Opportunities and limits of ozonation as pre-treatment method for production waste water from pharmaceutical manufacturing

Zur Erlangung des akademischen Grades eines
Dr.-Ing.
von der Fakultät Bio- und Chemieingenieurwesen
der Technischen Universität Dortmund
genehmigte Dissertation

vorgelegt von
Dipl.-Ing. Fares Daoud
aus
Jülich, Deutschland

Tag der mündlichen Prüfung: 17.01.2022
1. Gutachter: Prof. Dr. Oliver Kayser
2. Gutachter: Prof. Dr. Thomas Wintgens

Dortmund 2022

Acknowledgements

I would like to express my sincere appreciation and gratitude to all the people who accompanied and supported me in completing my thesis.

In the last ten years, I had worked intensively on my thesis and very often, my wife Nina and my two sons Noah and Luis suffered from my inattentiveness. However, they were all very understanding. Special thanks go to my beloved family for their longstanding support.

I would like to thank my supervisor Prof. Oliver Kayser (Department of Biochemical and Chemical Engineering, Technical Biochemistry, TU Dortmund) for his support within working on my PhD thesis. His support helped me with the writing of the publications and my dissertation. I am very grateful for the trust he has placed in me over the past years.

I thank Prof. Michael Spiteller (Institute INFU, Faculty of Chemistry and Chemical Biology, TU Dortmund) and to Dr. Sebastian Zühlke (Center for Mass Spectrometry, Faculty of Chemistry and Chemical Biology, TU Dortmund) for their assistance. Both supported me with all analytical topics and the identification of transformation products. Bastian Jansen and David Pelzer also completed two master theses under both supervisions. The master theses provided new information and aspects that had been of high interest for me.

I thank Bayer Corp. for their support and the funding of the laboratory equipment for all ozonation experiments. Special thanks go to Dr. Klaus Jelich and Volker Klotzki (both Bayer Corp. Wuppertal, Germany) for promoting me and allowing me to conduct ozonation experiments in the waste water treatment plant Rutenbeck. Moreover, I also sincerely thank my supervisors Dr. Wilfried Kopp (Bayer Corp., Dormagen, Germany) and Dr. Tobias Luecke (Bayer Corp., Leverkusen, Germany).

I warmly thank to all team members from my former department E&K, especially to Frank Niemeyer and Kevin Rosinsky. Both had spent a lot of time on conducting and monitoring the ozonation trials.

I would also like to thank my parents and brother for all their trust, encouragement, and support throughout my life. I would especially like to thank my father, Dr. Hassan Daoud. He accompanied

me with advice during my promotion and was my biggest supporter. I am glad, that he was able to witness my disputation. Tragically, he passed away on March 5th, 2022.

I also thank all my friends, family, and colleges who have wondered for over ten years whether “the story will ever end”.

Table of Contents

Summary

During production of active pharmaceutical ingredients, process waste water is generated at several stages of manufacturing. Whenever possible, waste water will be processed by conventional waste water treatment plants. However, due to low biodegradability of pharmaceuticals or toxicity towards aquatic organisms, some waste water streams must be processed differently. For those, incineration constitutes the current method of choice. A main disadvantage of incineration is high consumption of primary energy sources leading to substantial emission of carbon dioxide (CO_2). Thus, ozone treatment followed by biological waste water treatment was tested as an alternative method. In 2009, preliminary laboratory experiments were conducted to evaluate the elimination of DTPA in process waste water. Based on the initial results, the responsible authorities granted approval for a large-scale ozonation. Additional experiments at laboratory scale were performed with different waste water streams to assess the elimination of the target compound and the generation of its main transformation products. These were determined by high performance liquid chromatography – high-resolution mass spectrometry (HPLC-HRMS). All waste streams originated from production facilities of Bayer Corp. in Wuppertal. Four waste water streams from the ciprofloxacin, moxifloxacin, rivaroxaban and DTPA production have been investigated. The obtained results demonstrated that the concentration of moxifloxacin and its metabolites can be effectively reduced (> 99.7%) prior entering the receiving water. Applying the same ozonation time the concentration of ciprofloxacin and its metabolites remained too high for safe discharge. The concentration of rivaroxaban in the ozonated waste water was effectively lowered under acidic conditions. DTPA concentration was reduced to levels assuring safe discharge into the receiving water. Additionally, the ecotoxicity of the ozonated waste water for all target compounds was investigated using three trophic levels. A comparison of CO_2 emissions showed that ozonation in some cases is an ecological alternative to incineration.

