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Application of LC-MS for the identification and quantification of pharmaceuticals and their transformation products during catalytic ozonation

Åbo Akademi University

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“To live in harmony with nature, we must know how to sing the same song as nature. To do that, we must understand nature. Good intentions aren’t enough. Science might be – if we use it wisely.”

Terry Pratchett

Preface

This work was performed at the Laboratory of organic chemistry at Åbo Akademi in 2015-2019 and the thesis was finished in 2020. The doctoral studies were a part of the activities at Johan Gadolin Process Chemistry Centre (PCC). The work was financed by Maa- ja vesitekniikan tuki, Stiftelsen för Åbo Akademi, Victoriastiftelsen and Svenska litteratursällskapet.

I would like to thank my supervisors Leif Kronberg and Patrik Eklund. Thank you, Leif, for introducing me to the field of environmental organic chemistry and for providing me with the opportunity to start with this work. You retired when I was in the middle of my thesis work, but even after that you were willing to help me with my work. Thank you, Patrik for being my supervisor even though your field of interest is synthetic chemistry, not environmental chemistry. You have been a great help by providing a different perspective on the work; by helping with publications and with navigating bureaucracy.

I would like to thank Soudabeh Saeid for being an excellent collaborator. Without your help, my work would have been entirely different. My work was in a way an expansion on the work you had already started.

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Åbo, January 2021

Matilda Kråkström

Abstract

The presence of pharmaceuticals in the environment is of concern due to their harmful effect on aquatic organisms. Because of this, it is important that they are effectively removed in wastewater treatment plants. Conventional treatment plants are unable to completely remove pharmaceuticals, so new, more effective treatment methods are needed. One promising method is catalytic ozonation. In this thesis the removal of pharmaceuticals by catalytic ozonation is investigated.

It is not enough to remove pharmaceuticals in wastewater since complete mineralization of organic compounds is very rarely achieved during water treatment. Instead, pharmaceuticals are transformed into new products. These products can even be more toxic to aquatic organisms than the parent compound. In this thesis, the transformations which takes place during ozonation are studied for four pharmaceuticals: the pain-killers ibuprofen (IBU) and diclofenac (DCF), the anti-epileptic pharmaceutical carbamazepine (CBZ), and the antibiotic sulfadiazine (SDZ).

The transformation of the pharmaceuticals was studied with the help of liquid chromatography and gas chromatography coupled to ion trap-, triple quadrupole- and time-of-flight mass spectrometry. Some of the major products were also isolated and analyzed with nuclear magnetic resonance spectroscopy (NMR). Some of the major products formed during the ozonation of the pharmaceuticals were also quantified with LC-MS or LC-UV.

All of the selected pharmaceuticals could effectively be transformed through ozonation. Catalysts either slowed down or sped up the transformation. For IBU, copper-based catalysts enhanced the transformation, while platinum-based catalysts enhanced the transformation of DCF. The transformation of CBZ was slowed down by the addition of palladium-based catalysts, while copper-based catalysts enhanced the reaction. The transformation of SDZ was slowed down by both iron-based and copper-based catalysts.

For IBU, 12 different transformation products could be detected. The structures of six of the products could be confirmed with the help of authentic samples. The main product formed resulted from hydroxylation and subsequent oxidation to a ketone. The detected products only accounted for 6 % of the transformed IBU, indicating that IBU is transformed into smaller, more polar compounds which could not be detected with LC-MS or GC-MS.

For DCF 14 different products could be detected and the structure of three of them could be confirmed with the help of reference samples. Three of the DCF products were quantified. The major product was formed from DCF via hydroxylation. Only 20 % of the transformed DCF could be accounted for.

For CBZ, 15 different products were detected. Three of the products were isolated and the structures were confirmed with NMR. CBZ was mainly transformed via a rearrangement reaction to 1-(2-benzaldehyde)-4-hydro-(1H,3H)-quinazoline-2-one (BQM), and further oxidized via direct ozonation to 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD). During non-catalytic ozonation, 74 % of CBZ was transformed into BQM and 83 % of BQM was

transformed into BQD. A significant amount of CBZ is also transformed via ring-opening leading to the formation of 2,2'-azanediyldibenzaldehyde.

For SDZ, 16 different products were detected. The structure of one product was confirmed using an authentic reference samples and the structure of the major product was confirmed with NMR. SDZ was mainly transformed via a long series of steps leading to the formation of 8-nitropyrimido[1,2-a]benzimidazol-9-ol (SDZ-P15). The concentration of SDZ-P15 increased for 50 minutes, until it accounted for 30 % of the initial concentration of SDZ.

Sammanfattning

Närvaron av läkemedel i miljön är oroande på grund av den skada som de kan orsaka vattenlevande organismer. Därför är det viktigt att eliminera dem från avloppsvatten. Traditionella avloppsvattenreningsverk kan inte fullkomligt avlägsna läkemedel, så nya, effektivare reningsmetoder måste utvecklas. En lovande reningsmetod är katalytisk ozonering. Denna avhandling behandlar de reaktioner som sker när läkemedel ozoneras. De läkemedel som studeras är: de smärtstillande läkemedlen ibuprofen (IBU) och diklofenak (DCF), det anti-epileptiska läkemedlet karbamazepin (CBZ) och antibiotikan sulfadiazin (SDZ).

Alla läkemedlen kunde effektivt omvandlas genom ozonering. Tillsats av katalysatorer kunde antingen förbättra eller försämra transformationen. Kopparbaserade katalysatorer försnabbade transformationen av IBU, medan platinabaserade katalysatorer försnabbade transformationen av DCF. CBZ omvandlades snabbare i närvaro av kopparbaserade katalysatorer och långsammare i närvaro av palladiumbaserade katalysatorer. Närvaron av både järnbaserade och kopparbaserade katalysatorer ledde till en långsammare transformation av SDZ.

Transformationen av läkemedlen studerades med vätskekromatografi (LC), gaskromatografi (GC) och masspektrometri (MS). Några viktiga produkter isolerades och analyserades med Kärnmagnetisk resonans spektroskopi (NMR). Några av de viktigaste produkterna kunde också kvantifieras genom LC-MS eller LC-UV.

För IBU kunde 12 olika produkter detekteras. Produkten som bildades med högst koncentration när IBU ozonerades bildades genom hydroxylering följt av oxidering till en keton. Bara 6 % av IBU omvandlades till produkter som kunde kvantifieras. Det tyder på att en stor del av IBU omvandlades till mindre, mera polära föreningar som inte kunde detekteras med LC-MS eller GC-MS.

För DCF kunde 14 olika produkter detekteras, varav strukturerna för tre kunde bekräftas med referessubstanser. Tre av DCF produkterna kvantifierades. DCF omvandlades främst via hydroxylering. Bara 20 % av DCF omvandlades till produkter som kunde kvantifieras.

För CBZ kunde 15 olika produkter detekteras. Tre av produkterna isolerades och deras strukturer bekräftades med NMR. CBZ omvandlades främst via en omlagringsreaktion till 1-(2-benzaldehyd)-4-hydro-(1H,3H)-quinazoline-2-one (BQM). BQM oxiderades vidare till 1-(2-benzaldehyd)-(1H,3H)-quinazoline-2,4-dion (BQD). Under icke-katalytiska förhållanden omvandlades 74 % av CBZ till BQM och 83 % av BQM omvandlades till BQD. En stor del av CBZ omvandlades också till 2,2'-azanediyldibenzaldehyd.

För SDZ detekterades 16 olika produkter. Strukturen för en av produkterna bekräftades med en referenssubstans och en annan av de viktigaste produkterna isolerades och dess struktur bekräftades med NMR. SDZ omvandlades främst via en lång kedja av reaktioner till 8-nitropyrimido[1,2-a]benzimidazol-9-ol (SDZ-P15). SDZ-P15 var stabil och kunde inte vidare omvandlas genom ozonering.

List of publications

I. **Kråkström, M.**; Saeid, S.; Tolvanen, P.; Kumar, N.; Salmi, T.; Kronberg, L.; Eklund, P. Identification and quantification of transformation products formed during the ozonation of the non-steroidal anti-inflammatory pharmaceuticals ibuprofen and diclofenac (submitted)

II. **Kråkström, M.**; Saeid, S.; Tolvanen, P.; Kumar, N.; Salmi, T.; Kronberg, L.; Eklund, P. Ozonation of carbamazepine: product determination and reaction mechanisms, Environmental science and pollution research Environmental Science and Pollution Research **2020**, *27*, 23258-23269. doi.org/10.1007/s11356-020-08795-0

III. **Kråkström, M.**; Saeid, S.; Tolvanen, P.; Salmi, T.; Kronberg, L.; Eklund, P. Catalytic ozonation of the antibiotic sulfadiazine: Reaction kinetics and transformation mechanisms. *Chemosphere* **2020**, *247*, 1-12. doi.org/10.1016/j.chemosphere.2020. 125853

IV. Saeid, S.; **Kråkström, M.**; Tolvanen, P.; Kumar, N.; Eränen, K.; Peltonen, J.; Peurla, M.; Mikkola, J. P.; Maël, L.; Kronberg, L.; Eklund, P.; Salmi, T. synthesis and characterization of metal modified catalysts for decomposition of ibuprofen from aqueous solutions. *Catalysts* **2020**, *10*, 786. doi:10.3390/catal10070786

V. Saeid, S.; **Kråkström, M.**; Tolvanen, P.; Kumar, N.; Eränen, K.; Mikkola, J.P.; Kronberg, L.; Eklund, P.; Aho, A.; Palonen, H.; Shchukarev, A.; Salmi, T. Pt modified heterogeneous catalysts combined with ozonation for the removal of Diclofenac from aqueous solutions and the fate of by-products. *Catalysts* **2020**, *10*, 322. doi.org/10.3390/catal10030322

VI. Saeid, S.; **Kråkström, M.**; Tolvanen, P.; Kumar, N.; Eränen, K.; Mikkola, J. P.; Kronberg, L.; Eklund, P.; Peurla, M.; Aho A.; Salmi, T. Advanced oxidation process for degradation of carbamazepine from aqueous solution: Influence of metal modified microporous, mesoporous catalysts on the ozonation process. *Catalysts* **2020**, 10, 90. doi.org/10.3390/catal10010090

List of supporting publications

Sokolov, A.; **Kråkström, M.;** Eklund, P.; Kronberg, L.; Louhi-Kultanen, M. Abatement of amoxicillin and doxycycline in binary and ternary aqueous solutions by gas-phase pulsed corona discharge oxidation. *Chemical engineering journal* **2018**, *334*, 673-681.

Kruglova, A.; Gonzalez-Martinez, A.; **Kråkström, M**.; Mikola, A.; Vahala, R. Bacterial diversity and population shifts driven by spotlight wastewater micropollutants in low-temperature highly nitrifying activated sludge. *Science of the total environment* **2017**, *605-606,* 291-299.

Kruglova, A.; **Kråkström, M**.; Riska, M.; Mikola, A.; Rantanen, P.; Vahala, R.; Kronberg, L. Comparative study of emerging micropollutants removal be aerobic activated sludge of large laboratory-scale membrane bioreactos and sequencing batch reactors under low-temperature conditions. *Bioresource technol*ogy **2016**, *214*, 81-88.

Contribution of the author

Matilda Kråkström has contributed to the papers in this thesis as stated below:

I. Matilda Kråkström participated in the planning of the experiments, developed the analysis methods, analyzed the samples, evaluated the data and was responsible for writing the paper.

II. Matilda Kråkström participated in the planning of the experiments, developed the analysis methods, performed the product isolations, analyzed the samples, evaluated the data and was responsible for writing the paper.

III. Matilda Kråkström participated in the planning of the experiments, developed the methods, performed the product isolations, analyzed the samples, evaluated the data and was responsible for writing the paper.

IV. Matilda Kråkström performed the LC-MS analyses, evaluated the LC-MS data, and contributed to the writing of the paper.

V. Matilda Kråkström performed the LC-MS and LC-UV analyses, evaluated the LC-MS and LC-UV data, and contributed to the writing of the paper.

VI. Matilda Kråkström performed the LC-MS and LC-UV analyses, evaluated the LC-MS and LC-UV data, and contributed to the writing of the paper.

List of abbreviations

2-AP	2-aminopyridine
ACN	acetonitrile
AOP	advanced oxidation process
BQD	1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione
BQM	1-(2-benzaldehyde)-4-hydro-(1H,3H)-quinazoline-2-one
CBZ	carbamazepine
DCF	diclofenac
EIC	extracted ion chromatogram
ESI	electrospray ionization
GC	gas chromatography
H_2O_2	hydrogen peroxide
HRMS	high resolution mass spectrometry
IBU	ibuprofen
IS	internal standard
LC	liquid chromatography
MRM	multiple reaction monitoring
MS	mass spectrometry
MSn	multiple tandem mass spectrometry
m/z	mass-to-charge ratio
NMR	nuclear magnetic resonance
NSAID	Non-steroidal anti-inflammatory drug
·OH	hydroxyl radical
QTOF	quadrupole-time of flight
QqQ	triple-quadrupole
SDZ	sulfadiazine
TP	transformation product
UV	ultraviolet
WWTP	wastewater treatment plant

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1. Introduction

1.1. PHARMACEUTICALS IN THE ENVIRONMENT

1.1.1. Presence of pharmaceuticals in the environment

Since the 1970's, pharmaceuticals have been detected in environmental wasters (Nikolaou 2007). In environmental waters, pharmaceuticals are usually present in the ng/L range, however some pharmaceuticals, such as ibuprofen have been detected at concentrations exceeding 2 µg/L (Roberts 2006). In regions where wastewater treatment is lacking the concentrations of pharmaceuticals in surface water can be significantly higher. For example, the antibiotic sulfamethoxazole has been detected at concentrations of almost 14 µg/L (Ngumba 2016) and the antiviral lamivudine was detected at 167 µg/L (K'oreje 2016) in river waters in Kenya.

1.1.2. Effects of pharmaceuticals in the environment

Even though most pharmaceuticals are not persistent in the environment, they are always present due to their continual release. This leads to pharmaceuticals being "pseudo-persistent" (Barceló 2007). Pharmaceuticals are of concern as environmental contaminants because they have been specifically developed to have a biological effect (Barceló 2007). The effect of pharmaceuticals is difficult to establish because of the multitude of different organism in the environment and the difficulty of choosing relevant endpoints. While the purpose of human risk assessment is to protect individuals; environmental risk assessment deals with risks on the population level (Dorne 2007). Acute toxicity studies have shown that the concentrations of pharmaceuticals in the environment are not of concern, since the measured concentrations rarely excel the µg/L range, while the acute toxic concentrations are usually in the mg/L range (Fent 2006). Studies concerning chronic toxicity on the other hand have shown some reason for concern. For example, environmentally relevant concentrations of endocrine disrupters have a potential to affect populations due to the low concentrations needed to have a biological effect and the potential for them to effect reproduction (Dorne 2007). The contraceptive ethinylesradiol has a negative effect on fish reproduction already at low ng/L concentrations (Länge 2001, Parrott 2005). Another group of pharmaceuticals of particular concern is antibiotics. This is due to the rise of antibiotic resistance and the negative effect antibiotics can have on useful bacteria in the environment. The currently released concentrations of several antibiotics is thought to pose a high risk to the environment (Verlicchi 2012).

Another complicating factor is the mixture effect. Pharmaceuticals are not only present in the environment as individual compounds, but as a mixture. Together they can have an effect at concentrations at which none of the

pharmaceuticals have an individual effect (Fent 2006). For example, the anti-inflammatory drugs diclofenac, ibuprofen, naproxen and acetylsalisylic acid showed significant toxicity as a mixture at concentrations for which the individual pharmaceuticals did not have any effect (Cleuvers 2004).

1.2. PHARMACEUTICALS IN WASTEWATER TREATMENT

1.2.1. Presence of pharmaceuticals in wastewater

In domestic wastewater treatment plants, the concentrations of some pharmaceuticals, such as paracetamol, ibuprofen, tramadol and naproxen, can exceed 50 µg/L (Roberts 2006, Verlicchi 2012). The concentrations of pharmaceuticals in wastewater treatment plant effluent is lower, usually less than 1 µg/L, for individual pharmacueticals. However, some pharmaceuticals, such as bezafibrate, paracetamol and carbamazepine, have been detected at concentrations around 5 µg/L (Ternes 1998).

In industrial wastewater the concentrations of pharmaceuticals can be much higher: a wastewater treatment plant in New York which received water from a pharmaceutical formulation facility exhibited concentrations of almost 4000 µg/L of the pharmaceutical metaxalone (Phillips 2010). The concentration of ciprofloxacin in the pharmaceutical production wastewater in India exceeded 30 000 µg/L (Larsson 2007).

1.2.2. Wastewater treatment

Wastewater treatment is usually divided into preliminary, primary, secondary and tertiary treatment (von Sperling 2007, Hopcroft 2015). During preliminary treatment, coarse solids are removed. In primary treatment, settleable solids and part of the organic matter is removed. Secondary treatment is aimed at removing organic matter and nutrients mostly via biotransformation. In tertiary treatment, specific pollutants, such as toxic or non-biodegradable compounds, are removed (von Sperling 2007). The treatment processes can be divided into physical-, chemical- and biological processes. The physical processes include flocculation, flotation, sedimentation and filtration. The chemical processes include the addition of chemicals resulting in precipitation or disinfection. The biological processes include the use of bacteria to remove organic matter and nitrogen. (von Sperling 2007).

1.2.3. Advanced oxidation treatment

Since the removal of pollutants in conventional wastewater treatment plants is not complete, an additional treatment step is sometimes added. This is done for example if there are high concentrations of some specific pollutants in the wastewater, or if the waster will be recycled. The additional treatment methods are called tertiary treatments and include for example the absorption of pollutants into active carbon, separation via membrane processes and advanced oxidation processes (AOP) (Liu 2009). AOPs involve the generation of strong oxidizing agents in the form of reactive oxygen or free radicals. The most important radical is the hydroxyl radical (·OH) (Kanakaraju 2018).

There are several methods for forming oxidative agents in wastewater treatment. The Fenton reaction involves a combination of an iron catalyst and H_2O_2. UV light can be used to transform pollutants either directly or indirectly. Indirectly UV light in combination with H_2O_2 leads to the cleavage of the O-O bond in H_2O_2 and subsequently the formation of ·OH. Sonolysis involves the removal of pollutants with the help of ultrasound irradiation. The high intensity of acoustic cavity bubbles leads to the formation of OH, which can then react with pollutants. In electrochemical oxidation,·OH are formed with the help of electricity. The transformation of organic compounds can take place either via direct charge transfer or indirectly via the generation of reactive oxygen species at the surface of the electrode. Ionizing radiation can also be used to transform pollutants. This is accomplished via the formation of hydroxyl radicals and hydrated electrons (Kanakaraju 2018).

Ozonation is one of the most studied AOPs. The ozonation of pharmaceuticals can take place either as a direct reaction with ozone or as an indirect reaction with hydroxyl radicals that are formed when ozone decomposes in water. The reaction of pharmaceuticals with hydroxyl radicals is usually faster than the reaction with ozone. The apparent rate constants at pH 7 with hydroxyl radicals is of the order 10^9 ($M^{-1}s^{-1}$), while the constant with ozone varies between less than 0.8 for iopromide and 10^6 for sulfamethoxazole and diclofenac (Huber 2005).

1.3. TRANSFORMATION OF PHARMACEUTICALS

In the human body, pharmaceuticals are transformed via metabolism. In the environment, pharmaceuticals can be transformed via phototransformation and biotransformation. It is also possible for pharmaceuticals to be removed from water by sorbtion to solid particles (Fent 2006).

1.3.1. Phototransformation

During photolysis, the compounds can be transformed either directly, by absorbing light, or indirectly, via reactions involving natural photosensitizers such as nitrate and humic acids (Nikolaou 2007). During direct photochemical reactions, molecules absorb light leading to photoexcitation. Molecules in an excited state can then undergo different reactions while returning to the ground-state. The ability of pharmaceuticals to undergo direct photolysis depends on the structure of the compound. Only compounds which absorb light in the UV-visible light region can undergo direct phototransformation. One example of this is DCF. DCF absorbs sunlight, looses a proton and enters an excited singlet state. This is followed by an intersystem crossing and the loss of chlorine to form an excited triplet state. When the chlorine leaves, it will abstract a proton to form HCl concomitant with the formation of a new C-C bond (Figure 1.1.(a), Musa 2009). This reaction is not observed during other transformation methods, such as biotransformation or ozonation.

Compounds which do not absorb light in the correct region can be transformed via indirect phototransformation. So called photosensitizers can absorb solar energy which is transferred to the pharmceuticals, causing them to be transformed (Fatta-Kassinos 2011). Some naturally occurring species can also generate strongly oxidizing species such as hydroxyl radicals and singlet oxygen which are able to transform pharmaceuticals (Fatta-Kassinos 2011). One example of a pharmaceutical which undergoes indirect phototransformation is the analgesic drug paracetamol (PCM). PCM is mainly transformed via direct phototransformation, leading to direct phototransformation products, such as dimers, trimers and a product resulting from the breakage of the aromatic ring and N-dealkylation (Figure 1.1.(b)) or via reactions with triplet sensitizers to form similar products. PCM also reacted with hydroxyl radicals, leading to hydroxylation reactions and the formation of quinone imine products (Figure 1.1. (b), De Laurentiis 2014).

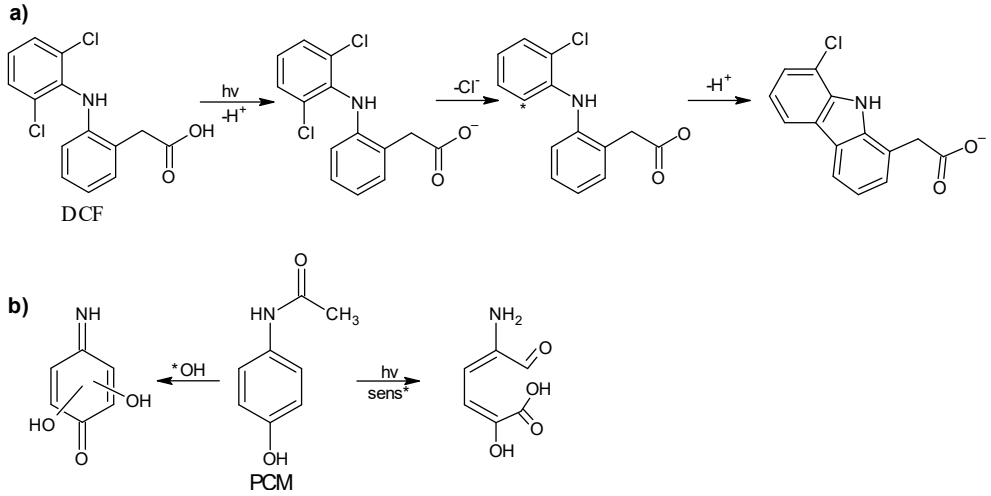


Figure 1.1. (a) direct phototransforamtion of DCF (b) indirect and direct phototransformation of PCM

The phototransformation of pharmaceuticals can sometimes lead to the formation of products which are more toxic than the parent compound (DellaGreca 2004). During the phototransformation of the steroid prednisolone seven products could be detected. All the products had a higher acute toxicity towards *D. magna* than the parent compound, although the concentration was several orders of magnitude higher than the concentrations expected to be found in the environment (DellaGreca 2004).

