efficient degradation. By contrast, CBZ and TMP were quite resistant toward UV photolysis (Fig. 4) and a combination of UV with H_2O_2 was essential in order to improve the removal efficiency of CBZ and TMP (Fig. 4).



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212 **Figure 3: Effect of UV irradiation on degradation of DCF and SMX (C_0 = 5 mg/L).**

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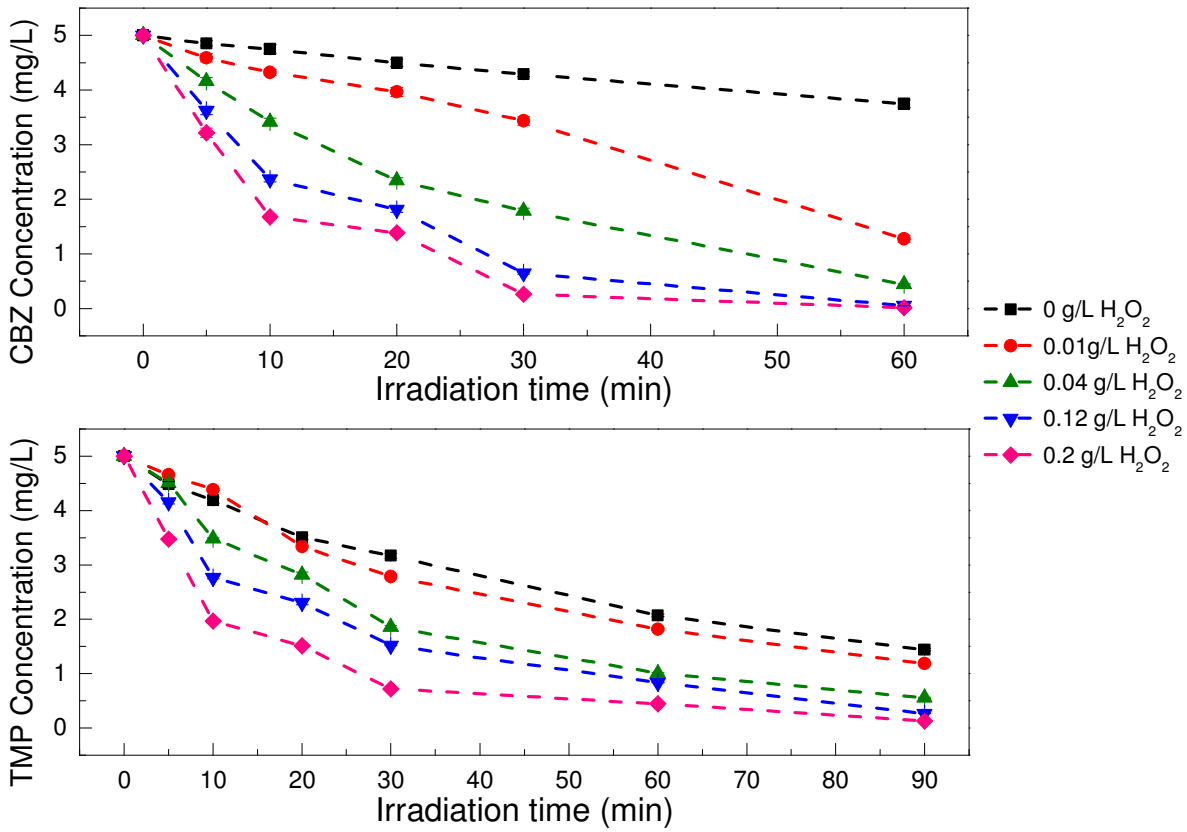


Figure 4: Effect of H_2O_2 concentration during UV irradiation on degradation of CBZ and TMP (C_0 = 5 mg/L).

The degradation of CBZ by UV photolysis and UV/ H_2O_2 advanced oxidation was investigated under a wide range of UV exposure times and H_2O_2 concentrations. CBZ removal by UV photolysis only (i.e. 0 g/L H_2O_2) was low, and only 25.2% of the initial CBZ concentration was lost after 60 min of UV exposure (Fig. 4). As expected, a much higher degradation rate for CBZ was achieved when the H_2O_2 concentration was increased. Adding H_2O_2 prior to UV irradiation produces highly reactive and nonselective hydroxyl radicals causing further degradation of the target compound [23]. The addition of 0.2 g/L of H_2O_2 prior UV exposure removed about 95% of the initial CBZ concentration after 30 min of irradiation with almost complete (99.7%) removal observed after 1 hour of UV exposure, demonstrating the effectiveness of the H_2O_2 addition for CBZ removal. Clearly, an increasing

228 H_2O_2 dose together with UV irradiation can achieve higher removal efficiency in a shorter
229 UV exposure time, compared with a low H_2O_2 dose.

230 The photodegradation of TMP by UV light and UV/H_2O_2 advanced oxidation was also
231 evaluated under a wide range of UV exposure time and H_2O_2 concentrations. TMP was found
232 to be moderately reactive towards UV photolysis with up to 71.2% of the initial TMP
233 concentration lost after 90 min of UV exposure alone (Fig. 4). Similar to CBZ, increasing the
234 concentration of H_2O_2 significantly improved the rate of TMP degradation. However, TMP
235 required a longer exposure time (90 min) compared to CBZ (60 min) during UV with 0.2 g/L
236 H_2O_2 in order to achieve over 97.4% removal of the initial TMP concentration, and TMP was
237 not completely removed after 90 min of UV irradiation even with the addition of 0.2 g/L
238 H_2O_2. Complete removal of TMP may be achieved by further increasing the irradiation time
239 and/or the H_2O_2 concentration. Therefore, the CBZ and TMP degradation rate were
240 controlled by the amount of the added hydrogen peroxide before UV exposure producing free
241 radicals. Overall, after a sufficient UV exposure time (i.e. 60 min for CBZ and 90 min for
242 TMP), there was no significant difference between the removal efficiency when H_2O_2
243 concentrations of 0.12 and 0.2 g/L were present. Therefore, a cost trade-off may exist
244 between H_2O_2 chemical consumption and UV exposure time for effective removal.