Zusammenfassung

Bei der Herstellung von pharmazeutischen Wirkstoffen fallen in mehreren Herstellungsstufen Prozessabwässer an. Abwasser wird nach Möglichkeit in konventionellen Kläranlagen aufbereitet. Aufgrund der geringen biologischen Abbaubarkeit von Arzneimitteln oder der Toxizität gegenüber Wasserorganismen müssen einige Abwasserströme jedoch unterschiedlich prozessiert werden. Für derartige Abwässer ist die Verbrennung die derzeitige Methode der Wahl. Der Hauptnachteil der Verbrennung ist der hohe Verbrauch an Primärenergieträgern, der zu einer erheblichen Emission von Kohlendioxid (CO_2) führt. Daher wurde die Ozonbehandlung mit anschließender biologischer Abwasserbehandlung als alternative Methode getestet. Im Jahr 2009 wurden vorläufige Laborexperimente durchgeführt, um die Eliminierung von DTPA in Prozessabwässern zu bewerten. Auf der Grundlage der ersten Ergebnisse erteilten die zuständigen Behörden 2011 die Genehmigung für die großtechnische Ozonierung. Zusätzliche Experimente im Labormaßstab wurden mit verschiedenen pharmazeutischen Abwasserströmen durchgeführt, um die Eliminierung der Zielverbindung und die Erzeugung der wichtigsten Transformationsprodukte zu bewerten. Diese wurden durch HPLC mit hochauflösender Massenspektrometrie (HPLC-HRMS) bestimmt. Alle Abfallströme stammen aus Produktionsanlagen der Bayer AG in Wuppertal. Es wurden vier Abwasserströme aus der Produktion von Ciprofloxacin, Moxifloxacin, Rivaroxaban und DTPA untersucht. Die erhaltenen Ergebnisse zeigten, dass die Konzentration von Moxifloxacin und seiner Transformationsprodukte vor Einleitung in das Fließgewässer wirksam reduziert werden kann (> 99.7%). Bei gleicher Ozonierungszeit blieb die Konzentration von Ciprofloxacin und seiner Transformationsprodukte zu hoch, um diese sicher einleiten zu können. Die Konzentration von Rivaroxaban im ozonisierten Abwasser wurde unter sauren Bedingungen wirksam reduziert. Die DTPA-Konzentration wurde auf Werte reduziert, die eine sichere Ableitung in das Fließgewässer gewährleisten. Zusätzlich wurde die Ökotoxizität des ozonisierten Abwassers für alle Zielverbindungen unter Verwendung von drei trophischen Stufen untersucht. Ein Vergleich der CO_2-Emissionen ergab außerdem, dass die Ozonisierung in einigen Fällen eine ökologische Alternative zur Verbrennung darstellt.

Chapter 1: Scope of the thesis

1.1 Goals and objectives

The aim of this work is to investigate an alternative disposal method for waste water from pharmaceutical production as a competitive process for incineration. Since there has been a large-scale ozonation facility in Wuppertal since 2004, only ozonation was investigated as the method of choice, even if other advanced oxidation processes (AOPs) could be more efficient. The ozonation system was originally installed to degrade excess sludge from the industrial sewage treatment plant. Contractual options for disposing of excess sludge massively reduced the profitability of the ozonation process. Bayer looked for other ways to use the ozonation facility and evaluated the options for treating other waste water streams from the manufacturing process.

Chapter 2 provides information on the occurrence of pharmaceuticals in waste water, waste water treatment in general and specific information on ozonation. Additionally, this chapter focusses on the required analytics and throws a glance at ecological aspects of different treatment methods.

Chapter 3 analyses the ozonation of ciprofloxacin and moxifloxacin containing waste water from original production. Occurring transformation products have been evaluated and the ecotoxicity of ozonated waste water has been investigated.

Chapter 4 analyses the ozonation of rivaroxaban containing waste water from original production. Occurring transformation products have been evaluated and compared to generated metabolites in human bodies and in animals.