1.3.2. Biotransformation

In humans and other animals, organic compounds are metabolized in order transform the compounds into pharmacologically inert compounds which are more easily excreted from the body (Thomas 2007). Metabolites are usually more water soluble than the parent compound and are thus more easily excreted in urine. Metabolism is divided into phase I and phase II metabolism. Most metabolic reactions take place in the liver.

During phase I, organic compounds are modified mainly via oxidation. Alkenes can be oxidized to oxiranes. This takes place for example during the metabolization of the anti-epileptic drug carbamazepine (Figure 1.2. (a), Thomas 2007). Alkynes can be oxidized to oxirenes. Oxirenes are usually unstable and rearrange to ketenes, which hydrolyze to carboxylic acids (Figure 1.2 (b), Lee 2011).

Pharmaceuticals containing primary alcohols, for example the high blood pressure medication losartan, can be oxidized to carboxylic acids (Figure 1.2 (c)). Secondary alcohols can be oxidized to ketones. This reaction takes place for example for hyrdoxylated ibuprofen metabolites.

Primary amines can be oxidized in several steps leading to nitro-compounds (Figure 1.2. (d), Thomas 2007). Secondary and tertiary amines amines can be N-dealkylated to an amine and an aldehyde or ketone. This reaction takes place for example for β-blockers (Figure 1.3. (a), Bussy 2013). Pharmaceuticals, such as the hydroxylated metabolites of ibuprofen and diclofenac, which contain two aromatic hydroxyl groups, or one hydroxyl group and an amine, can be dehydrogenetad to quinone-like structures (Figure 1.3.(b), Lee 2011).

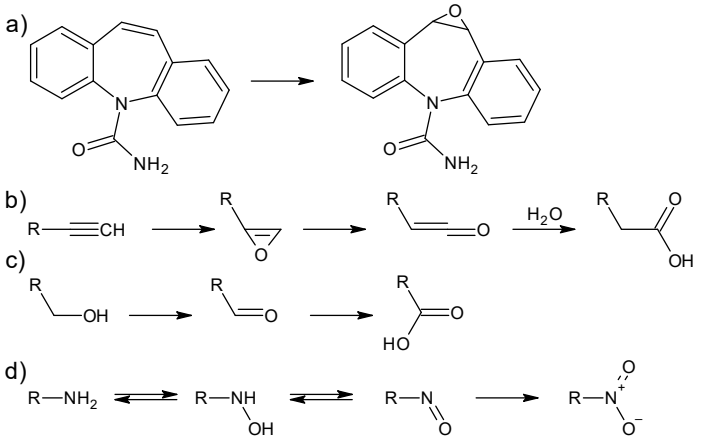


Figure 1.2. Oxidation of (a) the alkene in carbamazepine, (b) alkynes, (c) alcohols, (d) amines.

Pharmaceuticals, such as the pro-drug L-DOPA, which contain carboxylic acid groups can be decarboxylated (Figure 1.3. (c)). Pharmaceuticals which contain aldehydes, ketones, alkenes, nitro-groups, azo-groups or sulphoxides can be

reduced. One example of this is the nonsteroidal anti-inflammatory drug ketoprofen (Figure 1.3. (d), Alkatheeri 1999). Esters and amides can be metabolized through hydrolysis and epoxides through hydration. One example of hydrolysis is the painkiller fentanyl (Figure 1.3. (e), Wilde 2019).

Phase II metabolism involves conjugation. The conjugation reactions add acyl-, sulphate-, amino acid-, glucuronic acid- or mercapturic acid groups to the parent compound (Thomas 2007). The conjugation reactions mainly take place on hydroxyl groups or amine groups (Lee 2011).

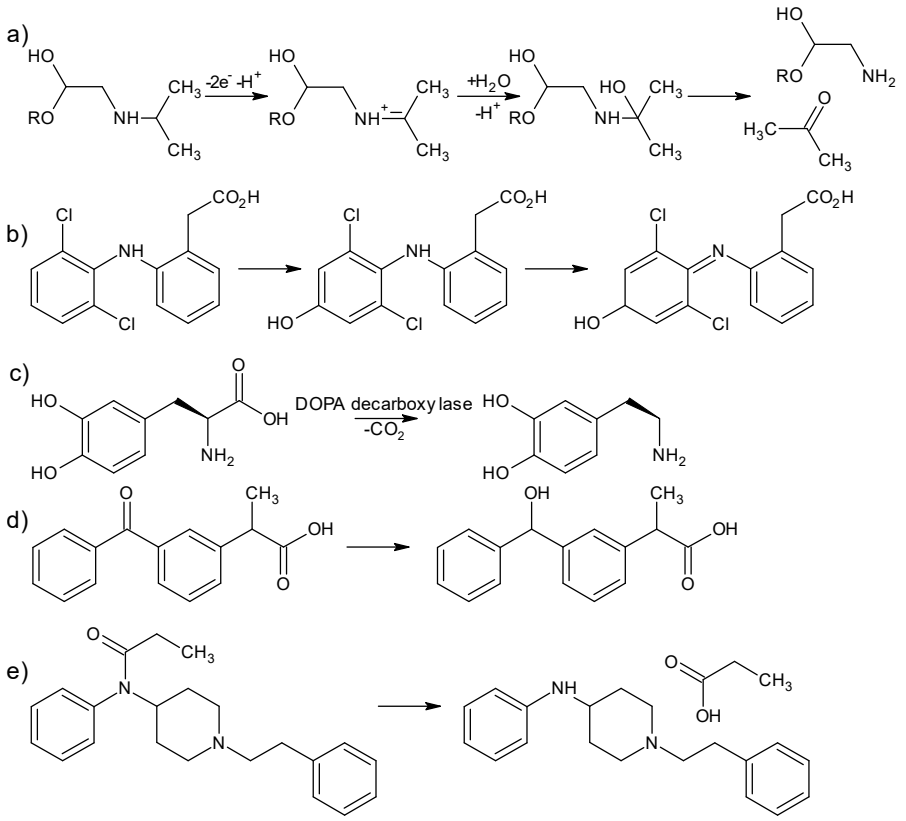


Figure 1.3. (a) N-dealkylation of β-blockers, (b) oxidation of diclofenac to form a quinone imine, (c) decarboxylation of L-DOPA, (d) reduction of ketoprofen, (e) hydrolysis of fentanyl.

In the environment, bacteria are able to biotransform some pharmaceuticals (Caracciolo 2015). Some pharmaceuticals, such as ibuprofen are easily biotransformed (Kunkel 2008), however others, such as carbamazepine (Li 2013) and erythromycin (Alexy 2004), are considered to be non-biodegradable. The biodegradability of pharmaceuticals depends on the structure of the compound. For example, the presence of sugar moieties increase biodegradability, while fluorinated compounds are less easily transformed (Onesios 2009). Biodegradation is also temperature dependent, and is faster during summer than during winter (Meierjohann 2016).

There is less information available about the transformation of pharmaceuticals by micro-organisms compared to mammals. Biotransformation takes place mostly in WWTPs where the concentration of micro-organisms is high. Environmental waters contain low concentrations of organic matter and consequently the microbial density is low. Due to the low concentration of pharmaceuticals in wastewater and environmental waters, the biotransformation of pharmaceuticals has to take place via co-metabolism. This means that the organic compound is altered, but the micro-organism does not gain assimilable carbon or energy from the compound. Co-metabolism requires the presence of other sources of carbon for the micro-organisms to survive (Lester 1999). Ibuprofen, Bezafibrate and Naproxen are not transformed when they are the sole source of carbon in a membrane bioreactor, however, they were ransformed when an additional source of carbon was added (Quintana 2005). Biodegradability can be divided into ready biodegradability and inherent biodegradability. Ready biodegradability means that a compound can be used as the only source of carbon and that it degrades extensively in 28 days. Inherent biodegradability means that the compound has a potential for biodegradability. Less than 20 % biodegradation means that the substance can be assumed to be persistent (Lester 1999). The biotransformation reactions which take place in micro-organisms are similar to the ones in humans. Additionally, aromatic rings can be broken through photo catabolism, resulting in mineralization (Saiskala 1998).

The biotransformation rate of pharmaceuticals varies greatly between different pharmaceuticals and different treatment plants. For ibuprofen, the biotransformation rate can be close to 100 %, while for diclofenac and carbamazepine the biotransformation is usually negligible (Joss 2006, Verlicchi 2012). The overall removal of ibuprofen, diclofenac and carbamazepine in wastewater treatment plants varies between -4 % and 100 %, -111 % and 90 % and -122 % and 80 % respectively (Verlicchi 2012). The large variation in removal rates depends on the specific conditions of the treatment plants. Higher temperature can lead to faster removal due to enhanced microbial activity (Castiglioni 2006). Different redox conditions (i.e. aerobic, anaerobic and anoxic conditions) can affect the removal. For example, ibuprofen is removed faster in oxic conditions than in anoxic conditions (Zwiener 2002). Longer hydraulic retention times usually leads to higher removal rates for pharmaceuticals since it increases the amount of time that the pharmaceuticals are in contact with the micro-organisms (Tauxe-Wuersch 2005). Higher sludge retention times (corresponding to the age of the micro-organisms in the sludge) can increase the removal of pharmaceuticals, since longer retention times increases the micro-organisms ability to transform pharmaceuticals (Ternes 2004). The negative removal rates that are sometimes observed can be explained by the deconjugation of glucuronated or sulphated metabolites, or the hydrolysis of hydroxylated or carboxylated metabolites (Verlicchi 2012). Also the release of pharmaceuticals which have been sorbed to particles could account for the increase in concentration in the effluent water (Verlicchi 2012).

1.3.3. Sorbtion

The sorbtion of pharmaceuticals depends on the characteristics of the target particles, the structure of the pharmaceuticals and the pH of the water. For example, some pharmaceuticals contain aromatic structures which can adsorbed by intercalation with clay minerals (Kümmerer 2009). Sorbtion is considered to be an important removal pathway only for certain pharmaceuticals, such as tetracyclines (Kümmerer 2009) and some fluoroquinolone antibiotics (Verlicchi 2012). Hydrophilic pharmaceuticals such as ibuprofen and naproxen are not sorbed to sludge to any significant extent (Reif 2008). The sorbtion of ibuprofen to the common minerals kaolinite and goethite varies between 3 and 6 % depenting on pH (Behera 2012). The sorbtion of mefenamic acid, propranolol, salbutamol and paracetamol at environmentally relevant concentrations is below 5 % in both sludge and sediment (Jones 2005).

1.4. DIRECT OZONATION OF ORGANIC COMPOUNDS AND PHARMACEUTICALS

During advanced oxidation treatment, other transformation methods than biotransformation and phototransformation are available. In this thesis, only the transformations which take place during ozonation will be discussed.

1.4.1. Ozonation of carbon-carbon double bonds

Ozone molecules can react with carbon-carbon double bonds through the Criegee mechanism (Bailey 1978, von Sonntag 2012). The ozone first forms a five-membered ring with the carbons in the double bond. The fate of this intermediate depends on the solvent. In aprotic solvents, the ring will be rearranged into a trioxolane (Bailey 1978). In water, on the other hand, a stabilized intermediate is formed (Figure 1.4). The ring is then opened, leading to the formation of an aldehyde and an α-hydroxyalkyl hydroperoxide (Bailey 1982). The α-hydroxyalkyl hydroperoxide is unstable and can transform in two ways. It can either lose water to form a carboxylic acid or loose hydrogen peroxide to form an aldehyde (Leitzke 2003). Which product is formed depends on the pH. For example, the α-hydroxyalkyl hydroperoxide which is formed during the ozonation of vinylphosphoric acid will lose water at pH 10.2 (when the molecule is fully dissociated) and hydrogen peroxide at pH 7 (when the molecule is mono-protonated) (Leitzke 2003).

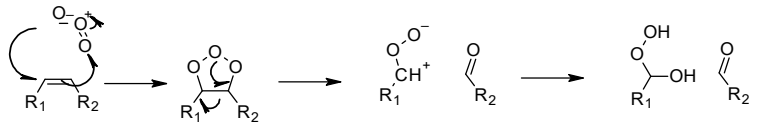


Figure 1.4. Formation of the stabilized Criegee intermediate in water.

Many pharmaceuticals contain a carbon-carbon double bond and are thus likely to react through direct ozonation. The antibiotic cephalexin contains an aromatic ring and a carbon-carbon double bond. The double bond reacts with ozone via the Criegee mechanism with the loss of hydrogen peroxide to form two ketone groups. The product is unstable and reacts further via the opening of the lactam ring. This product reacts with the primary amine to form a new six-membered ring. Over 30 % of cephalexin reacted through this pathway (Figure 1.5 (a), Dodd 2010).

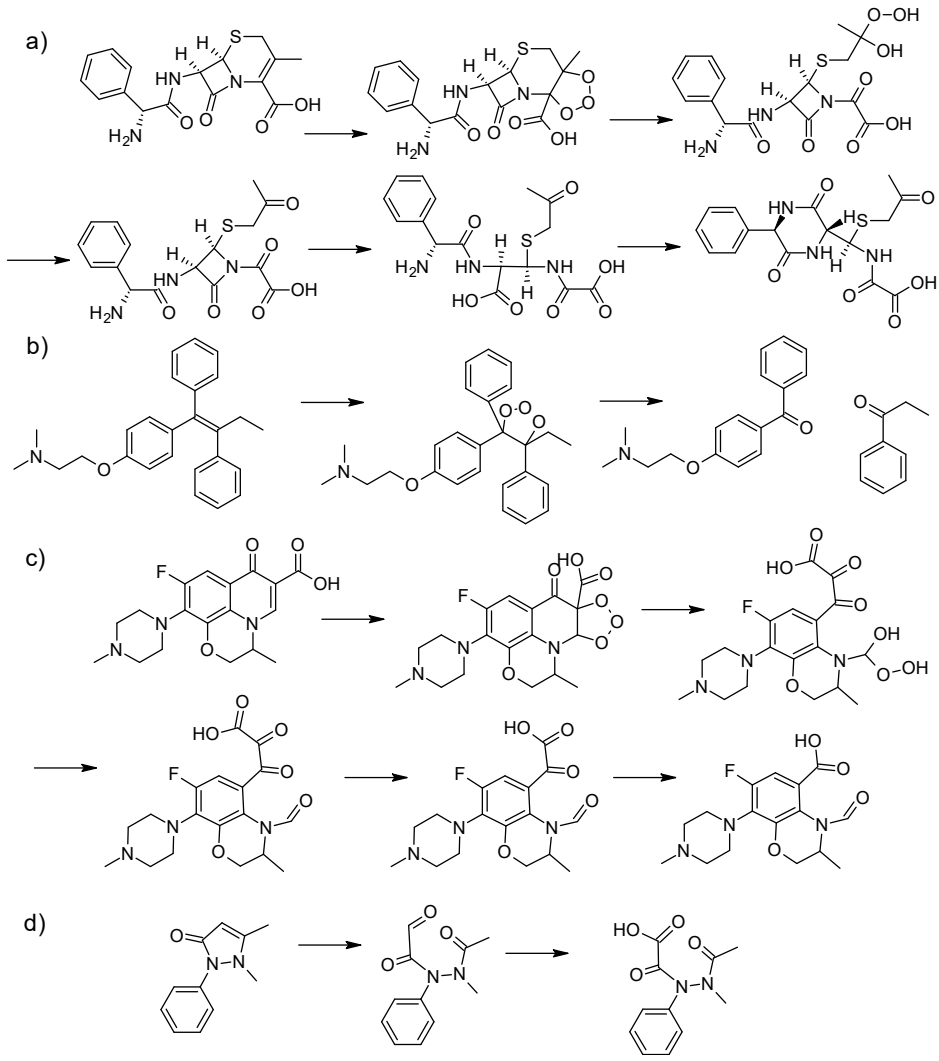


Figure 1.5. Ozonation of carbon-carbon double bonds in (a) cephalexin, (b) tamoxifen, (c) ofloxacin and (d) phenazone.

Tamoxifen, a drug used to treat breast cancer, contains three benzene rings and one carbon-carbon double bond. The double bond reacts with ozone to form an aldehyde and a ketone (Figure 1.5 (b)). The product was not quantified, however it was estimated that it was the main transformation product (Knoop 2018).

Ofloxacin is an antibiotic containing a quinolone ring. The carbon-carbon double bond in the hetero aromatic ring reacts with ozone to form an aldehyde and a ketone (Figure 1.5 (c)). The product is unstable and undergoes two subsequent decarboxylation reactions (Tay 2015).

Phenazone is a nonsteroidal anti-inflammatory drug containing a phenyl ring and a pyrazolone ring. The carbon-carbon double bond in the hetero aromatic ring reacts with ozone to form an aldehyde and a ketone (Figure 1.5 (d)). Also a product consisting of a ketone and a carboxylic acid is formed. This product could

be formed either via the oxidation of the primary product, or via the loss of H_2O from the α-hydroxyalkyl hydroperoxide (Favier 2015).

1.4.2. Ozonation of aromatic rings

Aromatic rings react only slowly with ozone, while the products are more susceptible to attack by ozone. The reaction is also not as selective as the reaction with carbon-carbon double bonds. The bonds in aromatic rings can react with ozone via the Criegee mechanism in the same way as carbon-carbon double bonds (Figure 1.6) leading to the breakage of the aromatic ring. Aromatic rings can also react with ozone in a different way, leading to the formation of a phenol (Figure 1.6, Bailey 1982).

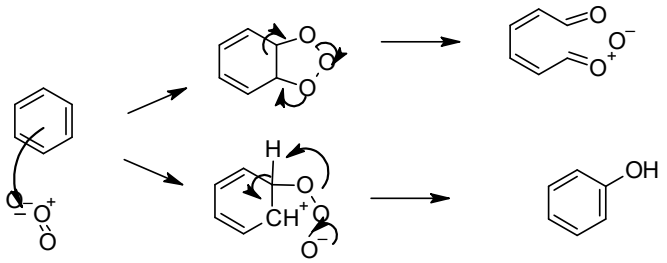


Figure 1.6. Direct ozonation of aromatic rings.

Bezafibrate is a lipid lowering agent containing two phenyl rings. During the ozonation of bezafibrate, one of the rings reacts with ozone, leading to breakage of the aromatic ring and the formation of an aldehyde and a ketone (Figure 1.7 (a), Sui 2016).

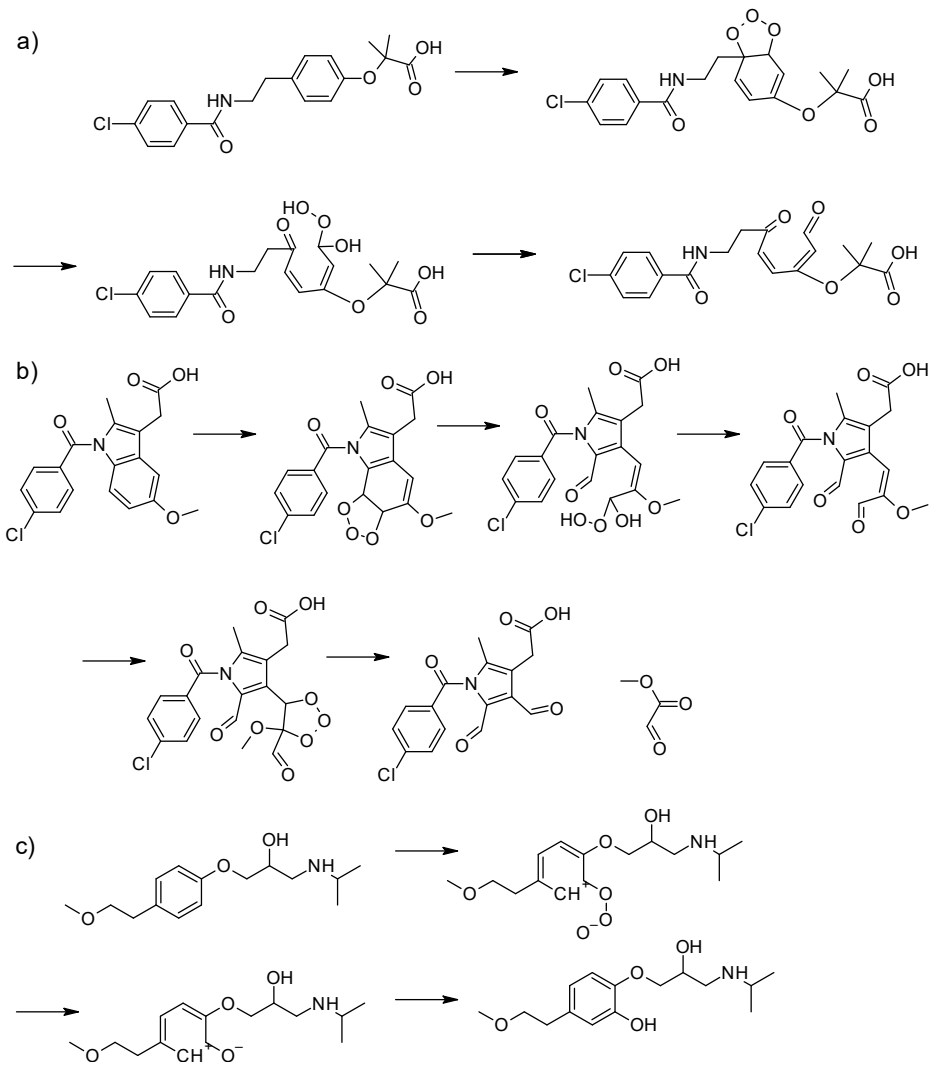


Figure 1.7. Ozonation of aromatic rings in (a) bezafibrate, (b) indometacine and (c) metoprolol.

The nonsteroidal anti-inflammatory drug indometacin contains a chlorobenzene ring and an indole ring. One of the carbon-carbon bonds in the indole ring reacts with ozone with the loss of hydrogen peroxide to form two aldehyde groups. The product which is formed contains a carbon-carbon double bond which in turn reacts with ozone, leading to the formation of an aldehyde and an ester (Figure 1.7 (b), Zhao 2017).

While the formation of phenol has been shown to result from the direct ozonation of aromatic compounds, it is also possible for phenols to be formed via the reaction of aromatic rings with hydroxyl radicals which are also present in water. It is therefore very difficult to determine if phenols are formed via direct or indirect ozonation. At pH 8, the formation of phenols from metoprolol is

thought to take place mainly via the reaction with hydroxyl radicals (Figure 1.7 (c), Benner 2009).

1.4.3. Ozonation of dihydroxybenzenes

While dihydroxybenzenes preferentially react via the Criegee mechanism, leading to ring cleavage, they can partially also be transformed into quinones. Dihydroxybenzenes initially react with ozone in a similar way as during the formation of phenol, however the intermediate structure loses water and oxygen to form quinone (Figure 1.8, Bailey 1982).

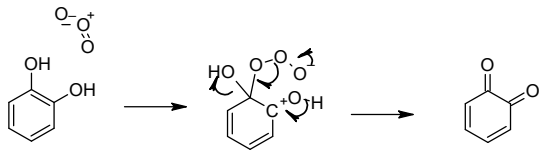


Figure 1.8. Ozonation of dihydroxybenzenes

Bisphenol A is an endocrine disrupting compound commonly used in the synthesis of plastics. It contains two phenolic rings. During ozonation, bisphenol A reacts via the Criegee mechanism, resulting in the breakage of the aromatic rings. It is also oxidized to a dihydroxybenzene, which subsequently forms a quinone (Figure 1.9 (a), Deborde 2008).