245 **3.2. Formation of degradation products**

246 **3.2.1 Diclofenac**

247 A total of fifteen photolytic degradation products were detected during UV photolysis of
248 DCF,. Eight of these (compounds # 7 – 12, 14, and 15) are reported here for the first time to
249 the best of our knowledge (Table 1). The DCF degradation products were eluted through the
250 LC-MS column at different retention times (RT) based on their hydrophobicity; all were at
251 lower RT than DCF (Fig. 5). All peaks presented in the LC chromatogram had their

molecular weight determined in the MS spectra. The majority of these degradation products were detected in both negative and positive scan mode, which was useful to confirm their correct molecular weight.

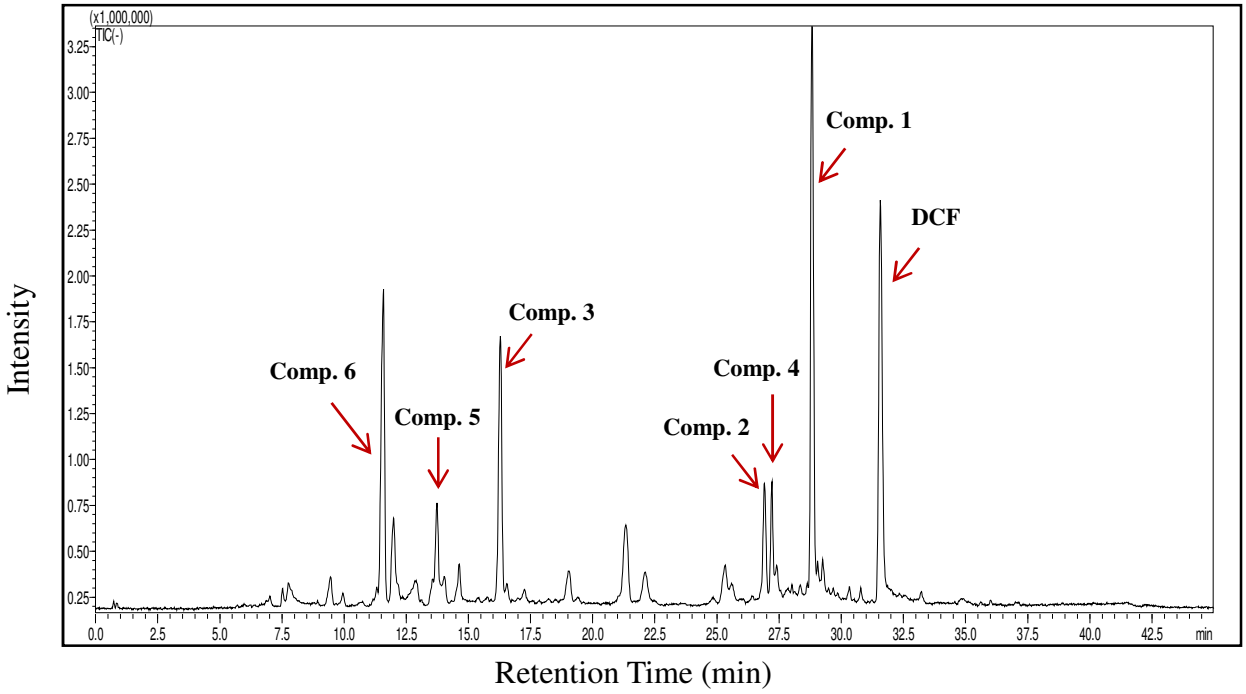
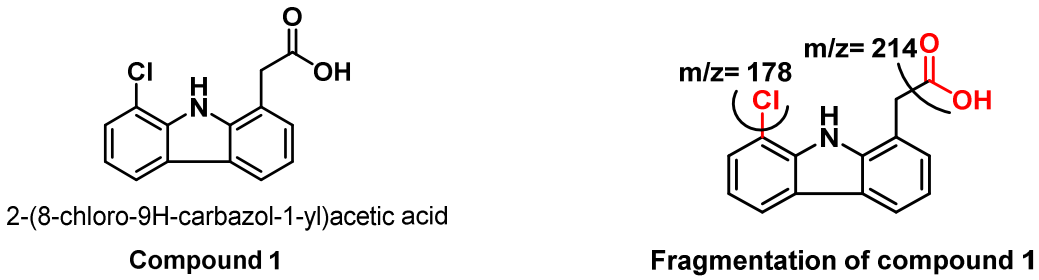


Figure 5: Typical LC-MS chromatogram for DCF and its major UV photolytic degradation products in negative scan mode (3 min UV exposure).

A number of the newly detected photolytic DCF degradation products #11, 12, 14, and 15 were proposed to be formed via dimerization. The formation of other dimer DCF degradation products during UV irradiation of DCF has been reported previously elsewhere [40, 43].

The irradiation of DCF using LP-UV mercury lamp leads to sequential elimination of the chlorine substituents within the DCF structure, followed by ring closure to form carbazole-1-acetic acid, which is considered the primary photolytic degradation product. The primary photolytic DCF compound #1 has been previously identified in a number of studies [43-46]. Additionally, Compounds # 2, 3, 4, 5, and 6 have also been reported as photolytic DCF degradation products [43]. Compound #13 has also been previously reported during DCF

279 photolysis elsewhere [47]. The fragmentation patterns obtained by MS of compound #1 have
280 confirmed the previously reported structures in the literature: its molecular weight was
281 determined to be 259 amu, with molecular formula $C_{14}H_{10}ClNO_2$. The collision-activated
282 dissociation of $[M-H]^-$ molecular ion of the main photolytic degradation product 2-(8-
283 chloro-9H-carbazol-1-yl) acetic acid represented the ions at m/z = 214 and 178, in agreement
284 with another study [48]. Mass losses from this photolytic degradation product were
285 accordingly reported to be 44(CO_2) and 80 (CO_2 and HCl) amu, respectively (Fig. 6).