Chapter 5 highlights the results from ozonation of DTPA containing waste water from original production. Transformation products have been investigated and large-scale results have been compared to lab scale ozonation experiments.

Chapter 6 focuses on the comparison of obtained results from the ozonation of four different pharmaceutical containing waste waters. Comparisons have been conducted with regard to rate constants, TOC reduction, ecotoxicity and ecological aspects.

Chapter 2: Introduction

Clean water is vital for humans and animals and essential for most biological and chemical processes in nature and in industry. Dealing with resources such as clean water is therefore of crucial importance for our future. Ensuring water quality to prevent water pollution from pharmaceuticals for future generations is of great interest to society. It is increasingly recognized that various substances tend to accumulate in the environment due to insufficient degradation in sewage treatment plants and surface water. This has far-reaching consequences for our aquatic systems. For some years now, many specialist journals and popular scientific publications have been pointing to the increasing accumulation of trace substances in the aquatic environment. This accumulation leads to an increased absorption of these substances in small organisms, but also in larger animals such as fish or smaller mammals. Relevant groups of trace substances are pharmaceuticals. The group of antibiotics, endocrine disruptors and antineoplastic agents is of relevance (Huber et al., 2005; Buffle et al., 2006). There is increasing evidence that these substances are more and more responsible for microbiological resistance, the feminization of higher organisms and other ecotoxicological effects on the aquatic system. Medicines in the environment have different origins. Notable sources are private households, hospitals, and manufacturers (Kuemmerer, 2008). Even if EU legislation in 1994 banned the disposal of unused pharmaceuticals with household waste, a significant number of pharmaceuticals still ends up in this disposal route or goes down the drain (Goetz and Keil, 2007; Bound and Voulvoulis, 2005). In addition, a certain percentage of the administered active drugs leave the body only partially metabolized (Heberer, 2002; H. Jones et al., 2005). Figure 2.1 shows the disposal routes for pharmaceuticals based on the studies by Schroeder (2003), Githinji et al. (2011), Kabasci et al. (2007) and Heberer (2002). All authors have emphasized different topics. Hence, some adjustments have been made to highlight the main topics of this work.

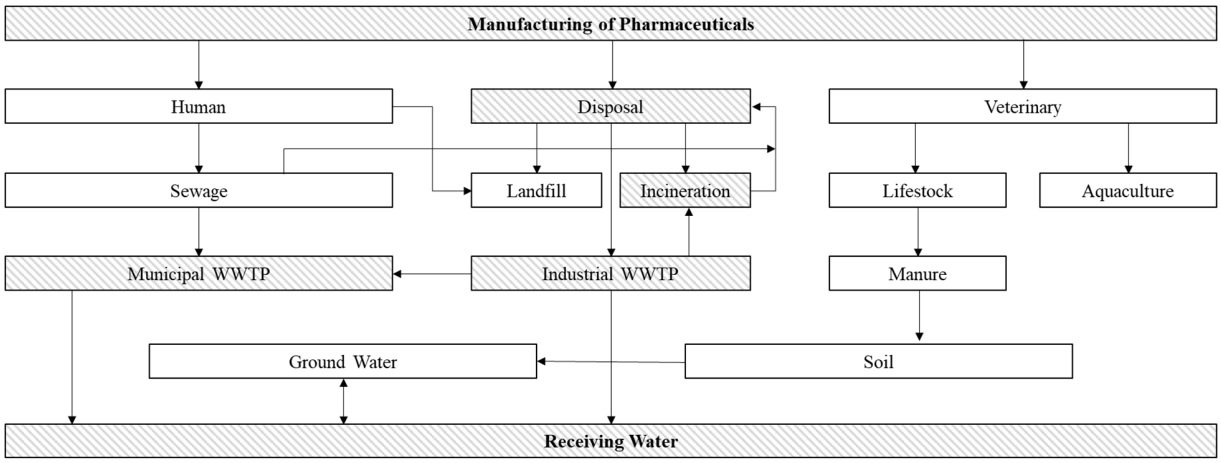


Fig. 2.1: Flow diagram for the introduction of medicinal products from human and veterinary medical applications into the environment (areas with a grey background are relevant for this work)