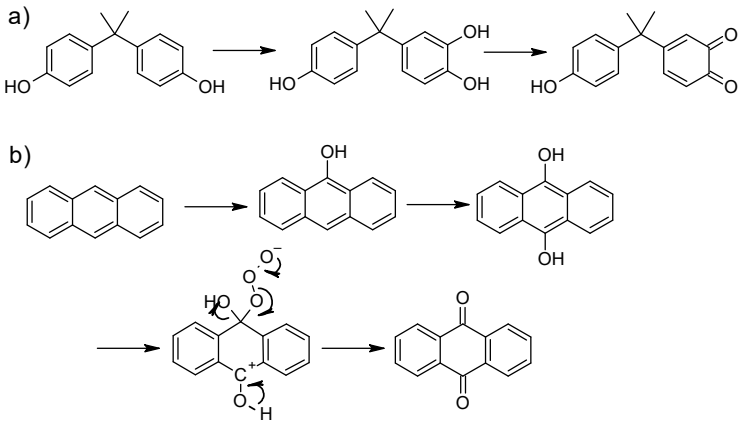


Figure 1.9. Ozonation of dihydroxybenzenes in (a) bisphenol A and (b) anthracene.

Anthracene consists of three fused benzene rings. Ozone attacks one of the carbons in the middle ring, leading to the formation of one alcohol group. Reaction with another ozone molecule leads to the formation of a diol. Reaction with a third ozone molecule leads to the formation of a dione (Figure 1.9 (b)). Anthracene can also react with ozone in a ring-cleaving reaction. This reaction does not take place via a Criegee mechanism. Instead, ozone forms a ring with the carbon atoms in positions 9 and 10 (Bailey 1964).

1.4.4. Ozonation of amines

Primary amines react with ozone to form amine oxides, hydroxylamines, nitroso groups and nitro groups. Amines can also loose ammonia with the simultaneous formation of an aldehyde. (Bailey 1982). Ozonation of amine also frequently leads to the cleavage of nitrogen-carbon bonds. This reaction has not been extensively studied, however, mechanisms involving hydroxyl radicals have been proposed (Figure 1.10 (a), Muñoz 2000). Also mechanisms involving radicals formed from ozone have been suggested (Figure 1.10 (b), von Gunten 2003, Lange 2006).

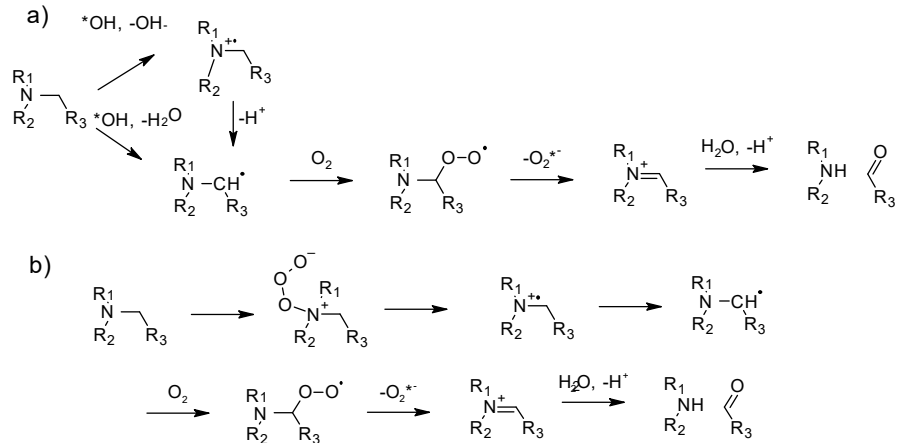


Figure 1.10. Mechanisms for the ozonation of amines proposed by (a) Muñoz 2000 and (b) von Gunten 2003.

While the formation of N-oxide is usually considered the predominant mechanism, there tends to be a small amount of dealkylated product formed. During the ozonation of N-alylated products, both trimethylamine and diethylamine were dealkylated. Etylamine lost ammonia. Due to the presence of hydroxyl radical scavengers, the reaction was though to take place via a radical reaction induced by ozone (Lim 2019).

For pharmaceuticals, the cleavage of carbon-nitrogen bonds during ozonation has been observed for clarithromycin (Lange 2006), levofloxacin (El Najjar 2013), ciprofloxacin (Dewitte 2008) and metoprolol (Benner 2009).

The ozonation of amines can also lead to the formation of N-oxides. These compounds. For example, the N-oxides of tiapride, tramadol and clarithromycin have been detected after ozonation (Merel 2017). Tramadol-N-oxide is biologically active and can be metabolized to re-form the original pharmaceutical (Wu 2002). It is also toxic to *Vibrio Fischeri* (Antonopoulou 2016).

1.4.5. Ozonation of imines

The direct ozonation of imine bonds has not been as extensively studied as the ozonation of carbon-carbon double bonds. Secondary aldimines react with ozone so that the ozone molecule attacks the carbon, leading to the formation of a ketone and an amide (Figure 1.11, Bailey 1982).

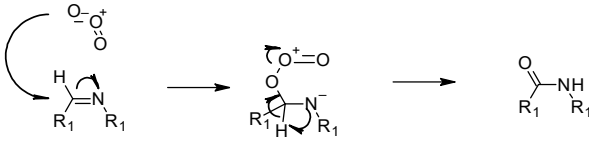


Figure 1.11. Ozonation of imines.

One example of this reaction is the ozonation of phenanthridine. The ozone molecule preferentially attacks the carbon-nitrogen bond, leading to the formation of an amine and a ketone (Figure 1.12). Ozonation of compounds containing a secondary ketimine (such as 1-Methylisoquinoline) did not lead to the formation of a similar product (Moriconi 1964).

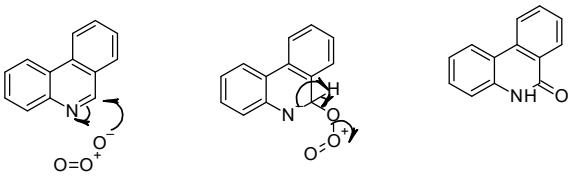


Figure 1.12. Ozonation of imine group in phenanthridine.

1.5. INDIRECT OZONATION OF PHARMACEUTICALS

In water, ozone is converted to hydroxyl radicals. The radicals are less selective than ozone and often react faster with organic molecules (Bailey 1982). The generation of hydroxyl radicals from ozone can be aided via the use of an alkaline pH, the addition of hydrogen peroxide or using ozone in combination with UV light (Gligorovski 2015). At low pH, the direct ozonation is dominant, whereas at high pH the indirect method is more important (Wang 2011). OH radicals mainly react with double bonds and aromatic compounds via addition reactions. With non-aromatic double bond, ·OH forms diols or saturated or unsaturated alcohols (Figure 1.13 (a)). With aromatic rings, a phenol is formed (Figure 1.13 (b)) (Gligorovski 2015).

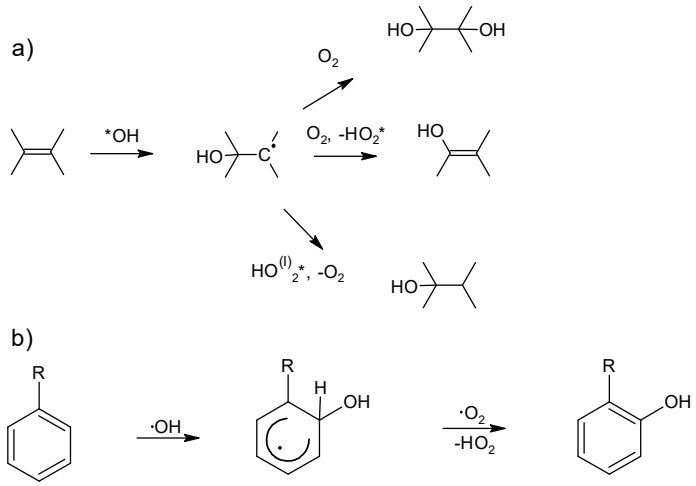


Figure 1.13. Indirect ozonation of (a) carbon-carbon double bonds and (b) aromatic rings.

When 2,6-dimethyl-aniline reacts with hydroxyl radicals, the amine is oxidized to a nitro group, the amine and/or nitro group is replaced by a hydroxyl group, hydroxyl groups are added, the dihydroxylated product is further oxidized to a quinone, and the ring was broken, leading to the formation of small carboxylic acids. No mechanisms were given for any of the reactions (Boonrattanakij 2009).

1.6. CATALYTIC OZONATION

The generation of hydroxyl radicals from ozone can be enhanced using catalysts. The catalysts can be homogenous or heterogeneous. In homogenous catalysis, metal ions present in the bulk solution react with ozone. The metal ions can also assist in the transformation of organic compounds via the formation of complexes. These complexes can then react with ozone (Wang 2011).

In heterogenous catalysis, the catalyst is solid. This means that the catalyst can be separated from the water and reused. However, the mechanism becomes more complex, involving several steps: transport of the reactant from the water to the catalyst surface, transporting the reactant to the interior of the catalyst, adsorption of the reactants onto the active sites, reaction to form adsorbed products, desorption of the products, transport of the products to the exterior of the catalyst and finally transport of the products to the bulk of the water. In heterogenous catalysis, the activity of the catalysts is related to the surface area. The surface area can be increased by using a catalyst support or a carrier. Catalyst supports also prevent the catalyst from deactivation by keeping the catalytic phase highly dispersed. By using a solid support for the catalyst, a homogenous catalyst can become heterogenous (Virkutyte 2010).

The most important properties of a catalyst are its surface area, porosity, particle size distribution, and particle density. During use, the catalyst might be deactivated by sintering, leading to a smaller surface area, leaching of the active component and poisoning (deposition of impurities on the catalyst surface). After deactivation, the catalyst can be regenerated or replaced (Virkutyte 2010).

For catalytic ozonation, the most common catalysts used are transition metals, metal oxides, activated carbon and supported metals (Virkutyte 2010). In heterogenous catalysis, solid metal oxides absorb ozone. The ozone is decomposed to surface-bound oxygen radicals and hydroxyl radicals. At low pH, the formation of surface bound oxygen, rather than free radicals, is thought to be the dominant reaction. Above pH 6, the formation of hydroxyl radicals is thought to be the dominant reaction. It is also possible that the adsorption of organic molecules, ozone or both to the metal surface can enhance the transformation of the organic molecules (Wang 2011). The limitations of catalytic ozonation are that catalyst recovery can be difficult and that there is still a lack of understanding of the catalytic ozonation mechanism (Miklos 2018).

1.7. MASS SPECTROMETRY

Mass spectrometry is a powerful tool used to analyze organic compounds based on their mass-to charge ratio. Mass spectrometry can be used for a broad range of applications, from the study the nature of chemical bonds, to the quantification of pesticides in food, the detection of explosives and the study of metabolites in the human body (Song 2018). A mass spectrometer consists of an ion source, a mass analyzer and a detector. Often, the mass spectrometer is coupled to a chromatography system (Gross 2011).

1.7.1. Ion sources

The purpose of the ion source is to create gas-phase ions (Ekman 2009, Gross 2011). The choice of ionization source depends on the purpose of the mass spectrometer. Mass spectrometers intended for the analysis of volatile, non-polar compounds usually use an electron ionization source. This type of mass spectrometer is usually coupled to a gas chromatography system. Mass spectrometers intended for the analysis of non-volatile, polar compounds usually use electro spray ionization. This type of mass spectrometer is often coupled to a liquid chromatography system. Other ionization sources, including chemical ionization, and inductively coupled plasma will not be discussed in this work.

Electron ionization
During electron impact ionization (EI), molecules in the gas phase are bombarded with electrons. This leads to the formation of positively charged radicals (Ekman 2009, Gross 2011). Electron impact ionization is a hard ionization method, meaning that the ionized molecules are very likely to fragment as a result of the ionization. EI is used in combination with gas chromatography to analyze small, volatile, nonpolar compounds. In the 1960s, GC-MS became the method of choice for the analysis of pharmaceuticals, replacing low resolution analysis methods such as infrared spectroscopy (IR) and liquid chromatography coupled to ultraviolet spectroscopy (LC-UV). GC-MS offers higher resolution than IR and LC-UV. Most pharmaceuticals, however, are polar and non-volatile, so extensive sample treatment, including derivatization, is needed (Prakash 2007). The advantage of GC-MS, compared to other MS methods, is that the mass spectra obtained with GC-MS are easily reproducible. This makes it possible to create libraries of the mass spectra of known compounds, facilitating the identification of unknown compounds.

Electrospray ionization
Molecules entering an electrospray ionization (ESI) chamber are in the liquid phase. Before entering the mass spectrometer, the compounds have to be ionized and the liquid has to be evaporated. The evaporation is accomplished with the help of heated gas. Usually nitrogen is used for this purpose. The application of

either a positive or negative charge to the needle aids in creating a large amount of positive or negative ions (Ekman 2009). When the liquid containing ions enter the spray chamber a so called Taylor cone will be formed. The tip of the cone will be enriched with either positive or negative ions, leading to the formation of charged droplets (Ekman 2009, Gross 2011). In the droplets there are ions with a certain charge. As the liquid is evaporated the ions will be forced close together until the repelling force between the ions is strong enough that the droplets disintegrate, leading to the formation of new, smaller droplets. This process continues until no liquid is left in the system. Electrospray ionization is a soft ionization method, meaning that it forms mostly intact pseudo-molecular ions (Ekman 2009, Gross 2011). Unlike EI, ESI can be combined with liquid chromatography (LC). ESI can also be used to analyze polar non-volatile compounds. In addition to simpler sample preparation, LC-MS is faster and offers higher resolution and greater sensitivity compared to GC-MS. Because of this, starting from the 1980s, GC-MS has largely been replaced by LC-MS in the pharmaceutical industry. (Prakash 2007).

1.7.2. Mass analyzers

The mass analyzer is used to separate ions based on their mass to charge ratio. The most commonly used mass analyzers are quadrupoles and time-of-flight analyzers. Also quadrupole ion-trap mass analyzers will be discussed in this thesis.

Quadrupoles
A quadrupole mass analyzer consists of four rods. A direct current potential is applied to two of the rods, while a radio frequency is applied to the two others (Ekman 2009, Gross 2011). The two pairs of rods have potentials with the same magnitude, but opposite sign. This makes it possible for the quadrupole to act as a mass filter, only letting ions with a certain mass-to-charge ratio through (Ekman 2009). By sequentially changing the field strength, it is possible to obtain a mass spectrum. The accuracy of the quadrupole is not very high, however the sensitivity is high (Ekman 2009). Quadrupoles can be attached in sequence in a so called triple quadrupole mass spectrometer (QqQ). These systems have first a quadrupole, followed by a reaction chamber, followed by a second quadrupole. In the reaction chamber, the ions can be made to fragment and in the second quadrupole the mass-to-charge ratio of the fragments can be detected (Gross 2011). Combination of several MS steps after each other is called tandem mass spectrometry, usually denoted MS^n. The QqQ is mainly used quantify organic molecules. In order to quantify organic molecules, the first quadrupole is used to select the pseudo-molecular ion (i.e. the $[M+H]^+$ or $[M-H]^-$ ion), the ion is fragmented in the second quadrupole and one or more of the major fragments is selected in the third quadrupole. This is called multiple-reaction-monitoring (MRM), and allows for a very high selectivity. While most other quantification methods, such as LC-UV, require complete chromatographic separation of all the

compounds in the sample, the selectivity of MRM makes it possible to quantify compounds even in complex matrices. In the early 21st century, triple quadrupole mass spectrometers were the most commonly used mass spectrometers in the pharmaceutical industry (Prakash 2007).

Time-of-flight mass analyzers

The separation of ions in a TOF analyzer is based on the differences in travel time for ions with different mass-to-charge ratios. At the start of the analysis, the ions are accelerated in such a way that all ions have the same kinetic energy, but the velocities will depend on the m/z values (Ekman 2009, Gross 2011). Ions with lower m/z ratio will travel faster than ions with higher m/z ratios (Ekman 2009, Gross 2011). The instrument has to be calibrated often using ions with known m/z values. A reflector is often applied. The reflector changes the path of the ions in order to increase the flight path and to compensate for the initial distribution of velocities (Ekman 2009, Gross 2011). The main advantage of TOF analyzers is the ability to obtain high resolution mass spectras (HRMS). With the help of HRMS the molecular formula of a compound can be determined with a high degree of certainty. This makes TOF analyzers useful for the identification of unknown compounds, such as metabolites (Prakash 2007).

Quadrupole ion trap analyzers

The quadrupole ion trap consists of a ring electrode and two end-cap electrodes. An RF potential is applied to the ring, while the end-caps are held at ground. Ions with specific m/z ratios can be trapped in the field depending on the level of the RF voltage. There is a collision gas in the trap in order to extract energy from the ions so that they stay in the trap. In order to produce a mass spectrum, ions with a range of m/z values are trapped. By changing the amplitude of the RF voltage, ions with increasing m/z values are able to leave the trap and reach the detector. This leads to the generation of a mass spectrum. It is also possible to trap only ions with a specific m/z ratio. These ions can then be fragmented and a mass spectrum of the generated ions can be produced. The process of ion isolation and fragmentation can be repeated multiple times, leading to the acquisition of MS^n spectra (Gross 2011). The possibility to obtain complex, multi-stage fragmentation patterns makes ion trap mass spectrometers useful for identifying unknown compounds, especially in combination with TOF instruments. Nowadays, ion trap mass spectrometers are usually used in combination with other analyzers such as TOF in hybrid instruments consisting of two different analyzers.

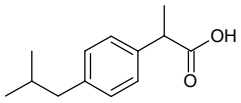
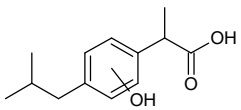
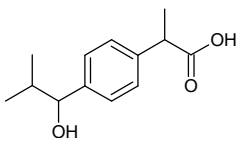
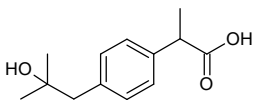
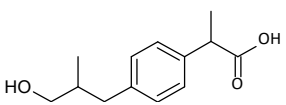
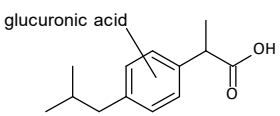
1.8. THE SELECTED PHARMACEUTICALS

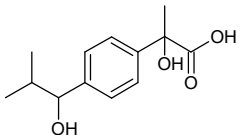
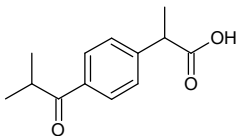
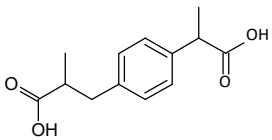
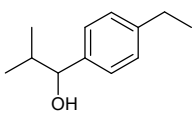
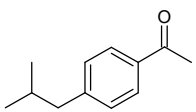
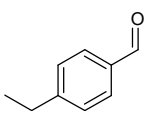
For this work, four different pharmaceuticals, ibuprofen (IBU), diclofenac (DCF), carbamazepine (CBZ) and sulfadiazine (SDZ) were studied. All the chosen pharmaceuticals are commonly detected in wastewater and in the environment. IBU was chosen as a starting compound to gain a basic understanding of the catalytic ozonation process. It is also of interest since it is the most commonly used pharmaceutical in Finland. DCF was chosen due to its toxic effect on wildlife. CBZ was chosen because it is difficult to remove from wastewater with conventional methods and it is very stable in the environment. SDZ was chosen because it is a commonly detected antibiotic which has not been extensively studied. Antibiotics are of special interest due to the potential of antibiotics in the environment contributing to the development of antibiotic resistance.

1.8.1. Ibuprofen

Ibuprofen (IBU) is a non-steroidal anti-inflammatory drug. It is the most used anti-inflammatory pharmaceutical in Finland (Suomen lääketilasto 2017). IBU is a chiral compound usually sold as a mixture of both enantiomers. IBU contains a carboxylic acid group and an aromatic ring. The structure of IBU and its major transformation products according to literature are presented in Table 1. In humans, the major phase I metabolites of IBU are carboxyibuprofen (CBX-IBU), 1-hydroxyibuprofen (1-OH-IBU), 2-hydroxyibuprofen (2-OH-IBU) and 3-hydroxyibuprofen (3-OH-IBU) (Hamman 1997, Kepp 1997). The major phase II metabolites are glucuronic acid conjugates of IBU and the phase I metabolites (Kepp 1997). Similar products were detected when IBU was transformed by fish, however in that case 1-OH-IBU and CBX-IBU were not detected (Brozinski 2013). During phototransformation, hydroxylated products were detected (Choina 2013, da Silva 2013). Also, products resulting from decarboxylation and the oxidation of OH groups to ketone groups were detected (Choina 2013, da Silva 2013, Iovino 2016). The products formed during advanced oxidation treatment processes are the same as those formed in phototransformation (Caviglioli 2002, Illés 2013, Li 2014). The transformation of IBU during ozonation treatment was studied by Huang (2015). The reaction mixture consisted of 5 mg/L IBU in ultrapure water and ozone (0.06 L/min) was added to the mixture. QTOF MS was used to detect six different products. IBU was transformed via the addition of OH groups, decarboxylation and oxidation of OH groups to ketone groups.

Table 1.1 IBU and commonly detected IBU products.

Name	Structure	Biotransformation	Phototransformation	AOPs
IBU				
OH-IBU			Choina 2013, da Silva 2013	Caviglioli 2002, **Huang 2015**[a], Illés 2013, Li 2014
1-OH-IBU		Kepp 1997		Caviglioli 2002
2-OH-IBU		Brozinski 2013, Kepp 1997, Hamman 1997		
3-OH-IBU		Brozinski 2013, Kepp 1997, Hamman 1997		
IBU-GLU		Brozinski 2013, Kepp 1997		

Name	Structure	Biotransformation	Phototransformation	AOPs
di-OH-IBU			da Silva 2013	Illés 2013, Li 2014
1-OXO-IBU			Choina 2013	Caviglioli 2002
CBX-IBU		Kepp 1997, Hamman 1997		
TP178			Choina 2013, Iovino 2016	Caviglioli 2002, **Huang 2015**[a], Illés 2013, Li 2014
TP176			Choina 2013, Iovino 2016	Caviglioli 2002, **Huang 2015**[a], Illés 2013, Li 2014
TP134			da Silva 2013	**Huang 2015**[a], Illés 2013, Li 2014

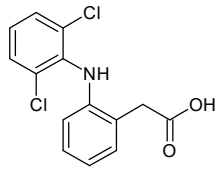
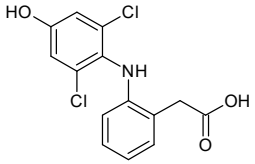
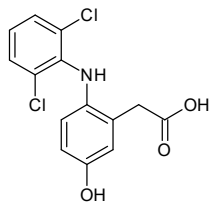
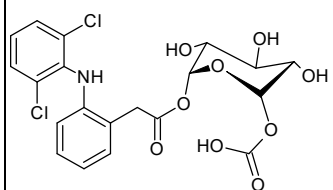
a. Ozonation products

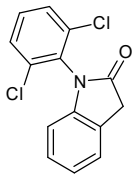
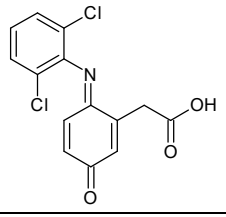
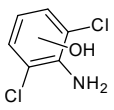
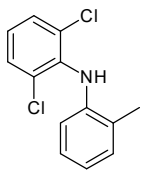
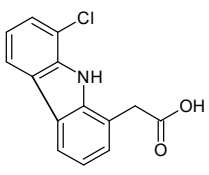
1.8.2. Diclofenac

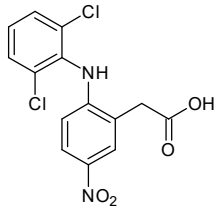
Diclofenac (DCF) is a non-steroidal anti-inflammatory drug. It is marketed as a prescription drug and as over-the-counter gels and sprays. DCF is of interest because it exhibits a high toxicity for example towards vultures (Oaks 2004). Because of this it has been added to the EU's first watch list, indicating its potential negative impact on the environment (European commission 2012). The structure of DCF contains two aromatic rings, a carboxylic acid group, an amine and two chlorine atoms. The structures of DCF and its major transformation products are presented in Table 2.