286 **Figure 6: The primary DCF UV photolytic degradation product [43-46].**

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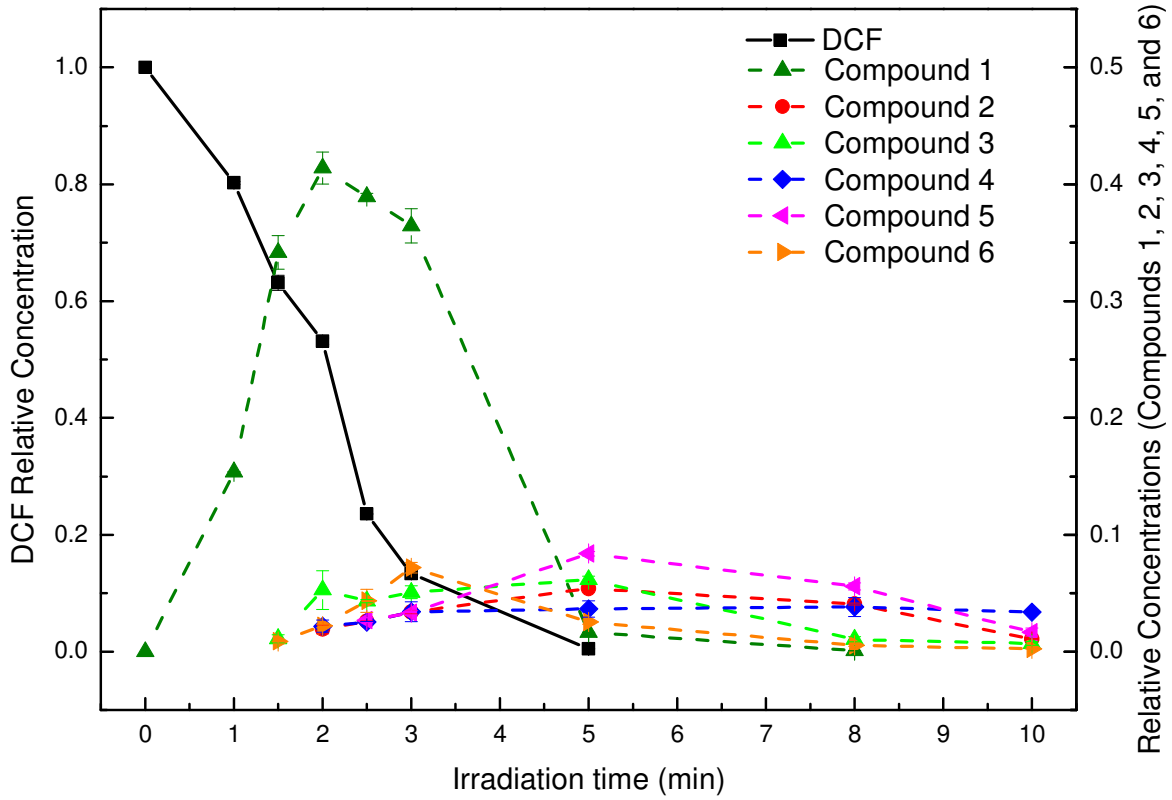

Figure 7: Evolution of main photolytic degradation products formed during degradation of 5 mg/L DCF by UV as determined by LC-MS.

Table 1: The degradation products produced from UV photolysis of DCF as identified by LC-MS.

Compound	Retention time (min)	Molecular ion (*m/z*)		Molecular mass (g/mol)	Peak Abundance*
		$[M-H]^-$	$[M+H]^+$		
DCF	31.42	294.1	296	295	
1	28.83	258.1	-	259	L
2	26.95	479.2	481.2	480	M
3	16.24	240.2	242	241	M
4	27.24	493.1	495.1	494	M
5	13.71	523.1	525.1	524	M
6	11.55	254.1	256.1	255	M
7	9.40	226.1	-	227	S
8	9.92	256.1	258.1	257	S
9	11.90	284.1	286.1	285	S
10	14.00	212.2	-	213	S
11	19.05	465.1	467.2	466	S
12	21.02	493.2	495.2	494	M
13	22.03	224.2	-	225	S
14	25.37	479.2	-	480	S
15	30.80	511.1	513.2	512	S

*An indication for degradation products abundance (i.e. Large (L), Medium (M), and Small (S)). Peaks with S/N ratio of < 10 were ignored.

296 The evolution of DCF and its major photolytic degradation products was observed during UV
297 photolysis (Fig. 7). The DCF began to quickly degrade as soon as the UV light was activated
298 and its major degradation products were produced. Compound #1 reached its highest
299 concentration after 2 min of exposing the DCF solution to UV. About 40% of the 5 mg/L
300 DCF concentration was converted to compound #1. Once the DCF was almost completely
301 consumed, the concentration of compound #1 dramatically decreased and it was completely
302 removed after 8 min. Interestingly, compound #1 and 6 had similar trends, which could
303 indicate that these degradation products were formed directly from DCF whereas, the other
304 degradation products were produced via other pathways. The other four detected degradation
305 products were not present in the first minute of UV exposure time. They began to form after
306 1.5 - 2 min and were still detected close to their original concentrations, even after 10 min of
307 UV exposure, despite their relatively low concentration compared with compound #1.
308 Therefore, these secondary degradation products, which contain some dimers, seemed to be
309 highly resistant toward UV photolysis.

310 **3.2.2 Sulfamethoxazole**

311 The UV photolysis of SMX in pure water generated twelve photolytic degradation products
312 (Table 2). The majority of these degradation products (compounds #3 –12) were reported as
313 SMX UV photolytic degradation products for the first time in this study. Compounds #1 and
314 2 have been previously reported elsewhere [49, 50] (Fig. 8). Similar to DCF degradation
315 products, the detected SMX photolytic degradation products were eluted through the LC-MS
316 column at different retention times based on their hydrophobicity. All the photolytic SMX
317 degradation products were formed at low concentrations, except compound #1, which was
318 considered to be the primary photolytic SMX degradation product.

Table 2: The degradation products produced from UV photolysis of SMX as identified by LC-MS.