Regarding pharmaceutical manufacturers, Roegener (2010) reported that a study by the US Geological Survey concluded that water from waste water treatment plants, which are fed with waste water from pharmaceutical manufacturers, has active substance concentrations up to 1000 times higher than water from other waste water treatment plants. The study was limited to two New York wastewater treatment plants, so these results cannot be generalized. Larsson et al. (2007) reported on other important releases by Indian manufacturers in which ciprofloxacin and other fluoroquinolones that are toxic to microorganisms were determined. Nevertheless, according to Kuemmerer (2008), emissions are negligible due to strict regulations and corporate sustainability programs. However, during the manufacturing process, waste water occurs in several stages of production. Most of the waste water is biodegradable in industrial and / or municipal waste water treatment plants, but other hazardous waste water is subject to alternative treatment methods (Daoud et al., 2017). Waste water is very often incinerated as incineration is still the most efficient method of decomposing pharmaceuticals and reaction by-products. This thesis addresses the question whether part of the production waste water can be treated with ozone as an alternative to incineration.

2.1 Pharmaceuticals in the environment

The pollution of the aquatic and terrestrial environment by pharmaceuticals and their transformation products or metabolites has been of particular importance for some years since an accumulation in the environment can increasingly be observed. Pharmaceuticals decompose differently in the human body, in animals, in the environment or in industrial processes. Therefore, Kuemmerer (2008) used different terminology depending on the type of degradation. He used the term metabolites exclusively for degradation products of pharmaceuticals in the human body. The term transformation products, on the other hand, is used to describe products that are degraded in the environment or through industrial elimination processes. According to Heberer (2006), metabolites arise through biotransformation, especially in the liver, but also in the kidneys, spleen, intestines, blood, skin, etc. In addition, it should be considered that some substances, so-called active ingredients, are only effective after metabolic activation in the body (Kuemmerer et al., 2011). These substances are also called prodrugs.

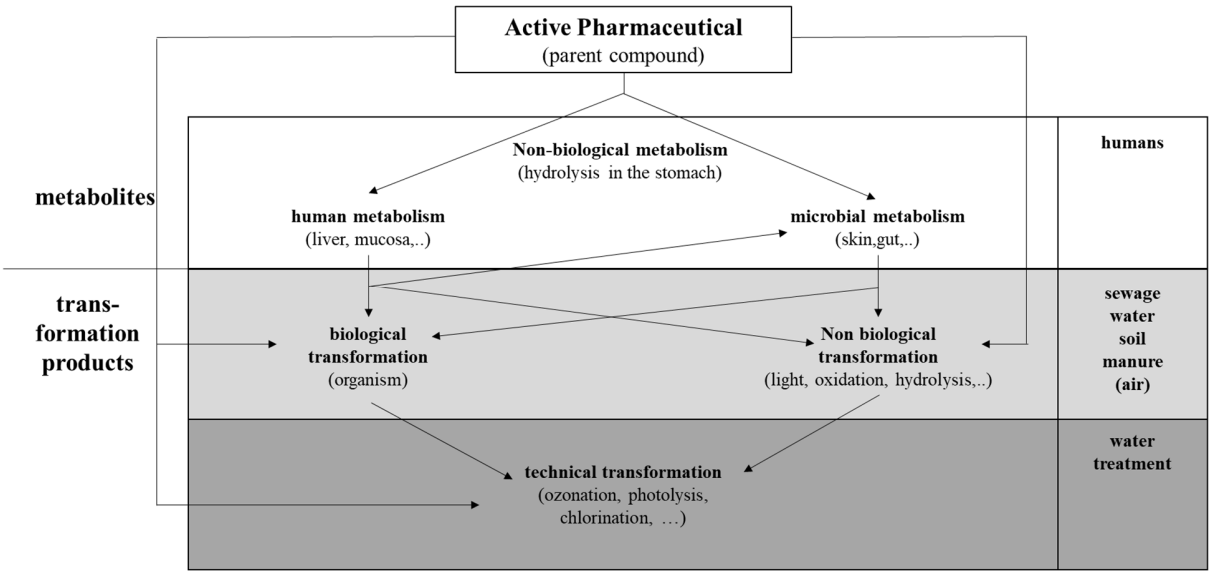


Fig. 2.2: Differentiation between metabolites and transformation products (Laengin et al., 2008)

Regarding the occurrence of drugs and their metabolites in the environment, Fig. 2.2 shows the entirety of all pharmaceuticals and their direct or indirect path into the environment. Even if it is generally assumed that the transformation products or metabolites have a lower activity than their

parent molecules, these also lead to environmental pollution and must be considered accordingly. Metabolites enter the aquatic environment mainly through the excretory organs of humans and animals.