During human phase I metabolism, DCF is transformed via hydroxylation and via a ring-closing reaction to DCF-amide (Stierlin 1979). The hydroxylated products can also be further oxidized to quinone imines (den Braver 2016). During phase II metabolism, DCF and its metabolites are glucuronated (Stierlin 1979). During microbial transformation, in addition to the human metabolites, a product with an additional NO_2 groups was detected (Kosjek 2008). During phototransformation, the most commonly detected products are ring-closing products such as RC-DCF and decarboxylated products such as DC-DCF (Agüera 2005, Eriksson 2010, Görner 2010). Advanced oxidation of DCF leads to the same products as phase I metabolization, additionally, smaller products such as 177-DCF and DC-DCF are formed (Monteagudo 2018, Yu 2013, Zhao 2018, Hartmann 2008). The transformation of DCF during ozonation treatment was studied by Coelho (2009). The reaction mixture consisted of DCF (200 mg/L) in ultrapure water. Ozone (0.07 g/min) was generated by an ozonator. Samples were prepared by SPE and analyzed by TOF MS. The structures were determined based on exact mass and fragmentation pattern. The ozonation products included unspecified isomers of OH-DCF, QI-DCF, hydroxylated DCF-amide, 177-DCF and 2,6-dichloroaniline.

Table 1.2. DCF and commonly detected DCF transformation products.

Name	Structure	Biotransformation	Phototransformation	AOPs
DCF				
4´-OH-DCF		Stierlin 1979, den Braver 2016, Bouju 2016		**Coelho 2009[a,b],** Monteagudo 2018, Yu 2013
5-OH-DCF		Stierlin 1979, den Braver 2016		Monteagudo 2018, Yu 2013, Zhao 2018
DCF-GLU		Stierlin 1979		

Name	Structure	Biotransformation	Phototransformation	AOPs
DCF-amide		Stierlin 1979, Bouju 2016		Monteagudo 2018, Hartmann 2008
5-QI-DCF		den Braver 2016		**Coelho 2009[a,b]**, Monteagudo 2018, Yu 2013, Zhao 2018
177-DCF				**Coelho 2009[a]**, Monteagudo 2018, Yu 2013, Hartmann 2008
DC-DCF			Agüera 2005	Zhao 2018, Hartmann 2008
RC-DCF			Agüera 2005, Eriksson 2010, Görner 2010	

Name	Structure	Biotransformation	Phototransformation	AOPs
NO_2-DCF		Kosjek 2008		Monteagudo 2018, Hartmann 2008

a. Ozonation product
b. exact isomer was not determined

1.8.3. Carbamazepine

Carbamazepine (CBZ) is used to prevent and control seizures. It can also be used to relieve nerve pain and to treat schizophrenia and bipolar disorder. CBZ is of interest due to its high stability in nature (Björnelius 2018).

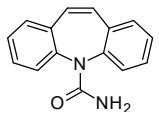
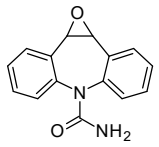
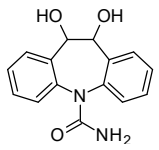
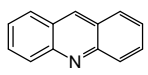
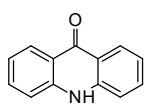
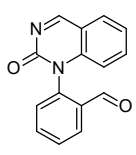
Metabolization of CBZ in humans leads to the formation of an epoxide, a dihydroxylated product, acridine and acridone. In bacteria and fungi, the same products were detected. During the transformation by white-rot fungi, 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD) and BaQD were also formed (Golan-Rozen 2015, Li 2013). It was suggested that these products were formed due to the presence of free radicals under oxidative conditions (Golan-Rozen 2015). During phototransformation and advanced oxidation treatment, the same products are formed as during biotransformation, however, more commonly, the rearrangement products BQM, BQD, BaQM and BaQD are detected (De Laurentiis 2012, Yang 2016, Liu 2012, Hu 2009, Azaïs 2017, Hübner 2014, McDowell 2005).

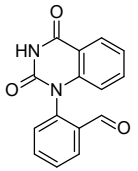
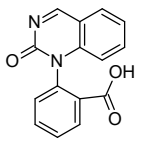
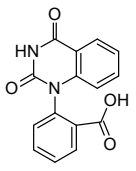
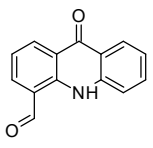
The transformation of CBZ during ozonation has been extensively studied. McDowell (2005) added ozone to a solution consisting of CBZ in pure water. They analyzed the products with GC-MS, LC-MS and NMR. They detected three products: BQM, BQD and BaQD. The structures of BQM and BQD were determined with NMR. The reaction rate for the formation and transformation of BQM and BQD were also determined.

Hübner (2014) used an ozonator do study the ozonation of CBZ in pure water. The structures of BQM, BaQM, BQD, BaQD and nine additional products were determined by TOF MS. In addition to the BQM pathway, CBZ was transformed via the addition of OH groups, the formation of an epoxy group and rearrangement products such as acridone and TP223. Additionally, The main products were quantified with QqQ MS. Between 50 and 60 % of CBZ was transformed into BQM.

Azaïs (2017) used an ozonator do study the ozonation of CBZ in phosphate buffer (pH 2). Products were identified with TOF MS. In addition to hydroxylated products, BQM, BaQM, BQD and BaQD, three products resulting from the breakage of the bond between nitrogen and the aromatic ring in BQM were detected.

Table 1.3. CBZ and commonly detected CBZ transformation products.

Name	Structure	Biotransformation	Phototransformation	AOPs
CBZ				
CBZ-EP		Breton 2005, Jiang 2019, Li 2013, Golan-Rozen 2015	De Laurentiis 2012, Yang 2016	**Hübner 2014**[a], Liu 2012
diOH-CBZ		Breton 2005, Jiang 2019, Golan-Rozen 2015	De Laurentiis 2012, Yang 2016	**Azaïs 2017**[a], Hu 2009, **Hübner 2014**[a]
Acridine		Jiang 2019, Li 2013, Golan-Rozen 2015	De Laurentiis 2012	
Acridone		Jiang 2019, Golan-Rozen 2015		Hu 2009, **Hübner 2014**[a]
BQM			Yang 2016	**Azaïs 2017**[a], **Hübner 2014**[a], Liu 2012, **McDowell 2005**[a]

Name	Structure	Biotransformation	Phototransformation	AOPs
BQD		Golan-Rozen 2015	De Laurentiis 2012, Yang 2016	**Azaïs 2017[a]**, **Hübner 2014[a]**, Liu 2012, **McDowell 2005[a]**
BaQM				**Azaïs 2017[a]**, Hu 2009, **Hübner 2014[a]**
BaQD		Golan-Rozen 2015		**Azaïs 2017[a]**, **Hübner 2014[a]**, **McDowell 2005[a]**
TP223		Li 2013	Yang 2016	Hu 2009, **Hübner 2014[a]**

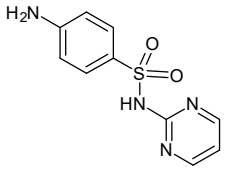
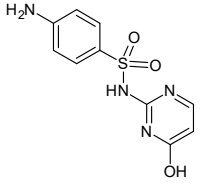
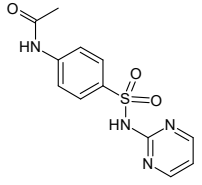
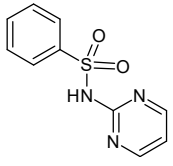
a. Ozonation products.

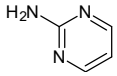
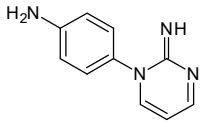
1.8.4. Sulfadiazine

Sulfadiazine (SDZ) is a sulfonamide antibiotic commonly used to treat urinary tract infections and toxoplasmosis. SDZ contains a 2-aminopyrimidine ring and an aniline ring connected by a sulfonyl group. It is important to study SDZ because of the potential for environmental bacteria to form resistance to the antibiotic. The structures of SDZ and its major transformation products are presented in Table 1.4.

SDZ is mainly metabolized into hydroxylated and acetylated products (Friis 1984, Lamshöft 2007). In bacteria, SDZ is also transformed into 2-aminopyrimidine (2-AP, Tappe 2013). During phototransformation, the main product is formed via rearrangement and the loss of the sulfonyl group (Periša 2013, Wang 2010). Also hydroxylated products and 2-AP are formed (Wang 2010). During advanced oxidation, the same rearrangement product is formed as during phototransformation (Neafsey 2010, Rong 2014). In addition, a product is formed via the loss of the amine group on the aniline ring (Rong 2014). The transformation of SDZ during ozonation had not previously been studied.

Table 1.4. SDZ and commonly detected SDZ transformation products.

Name	Structure	Biotransformation	Phototransformation	AOPs
SDZ				
OH-SDZ		Friis 1984, Lamshöft 2007	Wang 2010	Rong 2014
AC-SDZ		Friis 1984, Lamshöft 2007		
SDZ-P5				Rong 2014

Name	Structure	Biotransformation	Phototransformation	AOPs
2-AP		Tappe 2013	Wang 2010	Rong 2014
SDZ-P7			Periša 2013, Wang 2010	Neafsey 2010

1.9. SCOPE OF THE RESEARCH

The aim of the research was to study the transformation of selected pharmaceuticals during ozonation. The work was a collaboration with another PhD student who was studying the effect of catalysts on the transformation of pharmaceuticals. Because of this, the effect of catalysts on the transformation of pharmaceuticals will be discussed briefly in this thesis. A more extensive discussion of the catalysts can be found in papers IV, V and VI and in Saeid (2020).

For the identification of transformation products, liquid chromatography coupled to an ion trap mass spectrometer and a quadrupole time-of-flight mass spectrometer was used. For IBU, also gas chromatography coupled to mass spectrometry was used. The aim of the work was not only to tentatively identify products, whenever possible, the structures of the products were confirmed using commercially available reference samples. Some of the main reaction products were not commercially available. Instead, they were isolated from ozonated samples. The main products formed during the ozonation of CBZ was isolated using flash chromatography and the main product formed during ozonation of SDZ was isolated using semi-preparative HPLC. These products were studied with NMR in order to confirm the structures, and quantification methods were developed using LC-UV. The pharmaceuticals and their main transformation products were quantified using UV or multiple-reaction-monitoring methods.

In paper I, II and II, the transformation products formed during the ozonation of IBU and DCF, CBZ and SDZ, respectively, were identified and quantified. Papers IV, V and VI deal with the catalytic transformation of IBU, DCF and CBZ, respectively.

2. Methods

2.1. OZONATION

The catalytic and non-catalytic ozonation experiments were performed in a semi-batch double jacket glass reactor. Ozone was generated by an ozone generator (Absolute Ozone, Nano model, Edmonton, AB, Canada) from a feed gas containing a mixture of oxygen and nitrogen. The reaction temperatures in the experiments varied between 5°C and 50 °C. The reaction time was between 10 minutes and 3 hours. Stock solutions of the pharmaceuticals were prepared in ethanol (IBU and SDZ), water (DCF), methanol (CBZ) or acetonitrile (BQD). For the experiments, the pharmaceuticals were dissolved in 1L of de-ionized water to give a final concentration between 6mg/L and 30 mg/L.

2.2. QUANTIFICATION OF PHARMACEUTICALS

The pharmaceuticals were quantified using LC-UV (IBU, DCF and CBZ) or multiple-reaction-monitoring (MRM) (SDZ). Due to the high concentrations used in the experiments and the simple matrices IBU, DCF and CBZ could be analyzed using LC-UV. LC-UV was used whenever possible since no sample preparation was needed. SDZ could not be quantified using LC-UV because the SDZ chromatographic peak could not be separated from the products, so an MRM method was developed instead.

For the LC-UV analyses for DCF and CBZ, the chromatographic separation was performed using an Agilent 1100 binary pump equipped with a vacuum degasser, an autosampler, a thermostatted column oven set to 30 °C, a variable wavelength detector, and a Waters atlantis T3 C18 column (2.1 × 100 mm, 3 µm) with a pre-column made from the same material. The eluents were 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B). The flow rate was 0.3 ml/min and the injection volume was 30 µL. The detector was set to 254 nm. For quantification, a seven-point calibration curve was prepared in water by diluting the stock solution. The ozonated samples were injected without sample preparation. The gradients used are listed in table 2.1. For IBU, the same system was used, however an Ultra Techsphere ODS-5u C_{18}(4.6 × 250 mm, 53 µm) column was used. The eluent was 70:30 methanol: 0.5 % phosphoric acid (pH 1.8) with a flowrate of 1 mL/min. The injection volumne was 20 µL.

Table 2.1 the gradients used in the LC-UV methods

	% B	
Time	Diclofenac	Carbamazepine
0	30	0
1		0
2	30	
10	95	30
11	95	
11.1	30	
31	30	
24		95
25		0
35		0

For the LC-MRM analyses, an aliquot (450 µL) of the samples were transferred to clean vials and 50 µL of water containing 1 µg/mL of the internal standard sulfadiazine-D_4 was added. A 12-point calibration curve of concentrations between 0.5 and 5000 ng/mL of SDZ was prepared in water. The internal standard method was used for the quantification. An Agilent 6460 triple quadrupole mass spectrometer equipped with an Agilent Jet Spray electrospray ionization (ESI) source was used. SDZ was analyzed in positive ionization mode. The chromatographic separation was performed using an Agilent 1290 binary

pump equipped with a vacuum degasser, an autosampler, a thermostatted column oven set to 30 °C, and a Waters xbrigde C18 column (2.1 × 50 mm, 3 µm). The eluents were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The flow rate was 0.4 ml/min and the injection volume was 10 µL. The gradients used are presented in table 2.2.

Table 2.2. the gradients used in the LC-UV methods

	% B		
Time	Ibuprofen	Diclofenac	Sulfadiazine
0	5	10	5
0.5	5	10	5
3		30	
3.5	95		
4	95		
4.1	5		
4.5		95	
5			95
5.5	5	95	95
5.6		5	5
7		5	5

2.3. PRODUCT IDENTIFICATION WORKFLOW

Initially, the samples were analyzed using an ion trap mass spectrometer operating in auto MS^5 mode. The mass spectra for all the major peaks in the UV chromatogram were investigated. Since IBU was likely to transform into products which might be detected with GC-MS, but not LC-MS, IBU samples were also analyzed with GC-MS. The results were compared to previously published results. Published literature contained conflicting information concerning the fragments of some of the peaks, so they were tentatively identified based on the most likely fragmentation pattern. For previously unidentified products, the likely fragmentation patterns of potential products were determined and compared with the experimentally obtained results. Some of the tentatively identified products were available as reference samples. They were purchased and MRM methods were developed in order to confirm the structures of the products. Since some uncertainty remained concerning the identification of some major CBZ- and SDZ products, those products were isolated with either flash chromatography (CBZ) or semi-preparative LC (SDZ). NMR experiments were performed on the isolated products. Some products were further analyzed using QToF mass spectrometry in order to obtain high resolution mass spectra (HRMS). NMR, LC-MRM and HRMS could be used to conclusively identify three of the main products. Finally, high resolution mass spectrometry was performed in order to provide more certainty to the identification of the products.

2.4. PRELIMINARY STRUCTURE DETERMINATION

2.4.1. Ion trap MS

For preliminary structure determination, liquid chromatography coupled to ion trap mass spectrometry (LC-IT-MS) was used to obtain fragmentation patterns of the ozonation products. An Agilent 1100 LC/MSD ion trap mass spectrometer equipped with an electrospray ionization (ESI) source was used in full scan and MS^n scan modes was used. The instrument was operated in both positive and negative modes. Nitrogen was used as drying gas and argon was used as collision gas. Drying gas was held at 8 L/min and heated to 350 °C. The nebulizer pressure was set to 40 psi. The scan range was set to 50 - 600 m/z. The chromatographic method was the same as for the LC-UV method.

2.4.2. HRMS

DCF, CBZ and SDZ samples were analyzed with high resolution mass spectrometry in order to obtain the molecular formula of the ozonation products. High resolution mass spectra (HRMS) were obtained using a Bruker Daltonics micrOTOF quadrupole and time-of-flight mass spectrometer equipped with an electrospray ionization (ESI) source. The instrument was operated in full scan mode. Argon was used as drying gas and collision gas. The chromatographic separation was performed using an Agilent 1200 binary pump equipped with a vacuum degasser, an autosampler, a thermostatted column oven and a diode array detector. The column and chromatographic method were the same as for the LC-UV method. The mass spectrometer was operated in full scan mode.

2.4.3. GC-MS

IBU samples were further analyzed with GC-MS. An aliquot (10 ml) of samples taken after ozonation were freeze-dried, reconstituted with pyridine, silylated with HMDS and ClTMS and filtered through a 0,2 μm PTFE filter. The samples were analyzed the use of an Agilent Technologies 7890A gas chromatograph with a 5875C Series inert XL EI/CI MSD Triple-Axis Detector (GC–MS). One microliter samples were injected (splitless) using an injector 7683B Series (Agilent Technology). For the separation, a HP-5 MS column was used with helium as the carrier gas at the flow rate of 1 mL min^{-1}.

2.5. ISOLATION AND IDENTFICATION OF MAIN PRODUCTS

The main product formed during the ozonation of SDZ was isolated using semi-preparative LC. Semi-preparative LC was chosen due to the low concentration of the products in the samples. An aliquot of the ozonated sample (250 mL) was freeze dried and reconstituted in 1,5 mL of Milli-Q water. For product isolation, an Agilent 1100 LC equipped with an analytical scale fraction collector was used. The chromatographic separation was performed using an Agilent 1100 quaternary pump equipped with a vacuum degasser, an autosampler, a thermostatted column oven set to 30 °C, a Thermo Hypersil-Keystone BDS C18 column (100 × 500 mm, 5 μm) and a diode array detector monitoring 254 nm. Pure fractions were combined and freeze dried. The residue was dissolved in 850 μL deuterium oxide and transferred to an NMR tube. The samples were analyzed using a 500 MHz Bruker AVANCE-III NMR-system.

The main product formed during the ozonation of CBZ was isolated using an automated flash chromatography system. Flash chromatography was used since relatively high concentrations of the products in the ozonated samples. The main products, BQM and BQD and TP 225 were synthesized by dissolving 100 mg of CBZ in 1 L of water and ozonating the sample for 30 min. subsequently the sample was evaporated in a rotavapor. After evaporation, the sample was dissolved in chloroform and 200 mg Celite was added. The sample was evaporated and transferred to a Redi Sep Rf teledyre Isco cartridge. Chromatographic separation was performed using automated flash system (CombiFlash EZ prep, Teledyne ISCO) equipped with a silica column using ethyl acetate: petrol ether as the eluent system (gradient program). After separation, the fractions were evaporated and analyzed using a 500 MHz Bruker AVANCE-III NMR-system.

2.6. PRODUCT QUANTIFICATION

The commercially available products for IBU, DCF and SDZ were quantified using LC-MRM. While IBU and DCF could be quantified using LC-UV, MS was required to quantify the products since the concentrations of the products were much lower than the concentration of the parent compound. An attempt was made to develop one MS method for the quantification of both parent compounds and products, but due to the differences in concentration that was not possible. The commercially available SDZ product 2-aminopyridine (2-AP), could be included in the same method as SDZ, so they were quantified simultaneously. For IBU, the eluents were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Initially, formic acid was tested as eluent for the DCF products, but the two isomers of OH-DCF could not be chromatographically separated with formic acid, so 10 mM ammonium formate in water (A) and acetonitrile (B) were used instead. The internal standard method was used for quantification. The quantification was performed using a ten-point calibration curve prepared in water. Internal standard (100 ng/mL) was added to the calibration samples. The ozonated samples were prepared by adding 50 µL of internal standard solution (1000 ng/mL) to 450 µL of the sample. The transition used to quantify both 4'-OH-DCF and 5-OH-DCF was the same (m/z 310 -> 266), but the isomers were distinguished by their different retention times. The qualitative transitions were different for the isomers. The presence of the hydroxylated internal standard of the 4'-OH-DCF isomer further helped with distinguishing between the isomers.

The CBZ products BQM and BQD and SDZ product SDZ-P15 were quantified using isolated sampless. Since no internal standards were available, LC-UV was chosen for the quantification instead of LC-MS. The purified SDZ-P15 was dissolved in 800 µL deuterium oxide and 2 mg maleic acid dissolved in 50 µL deuterium oxide was added as an internal standard. A quantitative NMR experiment was run to determine the ratio of product to internal standard. Subsequently a calibration curve of SZD-P15 was prepared by dissolving the sample in water. Six-point calibration curves were prepared in water by diluting the standards. The same LC method was used for structure determination and quantification.

3. Results

The removal of pharmaceuticals from wastewater through non-catalytic and catalytic ozonation was investigated by measuring the concentrations of the selected pharmaceuticals at certain timepoints with either LC-UV or LC-MS. This thesis focuses mainly on the formation of transformation products. Detailed descriptions of the catalysts and the catalytic transformation of the pharmaceuticals can be found in papers IV-VI and in Saeid (2020).

The preliminary structure determination of pharmaceutical transformation products was performed using LC-IT-MS (all pharmaceuticals), GC-MS (IBU) and LC-Q-ToF-MS (DCF, CBZ, SDZ). Further, the exact structures of some major products were determined by comparison with reference samples (IBU, DCF and SDZ), synthesized samples (DCF) and products isolated from ozonated samples (CBZ and SDZ).

The products for which reference samples were available could be quantified. Quantification methods were developed using either LC-QqQ-MS, when internal standards were available (IBU and DCF), or LC-UV when no internal standards were available (CBZ and SDZ).

3.1. IBUPROFEN

3.1.1. Transformation of IBU (paper IV)

For the catalytic ozonation of IBU, different Cu-H-Beta catalysts were studied. Cu-H-Beta catalysts were chosen because they had previously been shown to be effective at transforming IBU (Saeid 2018). During non-catalytic ozonation, IBU could no longer be detected after 120 minutes of ozonation (Figure 3.1). The transformation was faster when a Cu-H-Beta catalyst was used. There was no significant difference between the different catalysts, however when the catalysts Cu-H-Beta-150-DP and Cu-H-Beta-EIM were used, IBU could no longer be detected after 30 minutes, while approximately 5 % of IBU was left after 30 minutes when the other catalysts were used. Cu-H-Beta-150-DP and Cu-H-Beta-150-EIM were similar catalysts, the only difference between them was the preparation method.