Compound	Retention time (min)	Molecular ion *(m/z)*		Molecular mass (g/mol)	Peak Abundance*
		$[M - H]^-$	$[M + H]^+$		
SMX	10.30	-	254.1	253	
1	2.91	-	254.1	253	L
2	1.96	-	99	98	S
3	5.00	-	296.1	295	S
4	10.00	266.1	-	267	S
5	13.43	266.1	-	267	M
6	11.18	258.2	260.1	259	S
7	12.50	423.1	-	424	S
8	15.02	282.1	284.2	283	S
9	17.62	282.2	284.1	283	S
10	19.70	-	284.2	283	S
11	21.00	346.1	-	347	S
12	22.38	282.2	284.2	283	S

*An indication for degradation products abundance (i.e. Large (L), Medium (M), and Small (S)). Peaks with S/N ratio of < 10 were ignored.

The UV photolysis of SMX in aqueous solution undergoes several reaction pathways, the most predominant involving cleavage of the nitrogen-oxygen bond and rearranging the isoxazole ring to form a five membered oxazole ring [49]. This compound is 4-amino-N-(5-methyloxazol-2-yl) benzenesulfonamide, which has been previously proposed elsewhere [49]. Furthermore, it has been reported that the hydroxyl radical may be produced during UV photolysis without any photochemical source [30]. This may contribute to the hydroxylation of SMX mainly on the benzene ring representing another photolytic pathway. Additionally, compound #1 may undergo hydroxylation similarly to the parent compound, SMX. The primary SMX photolytic degradation product, compound #1, eluted at 2.91 min on the reverse-phase LC-MS. In the ESI-MS spectrum a molecular ion at m/z = 254 in positive scan mode was detected. Therefore, its molecular weight was determined to be 253 amu, with molecular formula ($C_{10}H_{11}N_3O_3S$). The collision-activated dissociation of $[M + H]^+$ molecular ion of the primary SMX UV photolytic degradation product represented ions at m/z = 156, in agreement with a previous study [50]. Mass loss from the $[M + H]^+$ was

reported to be 98 amu, as a result of a cleavage in the sulfur-nitrogen bond within the structure of compound #1 (Fig. 8).

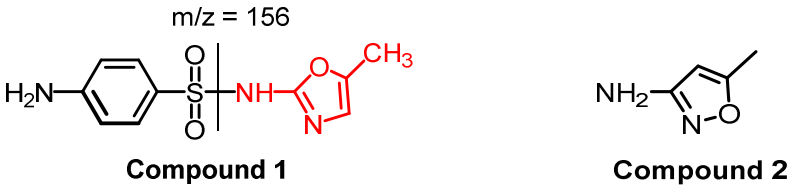

Figure 8: Major SMX UV photolytic degradation products [49, 50].

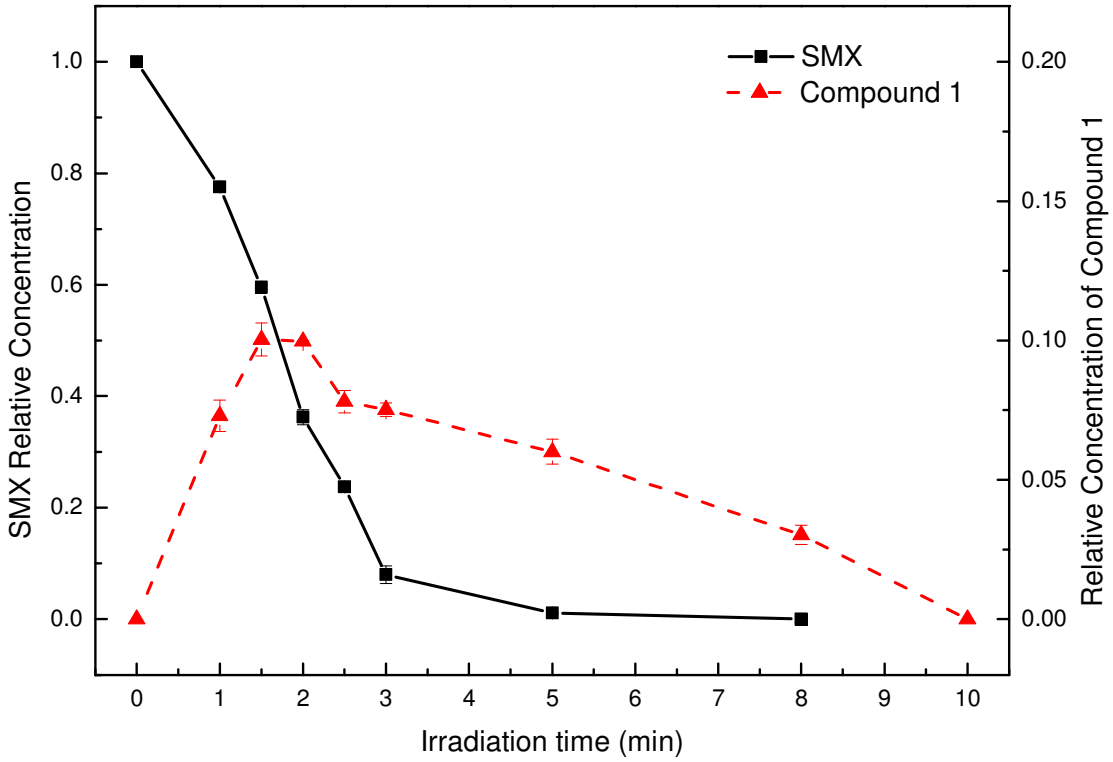

Figure 9: Evolution of the main degradation product formed during degradation of 5 mg/L SMX by only UV as determined by LC-MS.

The degradation of SMX and formation of its major photolytic degradation products was determined during UV photolysis (Fig. 9). SMX had only one major degradation product whilst all the other degradation products were formed at low concentrations. SMX began to sharply degrade as soon as it was exposed to UV, leading to the formation of SMX major photolytic degradation product, compound #1. This compound reached its maximum

concentration after 1.5 - 2 min of UV exposure time. Once the majority of SMX was degraded (3 min), compound #1 began to gradually degrade. SMX was removed to below its LC-MS detection limit (1 μg/L) at 8 min of UV exposure time. A near complete removal of compound #1 was achieved after 10 min of UV exposure. Therefore, UV photolysis under these conditions was capable of removing SMX and its major degradation products to below their LC-MS detection limit.