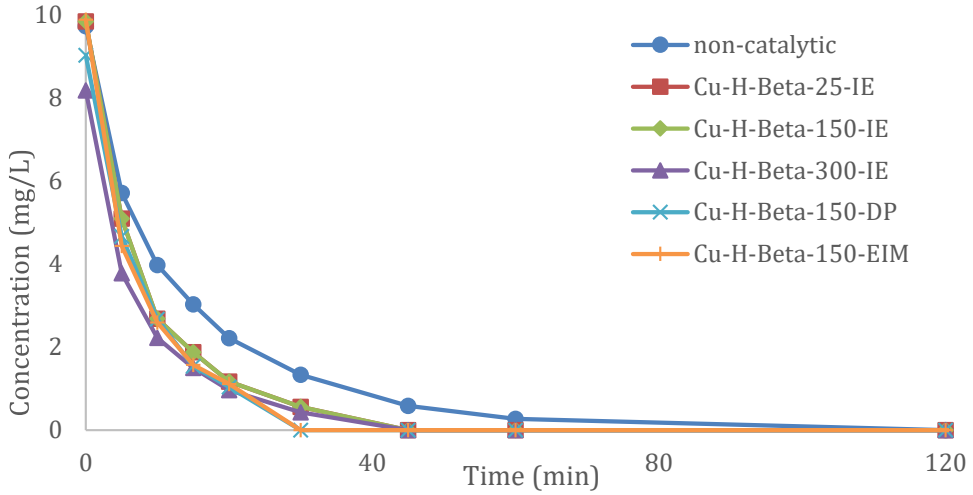


Figure 3.1. Transformation of IBU during catalytic and non-catalytic ozonation.

3.1.2. Identification of ozonation products (paper I)

The transformation of IBU during ozonation treatment has previously been studied. The most commonly detected products are presented in chapter 1.8.1. Of the products detected in this study, only TP192 had not previously been detected. Previous studies had not confirmed the structures of the products with reference samples, except the 1-OH-IBU isomer (Caviglioli 2002). In this study, reference samples were used to confirm the structure of 1-OH-IBU, two additional OH-IBU isomers and three other products.

The IBU ozonation products were determined using LC-IT-MS and GC-MS. The most common transformation of IBU was the addition of one or more hydroxyl groups. By comparison with reference samples, the positions of the hydroxyl groups for the three major isomers of singly hydroxylated IBU could be determined. The addition of hydroxyl groups took place mainly on aliphatic positions. The structure of one isomer of hydroxylated IBU was not ascertained, so the position of its hydroxyl group is unknown. Also a product containing two hydroxyl groups was detected. The positions of the hydroxyl-group were unknown, however it is likely that the 1- and α-positions are hydroxylated since those are the major products containing one hydroxyl group. After hydroxylation it was possible for the OH group in 1-OH-IBU (Figure 3.2) to be further oxidized to a ketone group. A reference sample was used to determine the structure of this product.

Additional products were formed via hydroxylation reactions, via the oxidation of hydroxyl groups to ketone groups and via decarboxylation reactions. Of these products, four were identified using LC-IT-MS (TP166, TP178, TP134 and TP176, Figure 3.2) and two were identified using GC-MS (TP190 and TP192, Figure 3.5). The exact structures of TP190 and TP192 could in this study be determined by comparison with reference samples. An additional isomer of TP192 could also be detected.

Based on our results, the ozonation pathway of IBU could not be ascertained. This was because many pathways could potentially lead to the formation of the observed products. For example, TP190 could be formed either from TP 178 of 1-OH-IBU. One potential pathway is presented in Figure 3.2.

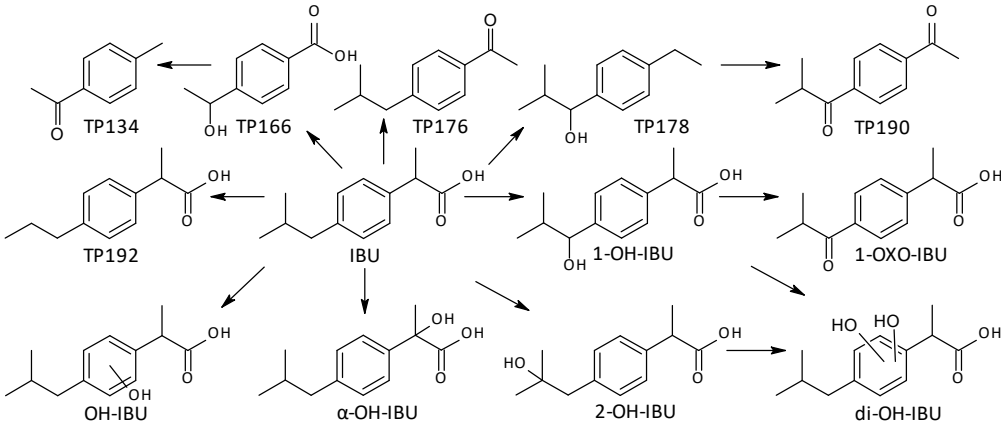


Figure 3.2. Suggested transformation pathway for Ibuprofen

3.1.3. Quantification of transformation products (paper I and IV)

An LC-QqQ-MS method was developed for the IBU transformation products α-OH-IBU, 2-OH-IBU, 1-OH-IBU, 1-OXO-IBU, TP190 and TP192 using reference samples. An internal standard (2-OH-IBU-d6) was also added to the method. All products could be detected in ozonated samples, however the concentrations of α-OH-IBU and TP192 were too low for reliable quantification. The main product formed during the ozonation of IBU was 1-OXO-IBU. The maximum concentration of 1-OXO-IBU appeared after 60 minutes, after which the concentration started to decrease (Figure 3.3). During non-catalytic ozonation, the two main isomers of OH-IBU which were formed: 1-OH-IBU and 2-OH-IBU, were formed simultaneously, however 1-OH-IBU was transformed faster than 2-OH-IBU (Figure 3.3). The concentration of TP190 increased to a maximum in 45 minutes, after which the concentration slowly started to decrease (Figure 3.3). The maximum concentration of 1-OXO-IBU accounted for only 3 % of the initial concentration of IBU. The maximum concentration of 1-OBO-IBU, 1-OH-IBU, 2-OH-IBU and TP190 accounted for 5 %, 0.5 %, 0.4 % and 0.2 % of the initial concentration of IBU, respectively. It was likely that a significant amount of IBU was transformed, e.g. via decarboxylation, to smaller, more polar compounds.

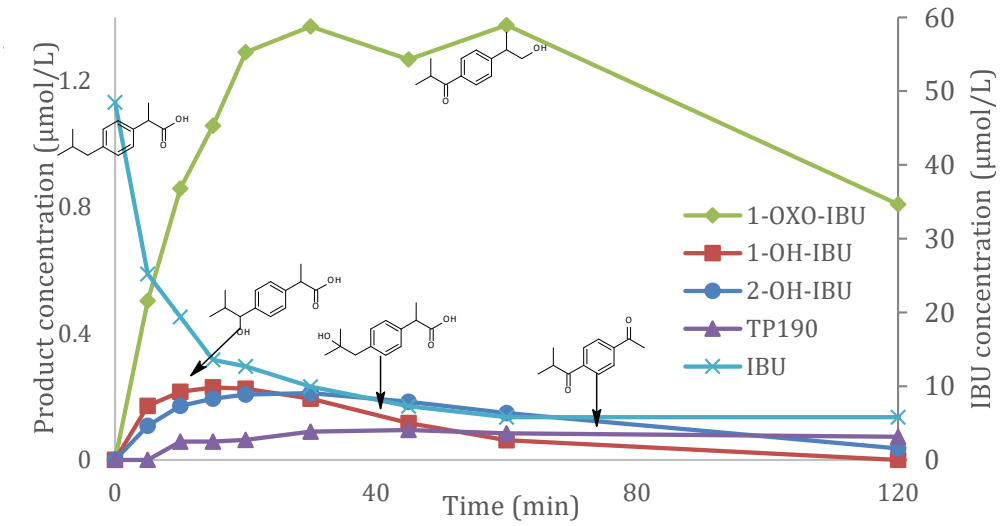


Figure 3.3. The ozonation of IBU and the transformation of the main products. Note the different scale for IBU.

In order to investigate the effect of catalysts on the formation and transformation of the main IBU ozonation products, different catalysts were acquired. The acquisition of the catalysts was described in paper IV. Most metal-catalyzed experiments lead to the formation of a lower maximum concentration of 1-OXO-IBU (Figure 3.4 (a)) and a lower final concentration of 1-OXO-IBU (at 180 minutes of ozonation time) compared to non-catalytic experiments. The exception was Fe-SiO_2-DP, for which both the maximum concentration and the final concentration were higher than during non-catalytic experiments. The lowest maximum concentration and the fastest transformation of 1-OXO-IBU could be detected when Cu-H-Beta-150-DP was used as a catalyst. Similar results were observed for 1-OH-IBU (Figure 3.4 (b)) and 2-OH-IBU (Figure 3.4 (c)). The highest concentration of TP190 was formed when Cu-H-Beta-150-DP was used as a catalyst, while the product could not be detected at all when Ni-H-Beta-150-DP was used as a catalyst (Figure 3.4 (d)).

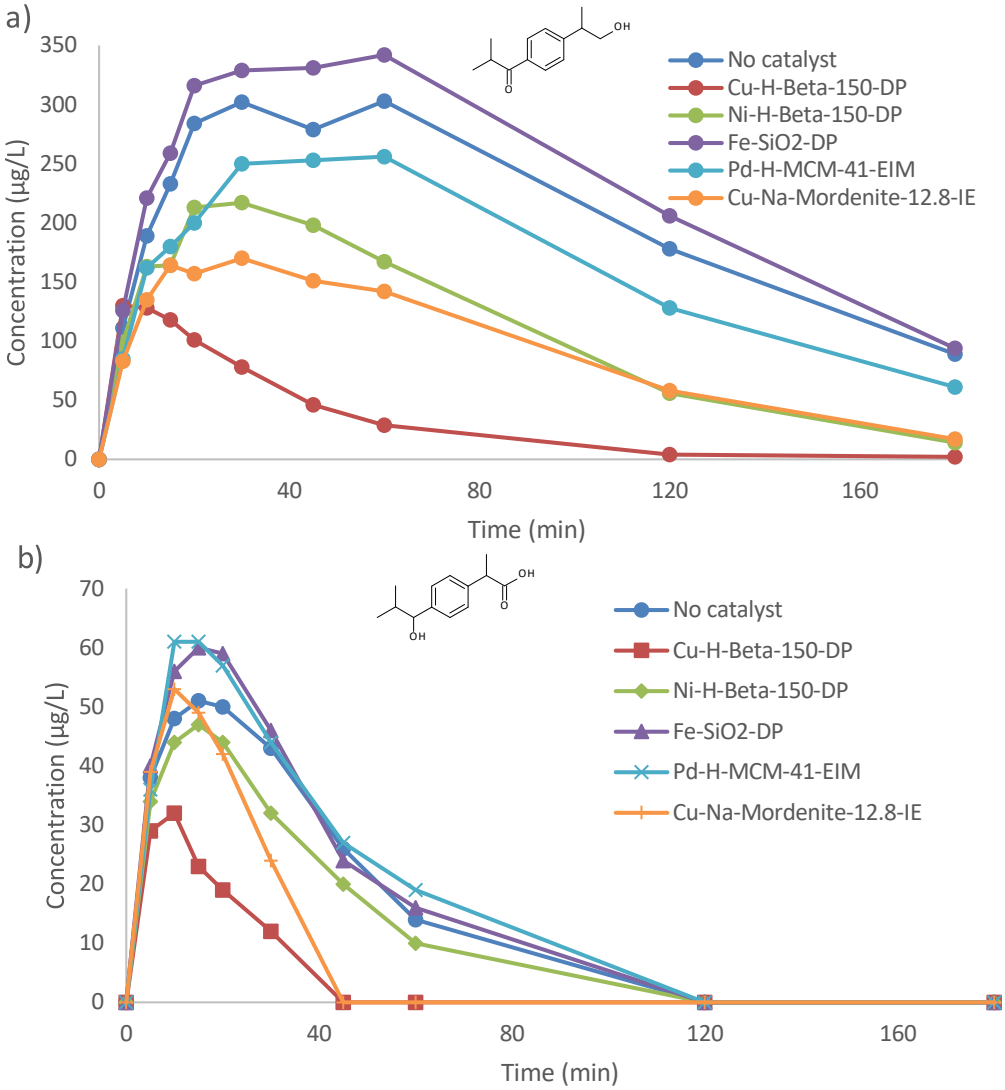


Figure 3.4. Concentrations of (a) 1-OXO-IBU (b) 1-OH-IBU in ozonation experiments catalyzed by different metals.

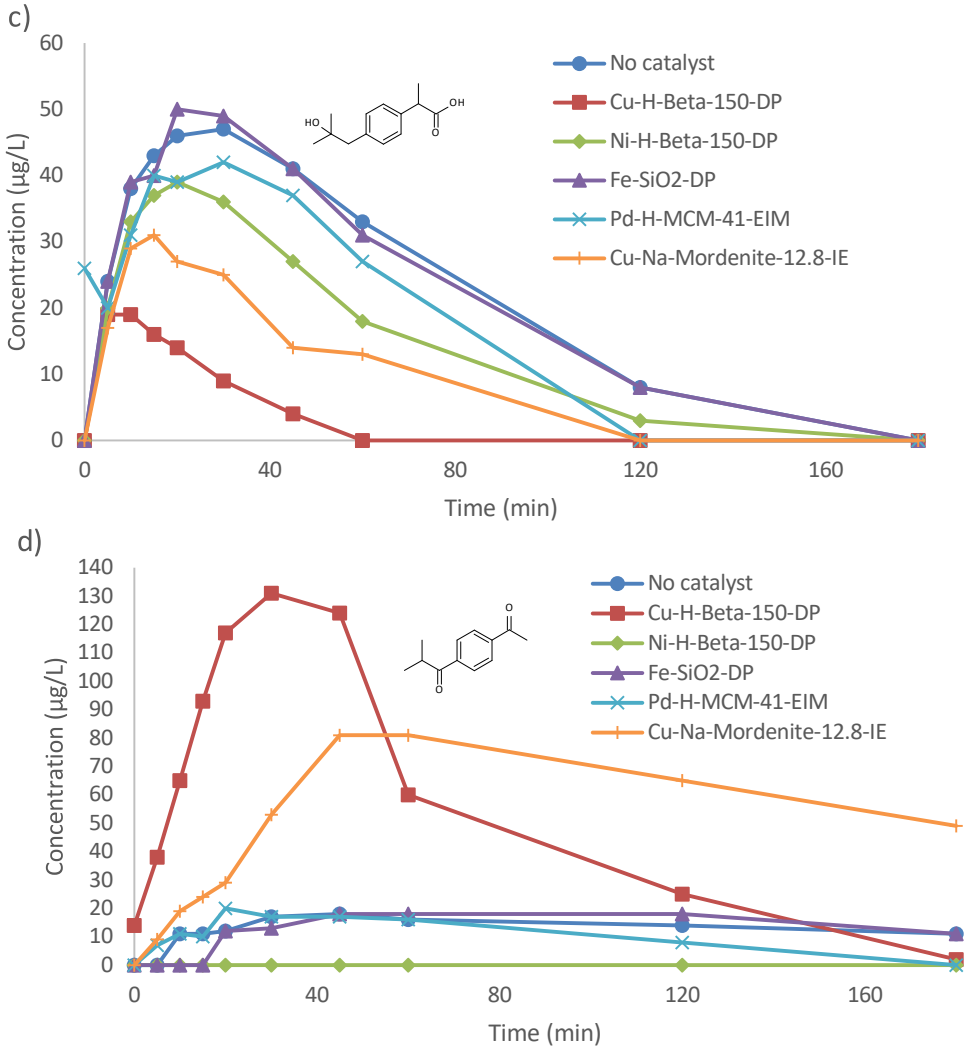


Figure 3.4. Concentrations of (c) 2-OH-IBU and (d) TP190 in ozonation experiments catalyzed by different metals.

Cupper was the most effective catalyst for the ozonation of IBU. The effect of different cupper-catalysts on the formation and transformation of IBU ozonation products was further investigating by synthesizing different cupper-catalysts. The synthesis of the catalysts was described in paper IV. When no catalyst was used, the maximum concentration of 1-OXO-IBU was 300 µg/L, while the maximum concentration of 1-OXO-IBU in the presence of a cupper-catalyst was 170 µg/L (Figure 3.14. (a))). The silicone/aluminum ratio did not affect the formation or transformation of 1-OXO-IBU, however using catalysts prepared via a deposition-precipitation method (Cu-H-Beta-150-DP) lead to more efficient transformation of 1-OXO-IBU compared to using catalysts prepared with ion-exchange methods (e.g. Cu-H-Beta-150-DP). The formation and transformation

of 1-OH-IBU and 2-OH-IBU follow a similar pattern to 1-OXO-IBU (Figure 3.5.(c)). The catalyst which most effectively transformed the IBU transformation products was Cu-H-Beta-150-DP. The pattern of formation and transformation of TP190 was inverse to that of the other products: when Cu-H-Beta-150-DP was used as a catalyst, the concentration of 1-OXO-IBU was the lowest, but the concentration of TP190 was the highest and inversely: during non-catalytic ozonation the concentration of 1-OXO-IBU was the highest and the concentration of TP190 was the lowest (Figure 3.5 (d)). The efficiency of transformation of the IBU transformation products in cupper-catalyzed experiments was probably because catalysts enhance the transformation of ozone into hydroxyl radicals which then react unselectively with IBU and its products.

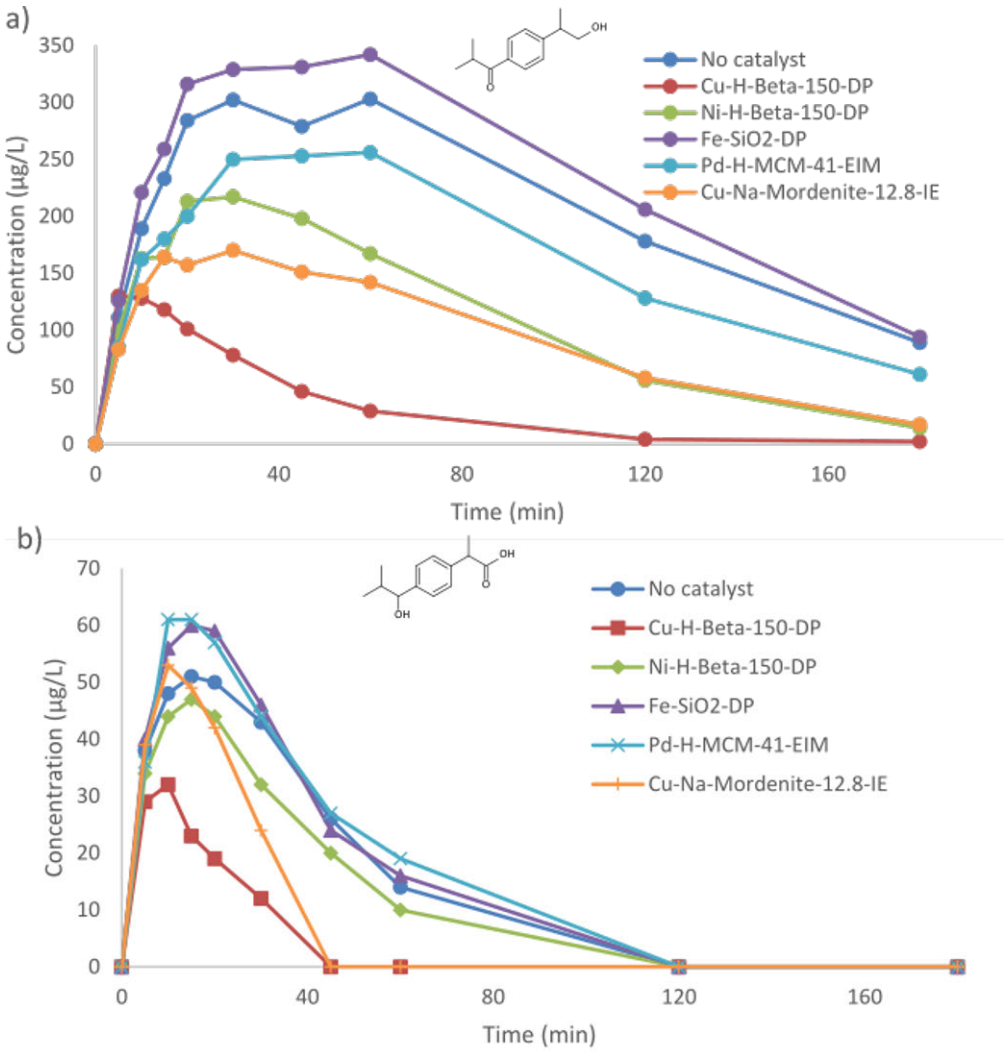


Figure 3.5. Concentrations of (a)1-OXO-IBU and (b) 1-OH-IBU in cupper catalyzed ozonation experiments.

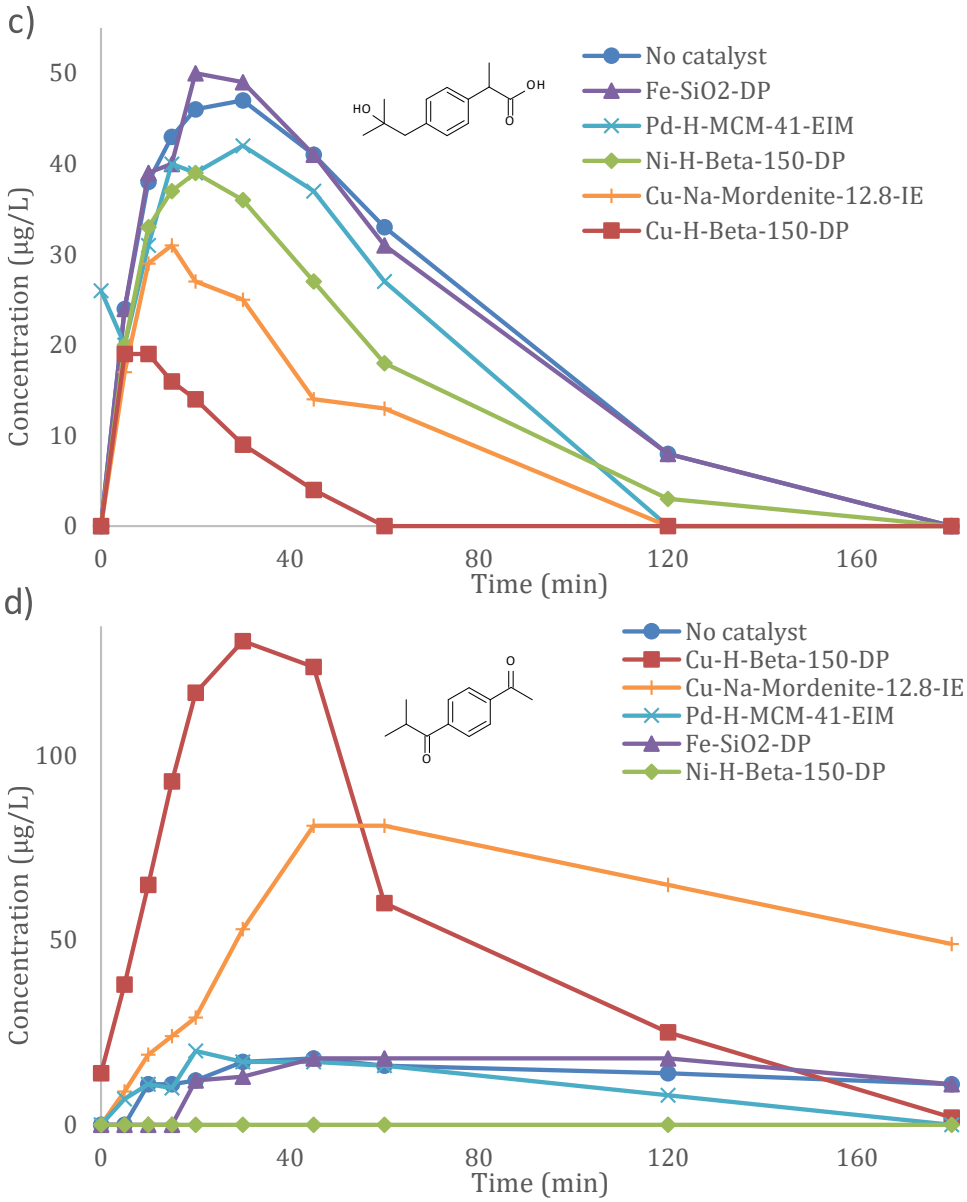


Figure 3.5. Concentrations of (c) 2-OH-IBU and (d) TP190 in cupper catalyzed ozonation experiments.

3.2. DICLOFENAC

3.2.1. Transformation of DCF (paper IV)

For DCF, a Pt-MCM was chosen because it enhanced the transformation of DCF. Initially, different weight percentages were studied to find the optimal catalyst composition. The platinum modified catalysts were compared to an unmodified H-MCM catalyst. The transformation of DCF was slowest when no catalyst was used (Figure 3.6 (a)). The transformation was fastest when 2 %wt Pt-MCM-22-100 was used. The optimal amount of catalyst to use was studied by adding 0.25 g, 0.5 g and 1 g of the 2 %wt Pt-MCM-22-100 catalyst to the reaction. Higher amounts of the catalyst led to faster transformation of DCF (Figure 3.6 (b)), but after 10 minutes, the lowest concentration of DCF was observed when 0.5 g of the catalyst was used. This indicated that the optimal amount of catalyst to use was 0.5 g.

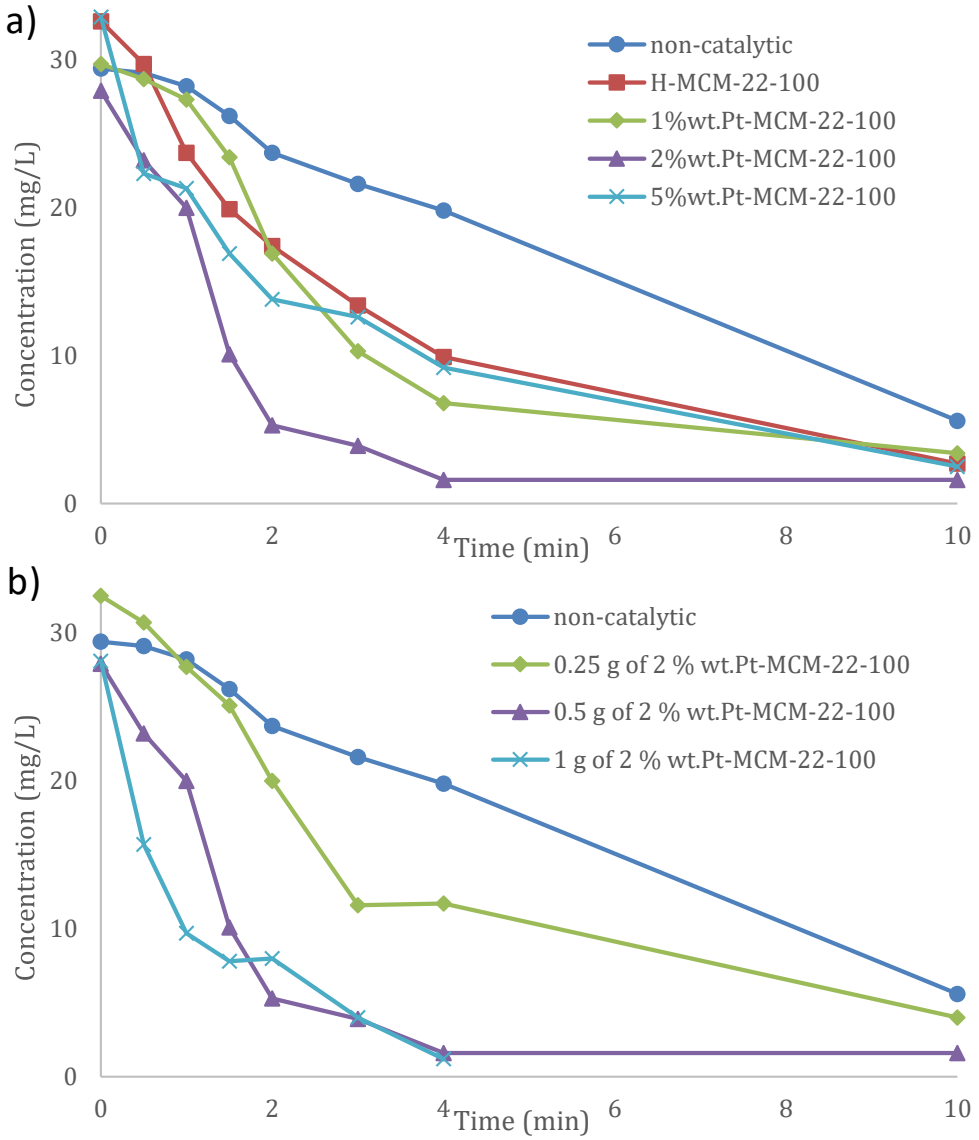


Figure 3.6. Transformation of DCF during ozonation with (a) different %wt of the Pt-MCM catalyst and (b) different amounts of the 2 %wt Pt-MCM catalyst.

3.2.2. Identification of ozonation products (paper I)

The DCF ozonation products were determined using LC-IT-MS and LC-Q-ToF-MS. The main DCF transformation reaction was the addition of one or more hydroxyl groups. The exact structures of two of the singly hydroxylated products could be determined using reference samples. Two additional hydroxylated isomers were detected for which no reference samples were available. The hydroxyl groups were mainly added to aromatic positions. One product containing two hydroxyl groups could be detected.

After the addition of hydroxyl groups, the product could further be oxidized to quinone-imines. In this study, one quinone imine product formed from OH-DCF (QI-DCF, Figure 3.7) and one quinone imine product formed from DCF with two additional OH groups (TP326) could be detected. The structure determination was based on LC-IT-MS and the position of the quinone imine moieties could not be determined. TP326 had not previously been detected.

DCF could undergo decarboxylation leading to the formation of TP282. The molecular formula of TP282 was determined with HRMS (error 1 ppm). This product could then be oxidized via the addition of an OH group to TP298 and further to the quinone imine TP296. The structure of TP298 and TP296 were determined with LC-IT-MS. TP296 and TP298 had not previously been detected.

The molecular formula of TP302 was determined with HRMS (error 3 ppm). TP302 was formed from DCF via the loss of CH_2COOH and the addition of two OH groups. During ozonation, the bond between nitrogen and one of the aromatic rings could break leading to the formation of TP178. The structure of TP178 was tentatively determined with LC-IT-MS. TP302 had not previously been detected.

The isotope ratio of TP328b indicated that both chlorine atoms were still present and the retention time (3.9 min) was significantly shorter than the retention time of DCF (20 min), indicating a loss of aromaticity. One likely structure for this product would be formed via the direct ozonation of one of the aromatic rings (Figure 3.6). It was not clear which ring was cleaved. TP328b had not previously been detected.

One product (NO_2-DCF) was identified based on comparison with a synthesized sample. This product is probably formed from a reaction with nitric acid. Nitric acid was produced from nitrogen gas present in the feed gas.

Based on the identified products, a DCF ozonation pathways is presented in Figure 3.7.

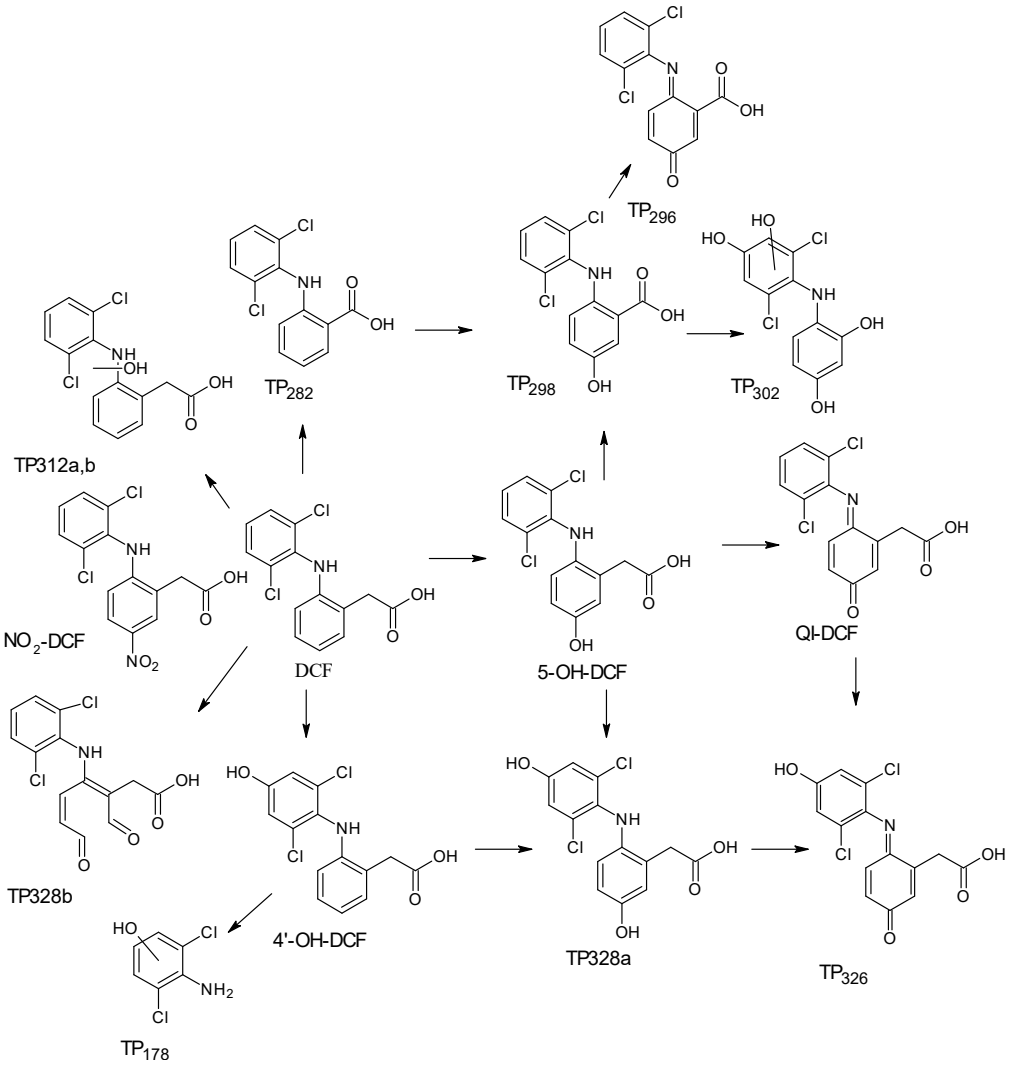


Figure 3.7. Proposed DCF ozonation pathways.

3.3.3. Quantification of transformation products (paper I and V)

Three of the available DCF products were quantified. In 10 minutes 80 % of DCF was transformed in non-catalytic experiments. The first of products to be formed was NO_2-DCF (Figure 3.8). The maximum concentration was observed after one minute, and after that the concentration started to decrease. The maximum concertation of NO_2-DCF corresponded to 3.5 % of the initial concentration of DCF. The first of the OH-DCF isomers to be formed was 4'-OH-DCF. The maximum concentration was observed after 4 minutes, and after that the concentration remained stable. The maximum concertation of 4'-OH-DCF corresponded to 8.5 % of the initial concentration of DCF. The second OH-DCF isomer, 5-OH-DCF was formed more slowly than 4'-OH-DCF. The concentration of 5-OH-DCF was still increasing at the end of the experiment. The maximum concertation of 4'-OH-DCF corresponded to 7.5 % of the initial concentration of DCF. The sum of the concentration the DCF products only adds up to 20 % of the transformed DCF. This indicates that a significant amount of DCF is transformed into other products, such as QI-DCF and small carboxylic acids.

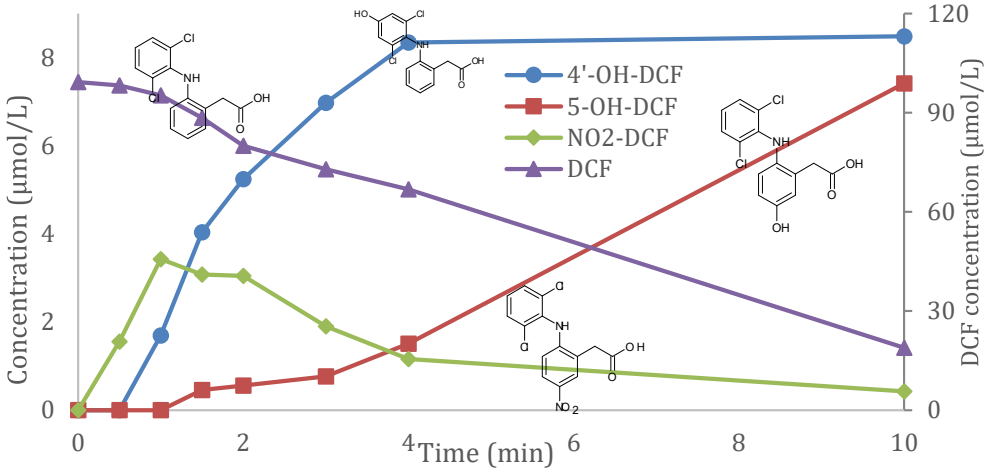


Figure 3.8. The ozonation of DCF and the transformation of the main products. Note the different scale for DCF.

For DCF, different platinum-based catalysts were used to investigate the effect of catalysts on the ozonation of DCF. Initially, catalysts with different weight % of platinum were investigated in order to determine the most effective metal %. An unmodified H-MCM catalysts was used to determine if the addition of the metal affected the transformation of DCF. Different amounts of the most effective catalysts were investigated. Details concerning the catalysts are presented in paper V.

Using a catalyst resulted in a lower maximum concentration of 4'-OH-DCF compared to non-catalytic experiments (Figure 3.9 (a)). When platinum was added to the H-MCM catalysts without metal, less 4'-OH-DCF was formed than

when the unmodified catalyst was used. The lowest maximum concentration of 4'-OH-DCF was observed for the 2% wt Pt-MCM-22-100 modified catalyst. When the H-MCM catalyst was used, the concentration of 4'-OH-DCF increased faster than in metal-catalyzed and non-catalyzed experiments. Similar results were observed for 5-OH-DCF (Figure 3.9 (b)).

The 2% wt Pt-MCM catalyst was further investigated by using different catalyst amounts (0.25, 0.5, 1g). All amounts of catalyst lead to a lower maximum concentration of 4'-OH-IBU than in the non-catalytic experiment (Figure 3.9 (c)). The maximum concentration of 4'-OH-DCF which was formed decreased when the amount of catalyst was increased. Similar results were observed for 5-OH-DCF, but when 1 g of catalyst was used, 5-OH-DCF could not be detected at all (Figure 3.9 (d)).

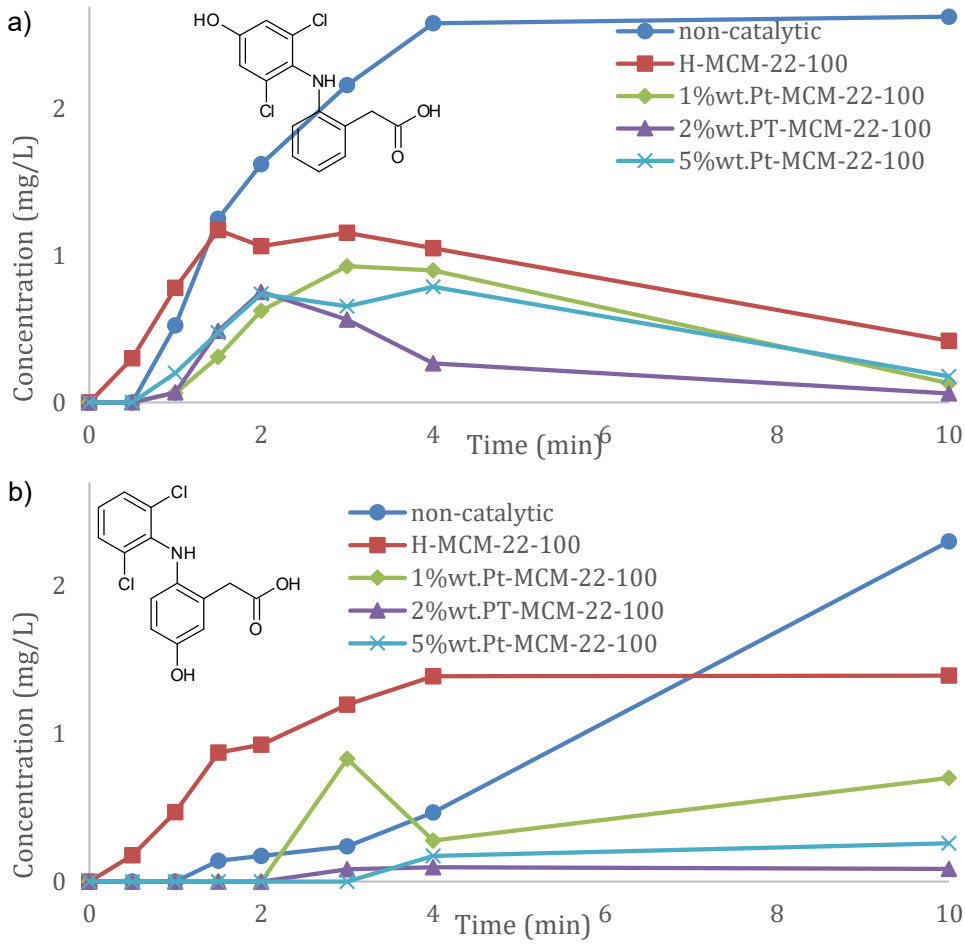


Figure 3.9. Concentration of (a) 4'-OH-DCF and (b) 5-OH-DCF during ozonation of DCF in non-catalytic experiments and in experiments catalyzed by different weight percentages of Pt-MCM-22-100 catalysts.

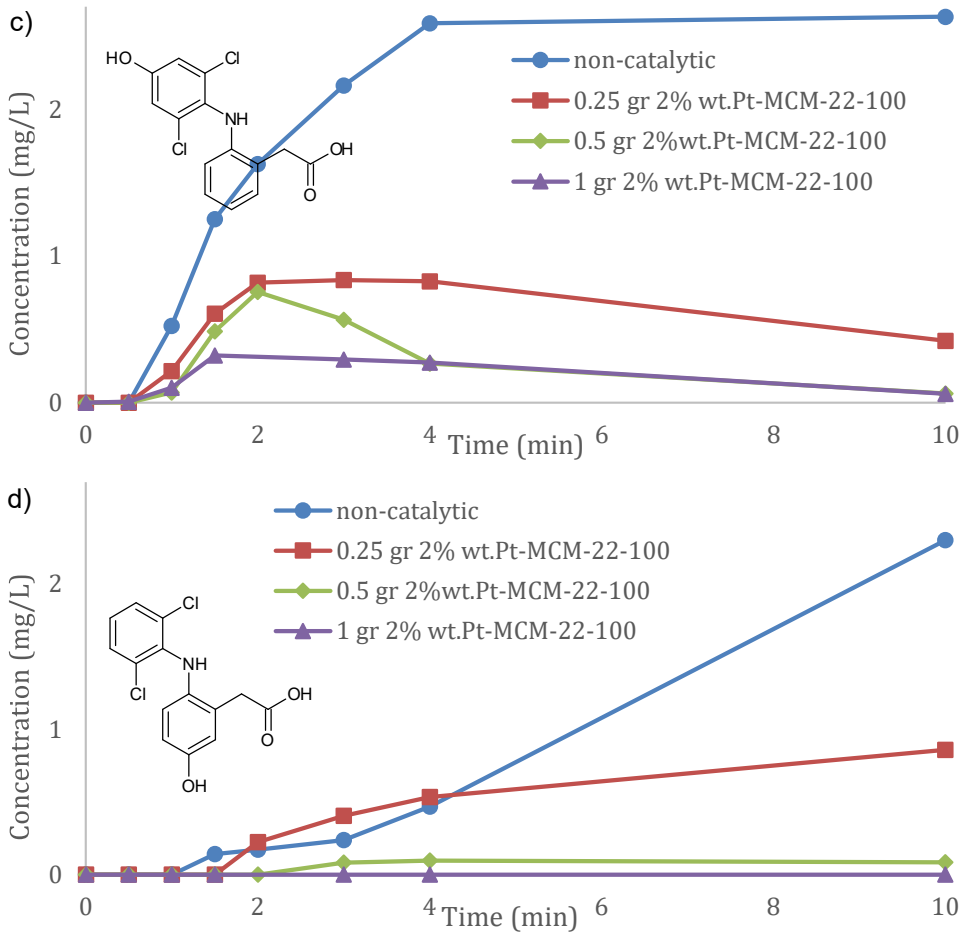


Figure 3.9. Concentration of (c) 4'-OH-DCF and (d) 5-OH-DCF during ozonation of DCF using different amounts of the 2 % wt Pt-MCM-22-100 catalyst.

3.3. CARBAMAZEPINE

3.3.1 Transformation of CBZ (Paper VI)

The transformation of CBZ during catalytic and non-catalytic ozonation was studied. A variety of different metal-based catalysts were used. The selection and acquisition of the catalysts are described in paper IV. CBZ could no longer be detected after 10 minutes of ozonation during both catalytic and non-catalytic ozonation (Figure 3.10). The addition of catalysts either enhanced or slowed down the transformation of CBZ. The slowest transformation was observed during ozonation catalyzed by Pd-H-Beta-300-EIM. When Cu-MCM-41-A-EIM was used as a catalyst, CBZ could no longer be detected after 2 minutes of ozonation. Of the catalysts which were tested, three (Pt-MCM-41-IS, Pd-H-Y-12-EIM and Pd-H-Beta-300-EIM) slowed down the reaction, two (Ru-MCM-41-IS and Cu-MCM-41-A-EIM) enhanced the reaction and one (Pt-H-Y-12-EIM) did not have any effect on the reaction. The catalyst could slow down the transformation of CBZ since CBZ was mainly transformed via direct ozonation, and some catalysts are able to transform ozone into hydroxyl radical, decreasing the concentration of ozone dissolved in the water.