3.2.3 Carbamazepine

The UV photolysis and UV/H_2O_2 of 5 mg/L CBZ in pure water produced more than eighteen CBZ degradation products. Eight of these degradation products (compounds #3, 9, 12, 13, 14, 15, 17, and 18) are reported for the first time in this study (Table 3). The detected degradation products were eluted through the LC-MS column at different retention times prior to introducing them to MS for m/z determination. The concentration of CBZ degradation products was dependent on the type of irradiation applied (e.g. UV only and UV/H_2O_2 amount). Firstly, a number of degradation products were formed at detectable concentrations during both UV only and also in combination of UV with varying amounts of H_2O_2 (i.e #5, 6, 10, 14, 15 and 16). Compounds #5, 6, 10, 14, 15 and 16 eluted at consistent retention times during all experiments. Additionally, the fragmentation patterns of compounds #5, 6, 10, and 15 also confirmed the formation of these degradation products during different treatment conditions. Markedly, the different amount of H_2O_2 played an important role in the evolution of different CBZ degradation products. For example, UV only and the combination of UV with 0.01 and 0.04 g/L of H_2O_2 produced degradation products #1, 2, 8, 11, and 12 (MW 179, 270, 222, 195, and 211 amu, respectively). Alternatively, three degradation products (#4, 7, and 9; MW 266, 252, and 268 amu, respectively) were detected only during the combination of UV with every amount of hydrogen peroxide. Lastly, UV with 0.04 g H_2O_2/L

or higher, resulted in the detection of compounds #3 and 13 only (MW 298 and 273 amu, respectively).

Evidently, the applied oxidation process (i.e. UV, UV with different H_2O_2 doses) plays an important role in the formation of particular degradation products. All of the identified degradation products were formed at only low concentrations during the experiments. For UV only, just 25.2% of 5 mg/L CBZ was degraded after 60 min of UV exposure, and only low concentrations of degradation products were measured. When combined with high H_2O_2 amounts, UV/H_2O_2 could be used to simultaneously degrade both the CBZ and its degradation products. Therefore, the concentrations of degradation products formed during UV/H_2O_2 were significantly lower than during UV only.

Table 3: The degradation products produced from UV photolysis and UV/H_2O_2 of CBZ as identified by LC-MS.

Compound	Retention time (min)	Molecular ion *(m/z)*		Molecular Mass (g/mol)	Type of irradiation					Peak Abundance*
		$[M-H]^-$	$[M+H]^+$		UV only	UV/H_2O_2 (g/L)				
						0.01	0.04	0.12	0.2	
CBZ	16.00	-	237.1	236						
1	4.00	-	180.2	179	√	√	√			L
2	10.71	-	271.2	270	√	√	√			S
3	10.93	-	299.2	298			√	√	√	S
4	11.53	265.1	267.1	266		√	√	√	√	S
5	12.10	-	251.1	250	√	√	√	√	√	S
6	12.30	267.1	269.1	268	√	√	√	√	√	S
7	12.56	251.2	253.2	252		√	√	√	√	S
8	12.79	-	223.1	222	√	√	√			M
9	13.06	-	269.2	268		√	√	√	√	S
10	13.62	251.2	253.2	252	√	√	√	√	√	S
11	13.83	-	196.1	195	√	√	√			M
12	14.72	210.2	212.2	211	√	√	√			S
13	16.09	272.2	-	273			√	√	√	S
14	18.53	242.2	244.2	243	√	√	√	√	√	S
15	19.51	240.2	242.2	241	√	√	√	√	√	S
16	21.35	-	226.1	225	√	√	√	√	√	S
17	22.15	256.2	258.2	257	√	√	√	√		S
18	22.84	-	194.2	193	√					S

*An indication for degradation products abundance (i.e. Large (L), Medium (M), and Small (S)). Peaks with S/N ratio of < 10 were ignored.

A number of studies have reported some of the CBZ degradation products under a wide range of reaction conditions [32-34, 51-53]. UV photolysis of CBZ is via two different reaction pathways [33], the majority of CBZ UV photolytic degradation products are produced through a dominant pathway of ring shrinkage. This process would firstly form 9-formylacridine-10(9H)-carboxamide (Compound #10) by hydroxylation at one position on the non-aromatic double bond of CBZ [33]. In this study, there were two different possible ways in which compound #10 may progress to form other degradation products. Firstly, hydroxylation and detaching of the $CONH_2$ lateral chain may have formed compounds #11 and #16. Secondly, the carboxyaldehyde group and $CONH_2$ lateral chain may have both detached and produced compound #1 [33]. For UV/H_2O_2, UV exposure to a solution containing H_2O_2 would generate $^{\bullet}OH$ which would form compound #7 and drive the degradation process [23] (Fig. 10).

The fragmentation patterns obtained by MS of compound #10 in this study confirm the previously elucidated structure in the literature [33]. The collision-activated dissociation of $[M+H]^+$ molecular ion of compound #10 produced ions at m/z = 210 and 180. Mass losses from the molecular ion were reported to be 43(-HNCO) and 73(-HNCO and $–H_2CO$) amu, respectively (Fig. 10). The collision-activated dissociation of $[M+H]^+$ molecular ion of compound #1, which was the major CBZ degradation product caused by UV photolysis, produced ions at m/z = 152, which is consistent with other literature [32]. Mass losses from the molecular ion were reported to be 28 amu. The collision-activated dissociation of $[M+H]^+$ molecular ion of the CBZ UV photolytic degradation product (compound #11) produced ions at m/z = 167, in agreement with a previous study [33]. Mass losses from the molecular ion were reported to be 29 amu.