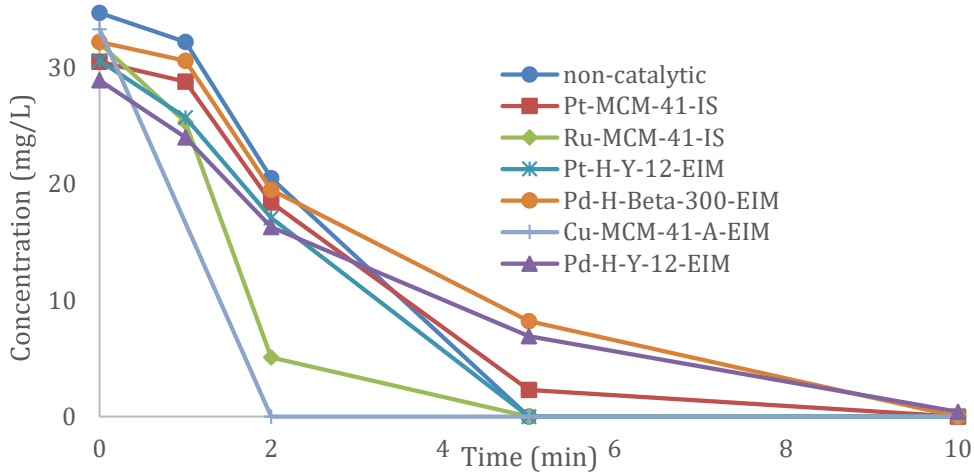


Figure 3.10. Transformation of CBZ during catalytic and non-catalytic ozonation.

3.3.2. Identification of ozonation products (paper II)

Most of the main products formed during the ozonation of CBZ had previously been identified (see chapter 1.8.3.), only the exact structure of TP225 was unknown. Additionally, four minor products were detected for the first time in this study.

The ozonation of CBZ mainly takes place via direct ozonation leading to the formation of rearrangement product BQM (McDowell 2005). In our study, BQM was isolated from an ozonated sample and the NMR spectrum of BQM was compared to the spectrum published in McDowell (2005). BQM was formed via the Criegee mechanism. CBZ reacted with ozone, forming a symmetrical product containing two aldehyde groups (Figure 3.11). This aldehyde was too unstable to be detected in this study. The amine group in the intermediate reacted with one of the aldehydes, eliminating water and forming BQM (Figure 3.11, pathway I).

BQM reacted further, either directly with ozone, or indirectly with hydroxyl radicals, to form BQD. BQD was initially detected using LC-IT-MS. The product was isolated, and the structure was confirmed by comparing its NMR spectrum to the spectrum published in McDowell (2005). The reaction mechanism leading to the formation of BQD was not known. However, it was likely that ozone reacts with BQM via a nucleophilic attack by ozone to the imine type carbon (Figure 3.11 (a) pathway (III), Bailey 1982). It was also possible that BQD is partially formed directly from CBZ (Figure 3.11 pathway (II). However, because BQD is formed significantly after BQM, this was not a major pathway.

The aldehyde groups in BQM and BQD could further oxidize to carboxylic acids, leading to the formation of BaQM and BaQD, respectively (Figure 3.12). BaQM and BaQD were identified based on their fragmentation pattern and by comparing the spectras with previously published results. BaQM and BaQD could then undergo ring-opening reactions leading to the formation of TP284 and TP300, respectively. TP300 could further undergo a ring-opening reaction leading to the formation of TP266. BaQD could also be oxidized to TP302, or decarboxylated to TP238. TP266, TP284, TP300 and TP266, TP302 and TP238 were identified based on their LC-IT-MS fragmentation patterns. TP238, TP266 and TP284 had not previously been detected.

During ozonation, the bond between nitrogen and the aromatic ring in BQM and BQD could break, leading to the formation of TP146 and TP162, respectively. TP146 and TP162 were identified based on their fragmentation pattern and by comparing their MS spectra with previously published results.

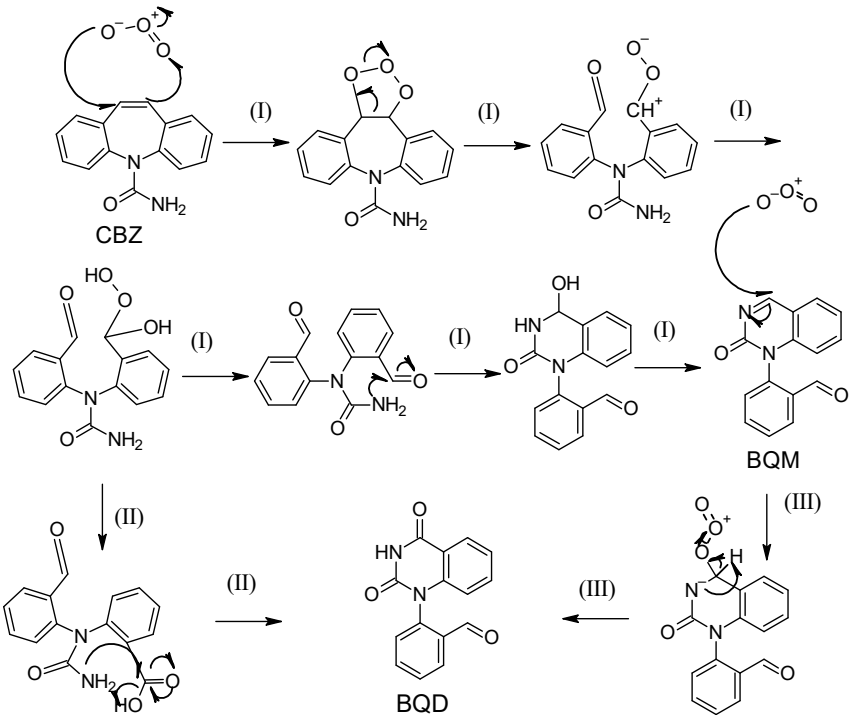


Figure 3.11. Transformation pathways leading to the formation of BQM and BQD.

Additionally to the two major products BQM and BQD, a major product with $[M+H]^+ = 226$ is often detected during oxidation of CBZ (Kosjek 2008, Hu 2009). In this study, this product (TP225) was initially detected using LC-IT-MS and LC-Q-ToF-MS. The HRMS revealed the molecular formula $C_{14}H_{11}O_2$ (error 9 ppm). Since the structure of this product had not been confirmed previously, the product was isolated. The structure of the compound was confirmed by $_1$H NMR, COSY, ^{13}C NMR, HMBC and HSQC experiments. The structure of TP225 was symmetrical. Based on the mass spectrum and the NMR data, it was concluded that the product is 2,2'-azanediyldibenzaldehyde (Figure 3.12 (b)).

One of the aldehydes in TP225 could oxidize to a carboxylic acid, leading to the formation of TP241. TP241 could undergo a ring-closing reaction leading to the formation of TP223. TP223 can finally also be oxidized to TP239. TP241, TP223 and TP239 were identified based on their fragmentation patterns and by comparison with published results (TP223 and TP239) A mechanism for these reactions is presented in Figure 3.12 (b). TP241 had not previously been detected.

The main transformation product BQD, was ozonated in order to further investigate the ozonation of CBZ. BQD was mainly transformed into four products with a shorter retention time than BQD: TP288, TP162 and TP300 and TP302. TP162 was also detected as a third-generation transformation product during the ozonation of CBZ. TP288, TP300 and TP302 were not detected during the ozonation of CBZ, likely since the experiments ended before these products were formed in high enough concentrations to be detected. TP288 could be

detected in both negative and positive modes, however the structure of this product could not be determined.

A suggested CBZ ozonation pathway is presented in Figure 3.12.

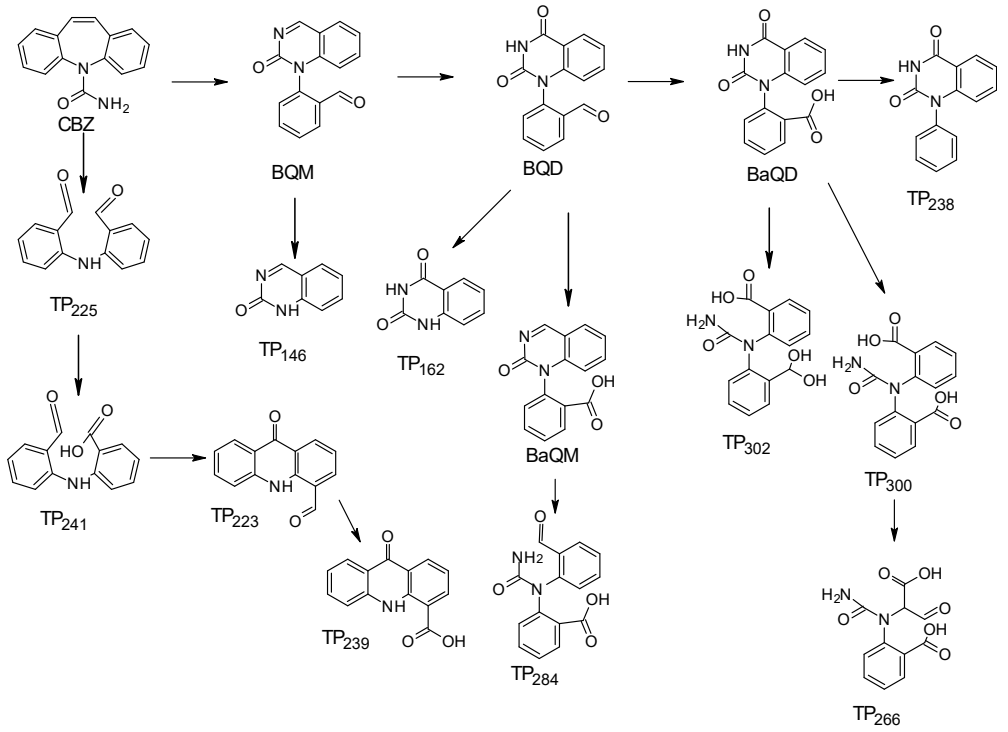


Figure 3.12. Proposed CBZ ozonation pathways.

3.3.3. Quantification of transformation products (paper II and VI)

None of the CBZ transformation products were available as commercial reference samples, so the main CBZ ozonation products, BQM and BQD, were isolated from an ozonated sample of CBZ and LC-UV quantification methods were developed. The molar concentrations of CBZ, BQM and BQD in an uncatalyzed ozonation reaction is shown in Figure 3.17. The initial concentration of CBZ was 139 µmol/L. The maximum concentration of BQM was 103 µmol/L, so 74 % of CBZ was transformed into BQM. BQM was much more stable than CBZ: CBZ could no longer be detected after 5 minutes, while there was still 3 µmol/L BQM left after 240 minutes of ozonation. At 120 min the concentration of BQD was 85 µmol/L, so at least 83 % of BQM was transformed into BQD. Also BQD was more stable than CBZ. When pure BQD was ozonated, 16 % if the initial concentration of BQD was still present after 240 minutes of ozonation.

The products TP225 was too unstable to be used to create a calibration curve, instead the concentration of TP225 which was formed was estimated by applying the calibration curves for CBZ and BQD to TP225. Both calibration curves gave the same concentration (RSD ≤ 20 %). Based on this it was estimated that 4 % of CBZ was transformed into TP225 (Figure 3.17).

The sum of the concentrations of CBZ and the three major products decreased with 16.5 % in the first two minutes and remained stable until 120 min. After 120 minutes, the concentration of BQD started to decrease, leading to a decrease in the sum of the three major products. The concentration of CBZ decreased to below the detection limit in five minutes, while both BQM and BQD were much more stable. The concentration of BQM decreased to 3.4 % of the maximum concentration in 235 minutes.

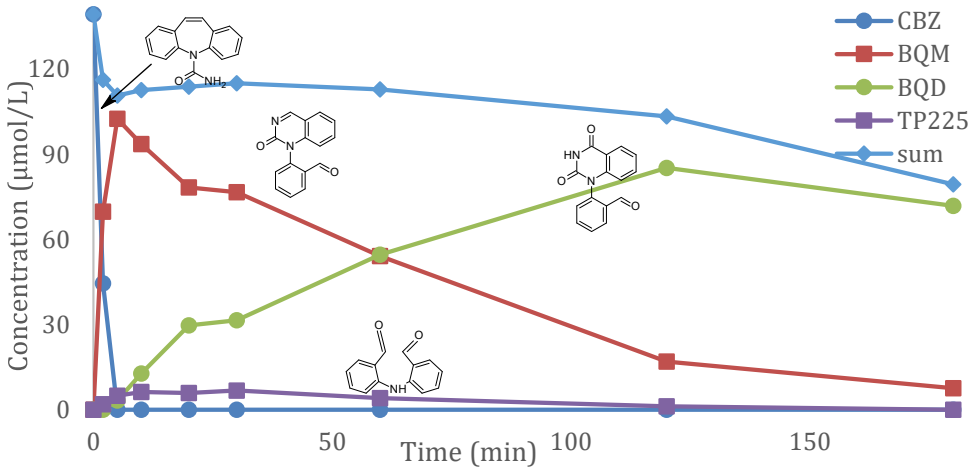


Figure 3.13. Molar concentrations of CBZ, BQM and BQD in a non-catalytic ozonation experiment.

The influence of temperature on the formation and transformation of BQM and BQD during non-catalytic ozonation was studied. Increasing the temperature to 50 °C slowed down the transformation of BQM, while there was no difference between experiments performed at 20 °C and 5 °C (Figure 3.14 (a)). While increasing temperatures generally decrease reaction times, this was not the case during ozonation. When the temperature increased, the concentration of ozone dissolved in water decreased. This led to slower transformation of BQM. Similar results were observed for BQD (Figure 3.14 (b)), however for BQD, the concentration of BQD was slightly higher at 5 °C than at 20 °C. This is likely because the concentration of ozone in the water is highest at 5 °C,

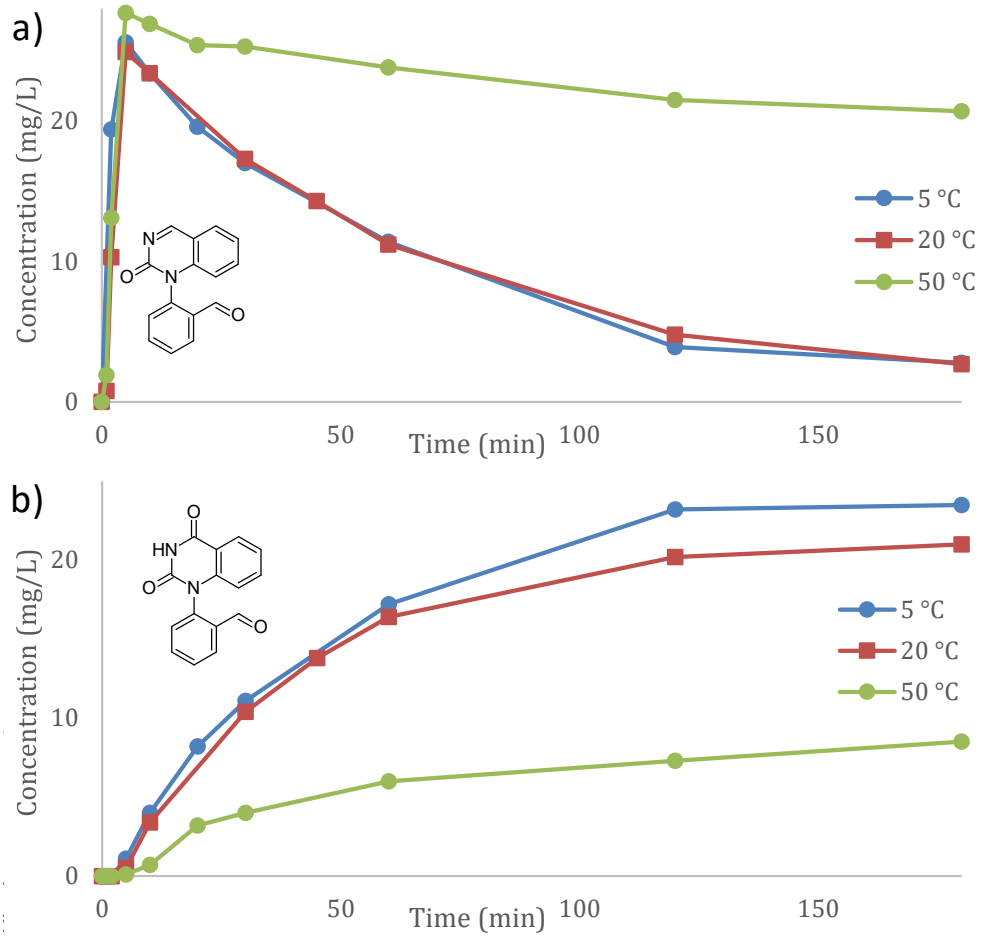


Figure 3.14. Concentration of (a) BQM and (b) BQD during non-catalytic ozonation experiments performed at different temperatures.

The influence of catalysts on the formation and transformation of BQM and BQD was investigated by adding different metal-bases catalysts to the reaction. The details concerning the catalysts are presented in paper VI. The presence of some catalysts, such as Ru-MCM-41-IS and Pt-H-Y-12-EIM, sped up the transformation

of BQM, while others, such as Pd-H-Y-12-EIM and Pd-H-Beta-300-EIM, slowed down the reaction (Figure 3.15 (a)). One explanation to how a catalyst would slow down the transformation of BQM is that the catalyst catalyzed the transformation of ozone into hydroxyl radicals. If the transformation of BQM involves direct ozonation, the decreased concentration of ozone in the water would slow down the reaction. Other catalysts might speed up the reaction by adsorbing BQM, ozone or both to the metal surface. After 120 min of ozonation, the BQM concentration was highest when Pd-H-Beta-300-EIM was used as a catalyst, while the concentration was lowest when Pt-H-Y-12-EIM was used as a catalyst.

The maximum concentration of BQM which was formed was similar for most experiments, except for the experiments using the catalysts which slowed down the transformation of CBZ the most (Pd-H-Y-12-EIM and Pd-H-Beta-300-EIM). When those catalysts were used, the maximum concentration of BQM was lower. Likely because BQM had time to transform further before the maximum concentration was reached.

The formation of BQD was always inversely proportional to the transformation of BQM (Figure 3.15 (a) and (b)). The highest concentration of BQD was formed when no catalyst was used, while only a very low concentration of BQD was formed when Pd-H-Y-12-EIM was used as a catalyst.

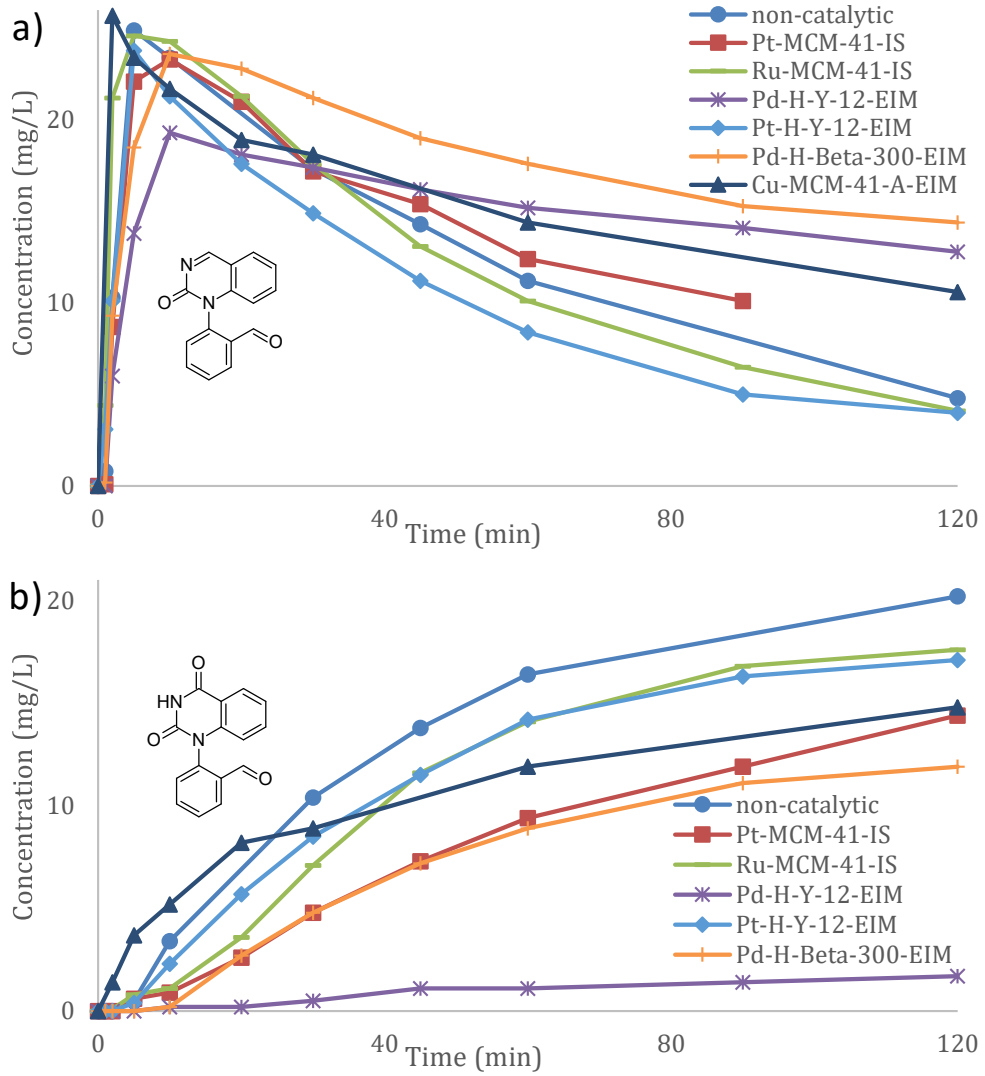


Figure 3.15. Concentration of (a) BQM and (b) BQD during catalytic ozonation.

3.4. SULFADIAZINE

3.4.1. Transformation of SDZ (Paper III)

For SDZ, only two catalysts were chosen, a Cu-H-Beta-150 catalyst and an iron-based catalyst. The transformation of SDZ during non-catalytic ozonation was efficient. Only a very low concentration of SDZ (0.2 % of the added SDZ) could be detected after 10 minutes of ozonation. The addition of catalysts slowed down the reaction (Figure 3.16). The reason for this was unknown, but might be the same as for CBZ: the catalysts might have decreased the concentration of ozone dissolved in the water.

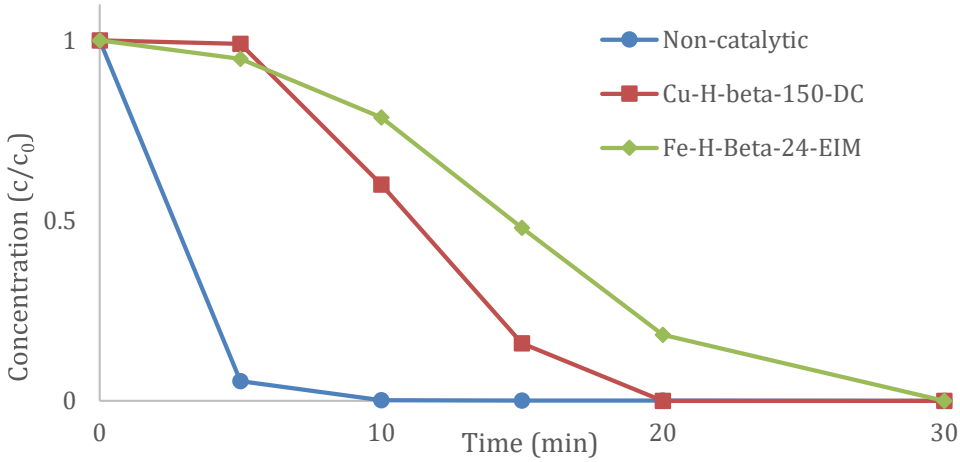


Figure 3.16. Transformation of SDZ during catalytic and non-catalytic ozonation.