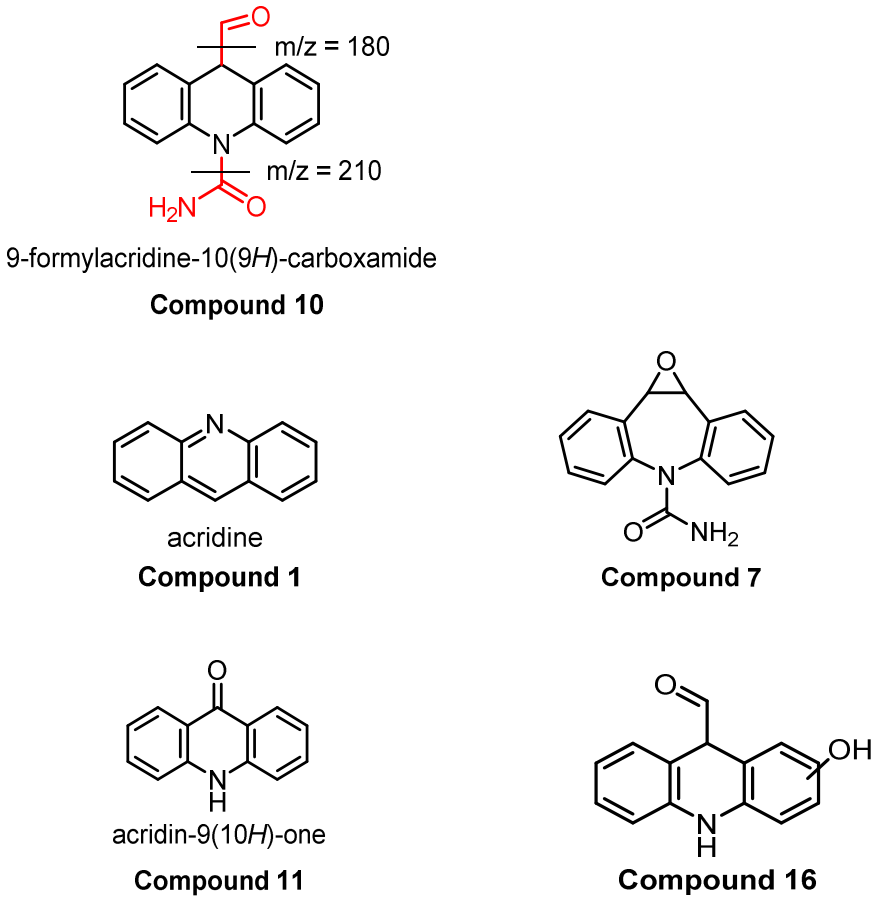

Figure 10: Major CBZ UV and UV/H_2O_2 degradation products [23, 33].

3.2.4 Trimethoprim

The UV photolysis and UV/H_2O_2 of 5 mg/L TMP in Milli-Q water produced more than 11 TMP degradation products, including five major ones (Table 4). Three TMP degradation products (#5, 7 and 11) are reported for the first time, including compound #5, which was a major degradation product found during UV with 0.04, 0.12, and 0.2 g/L of H_2O_2. Similar to CBZ degradation products, the formation of a particular TMP degradation product was dependent on the type of irradiation applied (e.g. UV only and UV/H_2O_2 amount). The identified TMP degradation products formed during UV photolysis could be categorised based on the applied conditions (i.e. H_2O_2 amount). Treatment with UV and 0.04, 0.12, and 0.2 g/L H_2O_2 resulted in detection of the majority of TMP degradation products (i.e. #3, 4, 5, 7, 10, and 11). On the other hand, the degradation products reported during both UV only and

also when combining UV with a low level (0.01 g/L) of H_2O_2 (i.e #1, 2, and 6) were only detected at these conditions. At the higher H_2O_2 amounts, compounds #1, 2, and 6 were not detected and therefore may have undergone further degradation. Alternatively, TMP degradation products #8 and 9, with the same molecular weight (i.e. 322 amu), were detected during UV only and UV with every H_2O_2 amount used except 0.2 g/L. Remarkably, four isomers at m/z = 323 were found, which was consistent with a previous study under different conditions [54]. Additionally, there were a number of very small peaks that had signal-to noise ratio less than 10 and did not meet the criteria for further analysis.

Table 4: The degradation products produced from UV photolysis and UV/H_2O_2 of TMP as identified by LC-MS.

Compound	Retention time (min)	Molecular ion *(m/z)*		Molecular Mass (g/mol)	Type of irradiation					Peak Abundance*
		$[M-H]^-$	$[M+H]^+$		UV only	UV/H_2O_2 (g/L)				
						0.01	0.04	0.12	0.2	
TMP	12.60	-	291	290						
1	13.87	-	305.2	304	√	√				L
2	9.20	-	307.2	306	√	√				L
3	10.20	-	323.2	322			√	√	√	L
4	10.40	-	323.2	322			√	√	√	L
5	11.28	-	321.2	320			√	√	√	L
6	8.46	-	295.2	294	√	√				S
7	9.55	351	353.2	352			√	√	√	M
8	13.24	-	323.2	322	√	√	√	√		S
9	15.75	-	323.2	322	√	√	√	√		S
10	16.41	-	307.1	306			√	√	√	S
11	20.08	-	275.2	274			√	√	√	S

*An indication for degradation products abundance (i.e. Large (L), Medium (M), and Small (S)). Peaks with S/N ratio of < 10 were ignored.

Hydroxylation and carbonylation are thought to be the most dominant pathways during UV and UV/H_2O_2, by which the hydroxyl groups could be connected to either rings of the TMP molecule [55]. Additionally, it has been reported that the UV photolysis of TMP would include hydroxylation, demethylation, and cleavage of the TMP parent structure [54]. For instance, compounds #2, 3, 4, 8, 9, and 10 showed addition of 16 or 32 amu to the TMP molecular weight. Carbonylation may occur at the methylene bond, for example, compounds #1 and 5. Compounds #1 and 2, which were found to be the primary TMP degradation products formed by UV photolysis, have been reported elsewhere during UV photolysis

under different conditions [54] (Fig. 11). Moreover, compounds #1, 2, 3, and 4 have also been reported during UV/H_2O_2 of TMP in artificial urine solutions [55]. However, compounds #1 and 2 were not detected during UV with 0.04, 0.12, and 0.2g H_2O_2/L in Milli-Q water. This may indicate that the addition of high amounts of H_2O_2 may cause reaction with TMP prior to significant UV exposure. The detection of compound #5, which had one additional oxygen atom to compound #1, may confirm the hydroxylation of compound #1 (Fig. 12).

The fragmentation patterns obtained by MS of the major TMP degradation products have confirmed the previously reported degradation products in the literature. The collision-activated dissociation of $[M+H]^+$ molecular ion of compound #1 produced ions at m/z = 289, 275, 259, 244, and 233. Mass losses from the molecular ion were found to be 16, 30, 46, 61, and 72 amu, respectively, with the first two fragments in agreement with another study [54]. The collision-activated dissociation of $[M+H]^+$ molecular ion of compound #2 produced ions at m/z = 289, 274, 259, 243, and 231. Mass losses from the molecular ion were accordingly reported to be 18, 33, 48, 64, and 76 amu, respectively, in agreement with another study [54]. The collision-activated dissociation of $[M+H]^+$ molecular ion of compound #3 and 4 produced ions at m/z 181. The collision-activated dissociation of $[M+H]^+$ molecular ion of compound #5 produced ions at m/z 303 and 273. Mass losses from the molecular ion were found to be 18 and 48 amu, respectively.

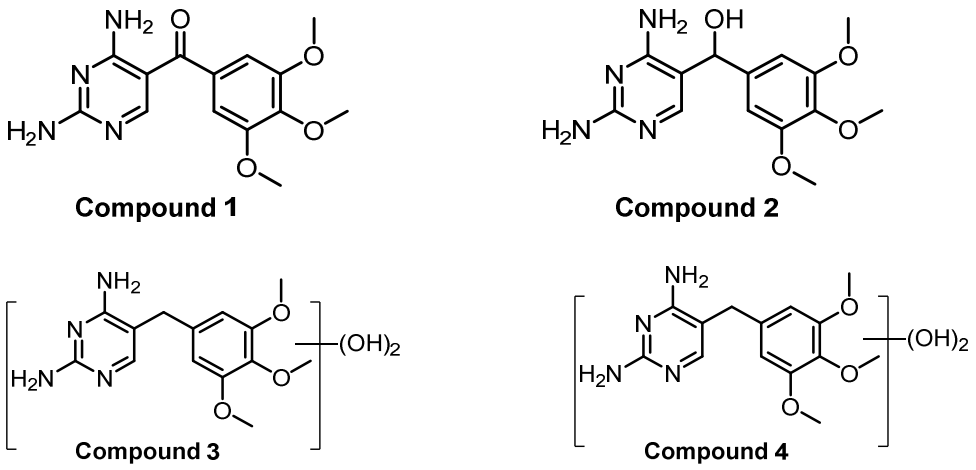

Figure 11: Major TMP UV and UV/H_2O_2 degradation products [54].

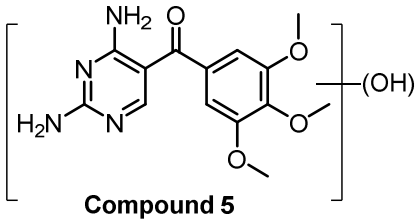

Figure 12: Proposed structure of compound #5.

The evolution of the main TMP degradation products during UV photolysis and UV with a different concentration of H_2O_2 was determined (Fig. 13). During UV photolysis, the major TMP degradation products were compounds #1 and 2, which were formed as TMP was gradually degraded (Fig. 13A). Approximately 75% of 5 mg/L TMP was removed after 90 min of UV exposure. However, the photolytic degradation products did not show any decrease in their concentrations, which may indicate their persistence to UV photolysis. With a combination of UV and H_2O_2 the degradation products (compounds #3, 4 and 5) displayed different evolutionary behaviour at each H_2O_2 concentration (Fig. 13B-D). The three degradation products were produced at similar concentrations for all H_2O_2 amounts used, however, the observed irradiation time varied significantly for each. The irradiation times to reach the highest concentration of degradation products were 60, 30 and 20 min, for H_2O_2 amounts of 0.04, 0.12 and 0.2 g/L, respectively. This was likely due to the faster TMP

degradation rate as a result of the higher H_2O_2 amount present in the solution during UV exposure. Once the majority of TMP had been degraded, the concentrations of the degradation products reduced with further irradiation time. Therefore, within 90 min irradiation time, compounds #3, 4 and 5 were highly susceptible to the oxidation process used. Results indicate that the highest H_2O_2 amount could achieve the highest removal of TMP, and also the degradation products showed the lowest final concentration.