3.4.2. Identification of ozonation products (paper III)

The ozonation of SDZ had not previously been studied in detail. Only a few transformation products had been detected and no ozonation products had been identified (see chapter 1.8.4.) In our study we found that a range of different products were formed during the ozonation of SDZ. These products could mainly be divided into two pathways: one where the N-(pyrimidin-2-yl) benzene sulfonamide skeleton was intact and one where SDZ underwent a rearrangement reaction. Additionally, the bond between nitrogen and one of the aromatic rings was broken, leading to the formation of 2-aminopyrimidine (2-AP). The structure of 2-AP was confirmed using a reference sample.

N-(pyrimidin-2-yl) benzene sulfonamide products

The SDZ ozonation pathways leading to the formation of products with an intact N-(pyrimidin-2-yl) benzene sulfonamide skeleton are presented in Figure 3.17 (a). In this pathway, SDZ was oxidized via the addition of one or more hydroxyl groups. Two isomers of SDZ with the addition of one OH group were detected. The exact positions of the OH groups could not be determined, however the fragmentation patterns indicated that both products had OH groups attached to the aniline ring (SDZ-P1, Figure 3.17 (a)). A product with three OH groups could also be detected ring (SDZ-P2, Figure 3.17 (a)).

The amine group in SDZ could also be oxidized to a nitro group (SDZ-P3, figure 3.17 (a)). SDZ-P3 could be further oxidized via the addition of an OH group to SDZ-P4 (Figure 3.17 (a)). Another product (SDZ-P5) was formed via the removal of the amine group from SDZ. SDZ-P5 was probably formed from a radical reaction of SDZ similar to the one Chignell noted for the photolytic cleavage of sulfanilamide (2008). An OH group could be added to SDZ-P5, leading to the formation of SDZ-P6. These products were identified based on their MS fragmentation patterns. Additionally, the mass spectra of SDZ-P1 and SDZ-P3 were compared with previously published results.

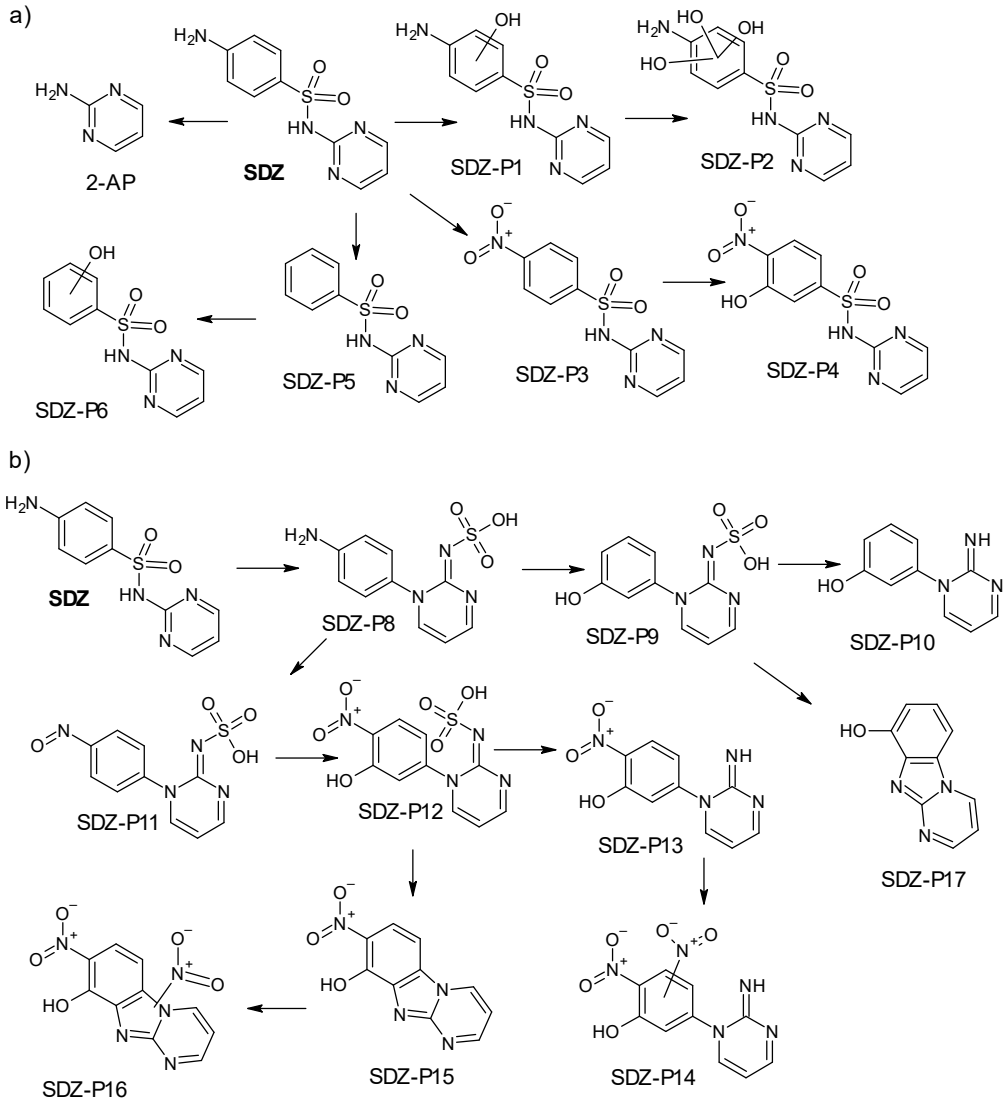


Figure 3.17. SDZ ozonation pathways (a) the N-(pyrimidin-2-yl) benzene sulfonamide pathway and (b) the rearrangement pathway

SDZ rearrangement products

When Sulfadiazine was ozonated, the compound underwent a rearrangement reaction (Figure 3.18). The rearrangement involved the formation of a new bond between the nitrogen atom in the pyrimidine ring and the aniline ring as well as the breaking the bond between the sulfonyl group and the aniline ring and the addition of a hydroxyl group to the sulfonyl group to form a sulfonic acid group. This leads to the formation of product SDZ-P8 (Figure 3.17 (b)) (Gao 2012). The rearrangement mechanism for this reaction has been studied previously (Gao 2012, Tentscher 2013). The rearrangement takes place via a Smiles type

rearrangement reaction (Figure 3.18) and that the reactions involves the formation of a radical.

In this study, it was noted that the $[M+H]^+$ peak of SDZ-P8 could not be detected. Instead, the base peak in the positive mass spectrum corresponded to the fragment created after the loss of the sulfonic acid group. The [M-H]- peak, on the other hand, could be detected. A similar pattern was noticed for SDZ-P9 and SDZ-P12.

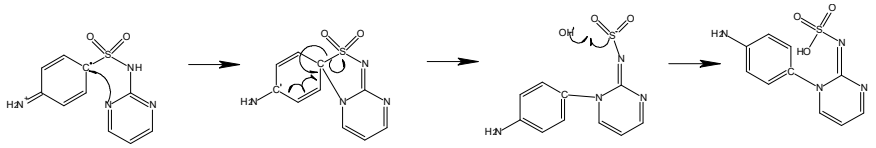


Figure 3.18. SDZ rearrangement mechanism (Tentscher 2013).

The NH_2 group in SDZ-P8 was lost in a similar way to the transformation of SDZ to SDZ-P5. Additionally, an OH group was added leading to the formation of SDZ-P9. The structure of SDZ-P9 was determined based on fragmentation pattern. In negative mode, the $[M-H]^-$=266 peak could be detected, but in positive mode the base peak was m/z = 188, indicating the loss of the sulfonic acid group. The molecular formula for SDZ-P9 was determined using HRMS (error 10 ppm in negative mode and 7.5 ppm in positive mode). The mass spectrum of SDZ-P10 was very similar to that of SDZ-P9, except that the negative mass spectrum did not contain the peak corresponding to the structure containing a sulfonic acid group. The retention time of the two structures were also very different (6.3 minutes for SDZ-P9 and 15.5 minutes for SDZ-P10) this indicated that SDZ-P10 was formed from SDZ-P9 via the loss of the sulfonic acid group. The structure of SDZ-P10 was determined based on fragmentation pattern and HRMS (error 0.1 ppm).

The NH_2 group in SDZ-P8 could also be oxidized to SDZ-P11 and SDZ-P12. SDZ-P11 was identified based on its fragmentation pattern. SDZ-P12 was identified passed on its fragmentation pattern and the HRMS of the base peak in positive mode m/z = 233, error 2 ppm. The $[M-H]^-$ peaks of SDZ-P11 and SDZ-P12 could not be detected, however it was assumed that the sulfonic acid group was still attached since the retention times of SDZ-P11 and SDZ-P12 were similar to that of SDZ-P9, rather than that of SDZ-P10. SDZ-P12 could lose the sulfonic acid group to form SDZ-P13. SDZ-P13 was identified based on fragmentation patterns and HRMS (error 14 ppm).

SDZ-P15 was the main product formed after SDZ had been ozonated for 240 minutes. The molecular formula for SDZ-P15 ($C_{10}H_6O_3N_4$) was determined with HRMS (error 2 ppm). The structure of SDZ-P15 was unclear, so SDZ-P15 was isolated from an ozonated sample with semi-preparative HPLC. The structure could then be determined based on NMR and MS fragmentation pattern. SDZ-P15 was likely formed via a ring-closing reaction to form SDZ-P12.

Two additional isomers of SDZ-P15 were detected. One isomer was identified based on fragmentation pattern and the other based on fragmentation pattern

and HRMS (error 3 ppm). The positions of the OH groups in the isomers could not be determined.

SDZ-P15 could be formed via a ring-closing reaction from either SDZ-P12 or SDZ-P13. Figure 3.19 shows the peak areas of SDZ-P12, SDZ-P13 and SDZ-P15 relative to the largest peak area (SDZ-P15 at 60 minutes). SDZ-P15 was formed inversely proportionally to SDZ-P12, so it was most likely that SDZ-P15 was formed from SDZ-P12. The sulfonic acid group in SDZ-P12 likely acted as a leaving group.

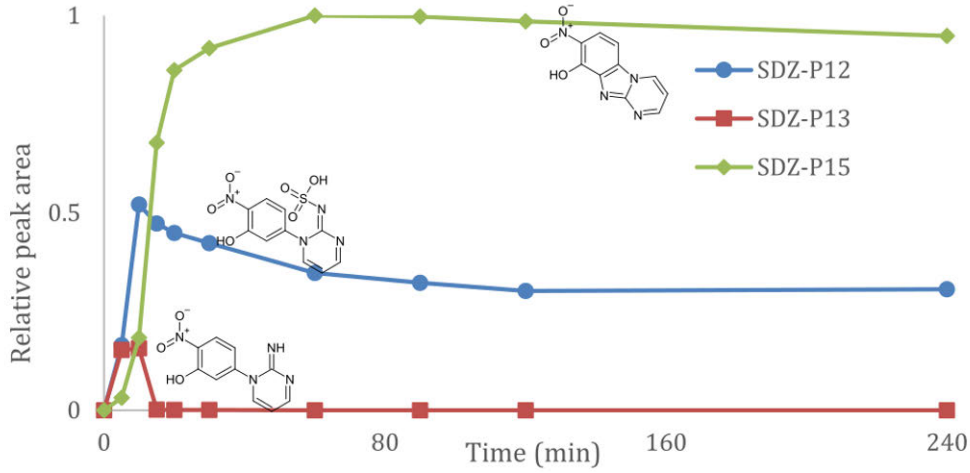


Figure 3.19. Relative peak areas of SDZ-P12, SDZ-P13 andSDZ-P15

SDZ-P17 had a similar fragmentation patten to SDZ-P15, but the HRMS suggested a molecular formula of $C_{10}H_7ON_3$ (error 10 ppm). This indicated that SDZ-P17 was also formed via a ring-closing reaction. The difference between the two structures was that SDZ-P17 did not contain a NO_3 group. The position of the OH group in SDZ-P17 was unknown, however it was likely that it was in the same position as in SDZ-P15. SDZ-P17 is most likely formed from SDZ-P9, similarly to how SDZ-P15 was formed from SDZ-P12.

Two additional products were detected. The molecular formulas for SDZ-P14 and SDZ-P16 were $C_{10}H_7O_5N_5$ (error 2 ppm) and $C_{10}H_5O_5N_5$ (error 2 ppm) respectively. The retention times of the products were very similar to, but slightly longer than the retention times of SDZ-P13 and SDZ-P15 (23 min and 24 min for SDZ-P13 and SDZ-P14, and 15 min and 16 min for SDZ-P15 and SDZ-P17). This indicated that SDZ-P14 and SDZ-P16 might be formed from SDZ-P13 and SDZ-P15 respectively via the addition of an NO_2 group. Nitric acid might have been present in the experiments due to the use of nitrogen in the feed gas.

3.4.3. Quantification of transformation products (paper III)

The SDZ transformation product 2-aminopyridine (2-AP) was available as a reference sample. A quantification LC-QqQ-MS quantification method was developed. The maximum concentration of 2-AP which was formed during non-catalytic ozonation was around 2 µM (Figure 3.20). This corresponded to 6 % of the original SDZ amount. The maximum concentration of 2-aminopyrimidine was reached after 10 min. The transformation of 2-AP was slow compared to SDZ. After 10 minutes only 0.2 % of SDZ remained, while 20 % of the maximum concentration of 2-AP remained after 240 minutes of ozonation. During catalytic ozonation, lower concentrations of 2-AP were formed (Figure 3.21 (a)).

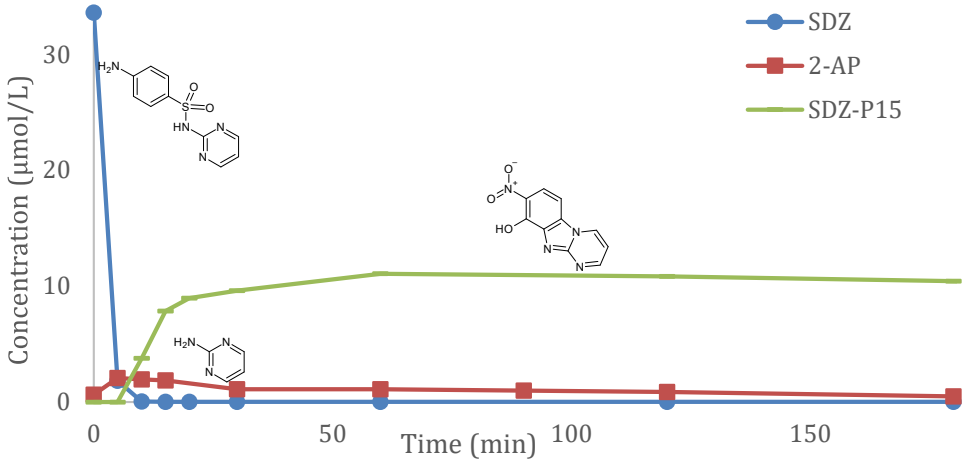


Figure 3.20. Concentration of SDZ, 2-AP and SDZ-P15 in a non-catalytic ozonation experiment.

The major product formed during ozonation of SDZ, SDZ-P15, was not available as a reference sample, so the product was isolated, and a quantification method was developed using LC-UV. During non-catalytic ozonation the concentration of SDZ-P15 increased to a maximum after 60 minutes after which the concentration remained stable (Figure 3.20)). The maximum concentration of SDZ-P15 was 11 µmol/L, so 28 % of SDZ was transformed into SDZ-P15. During experiments catalyzed by a cupper catalyst, the formation and transformation of SDZ-P15 was similar to the non-catalytic experiment, while the maximum concentration of SDZ-P15 was lower in experiments catalyzed by an iron catalyst (Figure 3.21 (b)). SDZ-P15 was much more stable than SDZ in both catalytic and non-catalytic experiments.

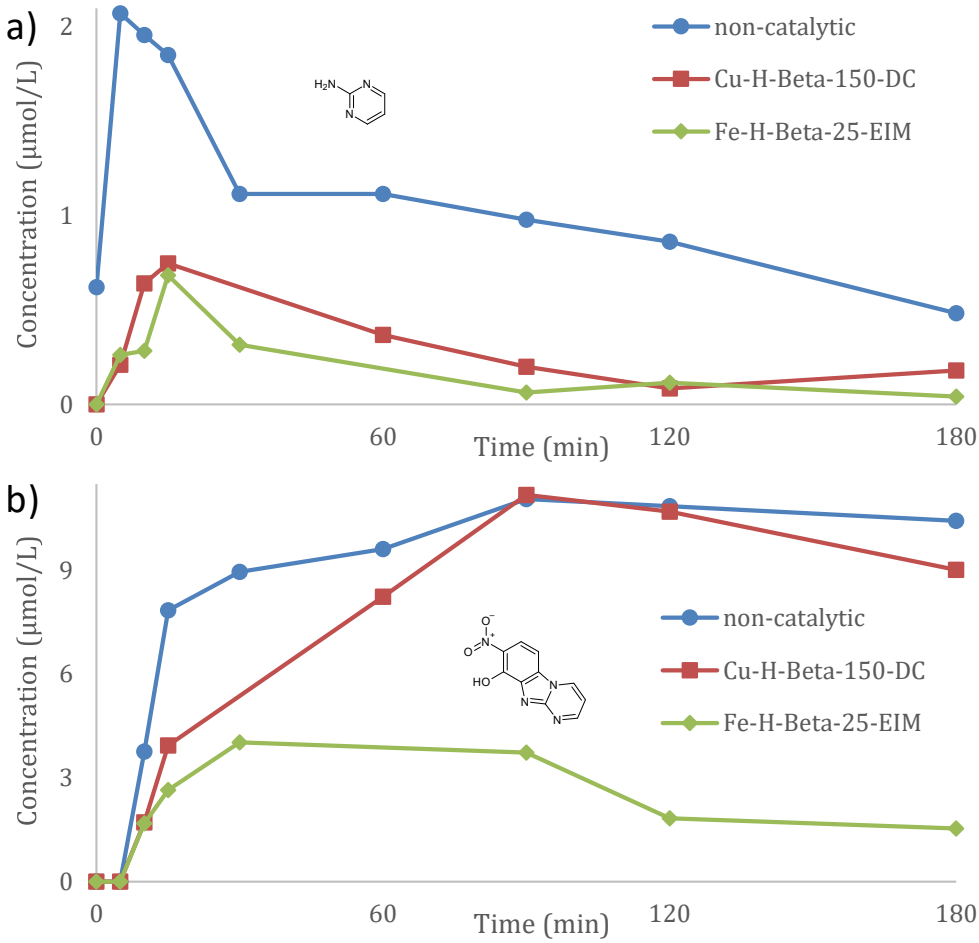


Figure 3.21. Concentration of (a) 2-AP and (b) SDZ-P15 in non-catalytic and metal catalyzed ozonation experiments.

4. Discussion

All four of the selected pharmaceuticals were transformed both through catalytic and non-catalytic ozonation. For IBU, DCF and CBZ it was possible to find catalysts which enhanced the transformation of the pharmaceutical, while some catalysts also slowed down the reaction. For IBU and CBZ, the most effective catalyst for enhancing the transformation were based on cupper, while platinum-based catalysts enhanced the transformation of DCF.

The catalyst which was most effective at enhancing the ozonation of IBU, Cu-H-Beta-150-DP, was also most effective at transforming the main ozonation products, 1-OXO-IBU, 1-OH-IBU and 2-OH-IBU. The maximum concentrations of these products were lower when Cu-H-Beta-150-DP was used, compared to other catalysts and non-catalytic ozonation. The fourth product which was studied, TP190, on the other hand was formed in higher concentration when Cu-H-Beta-150-DP was used.

Similar results were observed for DCF. When the catalyst which most enhanced the transformation of DCF (2 %wt Pt-MCM-22-100) was used, the concentrations of the main products were lower than in the other experiments.

For CBZ, the same pattern was not observed. The maximum concentration of BQM which was formed was not dependent on the catalyst. Instead, when the most efficient catalyst (Cu-MCM-41-A-EIM) was used, the highest concentration of BQM was observed. The maximum concentration of BQM was also reached faster than for less efficient catalysts. When Cu-MCM-41-A-EIM was used, the maximum concentration of QBM was reached after two minutes, during non-catalytic ozonation, the maximum concentration was reached after five minutes, and when the catalyst which slowed down the reaction the most (Pd-H-Beta-300-EIM) was used, the maximum concentration was reached after 10 minutes. BQM was transformed most efficiently when Pt-H-Y-12-EIM was used and Pd-H-Beta-300-EIM slowed down the reaction the most. Pt-H-Y-12-EIM did not have any effect on the transformation of CBZ and Pd-H-Beta-300-EIM slowed down the transformation of CBZ.

Due to the differences in the structures of the pharmaceuticals, there were large differences in the type of reactions noted during the ozonation experiments. The different reactions which were observed are summarized in Table 4.1. Hydroxyl groups were added to all the pharmaceuticals, or to their ozonation products. The addition took place on aliphatic carbons for IBU and possibly for DCF. Neither CBZ nor SDZ have any aliphatic carbons available for the addition of OH groups, however, after rearrangement, the CBZ ozonation products had aliphatic positions available for hydroxylation. The OH groups where added to aromatic carbons for DCF, SDZ and possibly for IBU. For CBZ the OH groups were added to an aldehyde, leading to the formation of a carboxylic acid.

IBU and DCF were decarboxylated, but CBZ and SDZ were not, since they did not contain any carboxylic acid groups. Some CBZ ozonation products did contain carboxylic acids, but no decarboxylated products of them were detected.

The oxidation of secondary alcohols was noted for IBU and DCF. Hydroxylated IBU products were oxidized to ketones and hydroxylated DCF products were oxidized to quinone-imine products.

The cleavage of a bond between nitrogen and an aromatic ring was noted for all the nitrogen containing compounds (DCF, CBZ and SDZ). NO_2 groups were added to aromatic rings during the transformation of DCF and SDZ.

The ozone-mediated cleavage off an aromatic ring was noted for DCF and CBZ. CBZ and SDZ also underwent reactions that are specific for the respective compounds. Ring-closing reactions took place for both CBZ and SDZ. CBZ could react directly with ozone, leading to the formation of BQM, while SDZ was rearranged to SDZ-P8. Finally, the primary amine in SDZ could be oxidized to a nitro group.

For IBU and DCF, the quantified products only accounted for a small fraction of the transformed pharmaceutical. This indicated that IBU and DCF were effectively transformed into products which could not be identified with the help of MS, such as small carboxylic acids. CBZ was mainly transformed into BQM and further into BQD. SDZ was mainly rearranged into SDZ-P8. SDZ-P8 was then further transformed into several products, mainly SDZ-P15. CBZ and SDZ were very slowly transformed into smaller molecules, compared to their respective parent compounds.

Table 4.1. Summary of ozonation reactions

Reaction	Ibuprofen	Diclofenac	Carbamazepine	Sulfadiazine
Hydroxylation	yes	yes	yes	yes
- aliphatic	yes	possibly	yes	n/a
- aromatic	possibly	yes	no	yes
Decarboxylation	yes	yes	no	n/a
Oxidation of OH groups	yes	yes	n/a	n/a
N-C bond breaking	n/a	yes	yes	yes
Addition of NO_2	no	yes	no	yes
Cleavage of aromatic ring	no	yes	yes	no
Rearrangement	no	no	yes	yes
Ring-closing reactions	no	no	yes	yes
Oxidation of NH_2 group	n/a	n/a	n/a	yes

5. References

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