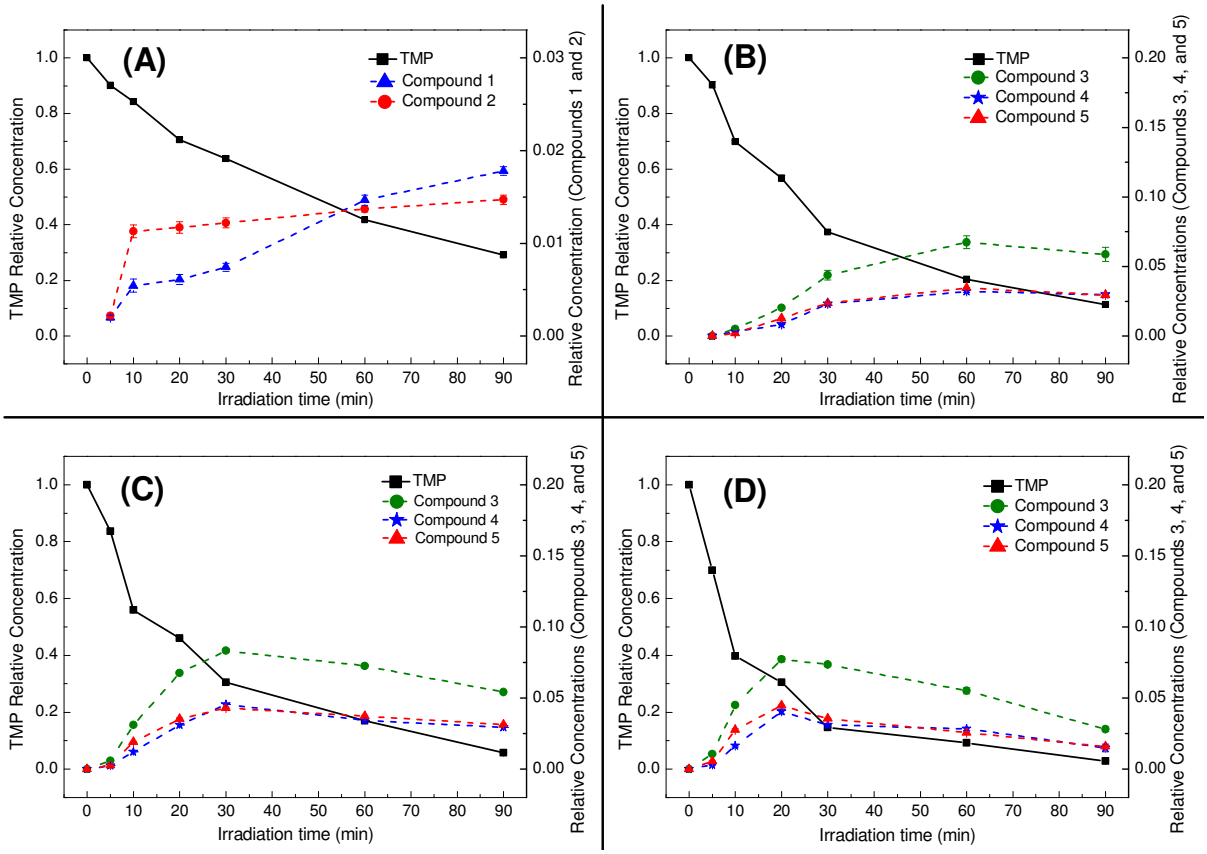

Figure 13: Evolution of main degradation products formed during degradation of 5 mg/L TMP by UV exposure with (A) 0, (B) 0.04 $_2$, (C) 0.12, and (D) 0.2 g/L of H_2O_2.

3.3. Photolytic solution toxicity

The toxicity of the four pharmaceuticals and their degradation product mixtures during UV photolysis and UV/H_2O_2 were evaluated (Table 5). None of the tested parent compounds were toxic in the BLT-Screen at the concentration tested (all <0.6 rTU at 5 mg/L), but most (DCF, CBZ and SMX) showed an increased level of toxicity after UV treatment, most likely as a result of more toxic photolytic degradation products in the solution. The toxicity of the DCF solution increased dramatically from <0.6 to 6.7-7.2 rTU after 3, 5, and 10 min of UV exposure, during a time window when the sample contained six degradation products at relatively high concentrations (Fig. 7). Applying a high H_2O_2 dose (i.e. 0.12 g/L) and a long UV exposure time (i.e. 20 min) to DCF effectively produced final degradation products with toxicity similar to the original solution (<0.6 rTU). The toxicity of the CBZ solution was increased to 1.7 rTU after 60 min of UV exposure. This CBZ solution contained 3.74 mg/L of CBZ and a number of photolytic degradation products with a relatively predominant abundance of compound #1. The combination of UV with 0.12 g/L of H_2O_2 for the removal of CBZ reduced the solution toxicity again to below the detection limit of the assay (<0.6 rTU). The SMX solution toxicity showed slightly elevated toxicity (0.7 rTU) after 3 min of UV exposure compared to below detection limit (<0.6 rTU) for untreated SMX solution. In contrast, the TMP solution showed no change in toxicity during UV exposure, or when combined with H_2O_2 (all <0.6 rTU). Therefore, although UV treatment may lead to the formation of toxic degradation products for DCF, in particular, but also CBZ and SMX, the combination with H_2O_2 effectively degraded them into less toxic degradation products.

Table 5: The solution toxicity of the parent pharmaceuticals and their degradation products under different reaction conditions. Toxicity is expressed as relative toxic unit (rTU), calculated as $1/IC_{20}$ in the BLT assay.

Pharmaceuticals	Experimental conditions		Toxicity in the BLT (rTU)
	UV exposure time (min)	H_2O_2 amount (g/L)	
DCF-5mg/L	0	-	<0.6
	3	-	6.7
	5	-	6.7
	10	-	7.2
	20	0.12	<0.6
CBZ-5mg/L	0	-	<0.6
	60	-	1.7
	60	0.12	<0.6
SMX-5mg/L	0	-	<0.6
	3	-	0.7
	3	0.01	<0.6
TMP-5mg/L	0	-	<0.6
	90	-	<0.6
	90	0.12	<0.6

4. Conclusion

This work provides further insights to the UV photolysis and UV/H_2O_2 of DCF, SMX, DCF, and TMP. DCF and SMX were easily degraded to below their LC-MS detection limit (1 μg/L) after only 8 min of exposure to UV photolysis, and there was no need to combine UV with H_2O_2 to improve their removal. However, the addition of H_2O_2 may be essential during UV photolysis of DCF to avoid the formation of toxic degradation products during UV alone. CBZ and TMP were quite resistant to UV photolysis, whereas, the combination of UV with H_2O_2 significantly improved the removal of CBZ and TMP, with higher H_2O_2 dose resulting in better removal. A number of new degradation products have been identified in this study for all of the four selected pharmaceuticals during UV and UV/H_2O_2 photolysis. Some of the identified CBZ and TMP degradation products during UV photolysis were different from those formed during UV/H_2O_2. The combined oxidation process using only UV and UV with different H_2O_2 plays an important role in the formation of CBZ and TMP degradation products. Some of the formed degradation product mixtures were more toxic than their parent

compounds, whereas others did not show any change in toxicity compared with their parents. This work clearly illustrates the need to conduct both chemical and toxicity analysis in parallel to provide a more comprehensive assessment of removal mechanisms. Further work is currently underway to enable a comprehensive identification of new detected degradation products using their fragmentation ions for structural confirmation.

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