Drugs used in human medicine are, according to Kuemmerer (2008), either excreted directly (not absorbed or not metabolized) or after their conversion or breakdown into so-called metabolites in the human or animal organism. This usually takes place in the form of conjugates, in which the compounds are bound to endogenous molecules as part of the biotransformation to improve their solubility and facilitate their removal.

2.1.1. Water laws

To answer the question of what degree of elimination should be achieved, it is worth looking at current European and US legislation. The State Water Act and the European Water Framework Directive are binding for Germany. According to the European Water Framework Directive, the water quality should reach a good "chemical status" and a good "ecological status". These laws set the rules for protecting the aquatic environment. For some years now, however, pharmaceutical legislation has also required that the effects of pharmaceuticals and their metabolites on the environment be assessed and submitted to the authorities when the pharmaceuticals are approved. In addition to the state authorities, there are also valuable information from national and international organizations such as the OECD (Organization for Economic Cooperation and Development). Information on medicinal products in water can be found, for example, in the Drinking Water Ordinance (German: Trinkwasserverordnung). The impurities tolerable in the "Ordinance to Amend the Drinking Water Ordinance" (German: Verordnung zur Novellierung der Trinkwasserverordnung) of May 21, 2001 were not changed in the last revision in 2011 (Bergmann et al., 2011). According to the guidelines of the Federal Environmental Agency (German: Umweltbundesamt, UBA), drinking water should always be free of foreign substances. This corresponds to the intentions of the Water Resources Act as well as the common enforcement practice of the NRW environmental administration and the Water Safety Concept of the WHO (2010). As the accuracy of analytical measurements improved, this requirement could no longer be met, so that the UBA now proposes a concentration of 0.1 µg/L per group of active substances as a health precautionary value for non-genotoxic substances (Fahlenkamp et al., 2008).

Additionally, the UBA provides health benchmarks (German: gesundheitliche Orientierungswerte; GOW) for selected organic ingredients (Pinnekamp et al., 2008). Target values can be found in "Requirements for the protection of surface water to secure the drinking water supply" 16.11.2007 (Drug residues 0.1 µg/L, X-ray contrast media 1 µg/L, chelating agents 5 µg/L).

Regarding pretreatment methods the Waste Water Ordinance (German: Abwasserverordnung AbwVO, 2004) regulates the requirements for sewage disposal in receiving waters. For a pretreatment process in the chemical industry appendix 22, Part C is binding. Following maximum dilution factor must be considered:

- Toxicity to fish eggs $G_{EI} = 2$
- Toxicity to daphnia $G_D = 8$
- Toxicity to algae $G_A = 16$
- Toxicity to luminous bacteria $G_L = 32$
- Mutagenic potential (umu test) $G_M = 1.5$

2.1.2. Approval of drugs

Various drug agencies such as the EMA (European Medicines Agency) and the US FDA (Food & Drug Administration) are addressing the increasing problem of detected concentrations of drugs in water bodies. For some years now, an environmental assessment has been required when submitting authorization applications for medicinal products with new active ingredients. In 1995, an environmental risk assessment for European authorization procedures was introduced into EU law. This was also adopted into German pharmaceutical law in 1998. However, there were no clear guidelines on how the environmental risk assessment for medicinal products for human use should be carried out. Since 2006 there has been a guideline prescribed by the EMA, which applies to all new authorization applications as well as to applications for additional indications or dosage forms of already authorized drugs (Fent, 2008). The EMA published guidelines for assessing potential environmental risks. This recommends a step-by-step approach with appropriate toxicity tests (EMA, 2006).

In a first step, the expected concentration in the aquatic environment is calculated. If the calculated value, the PEC (predicted environmental concentration), is below the action limit of 0.01 µg/L, it

is assumed that the drug does not pose a risk to the environment. In this case, the further investigation will be discontinued. Regarding the determination of the action limit, the FDA subsequently evaluated the data and decided to increase the action limit to 1 µg/L. If the PEC exceeds the action limit, phase II follows. However, if a very high potential for environmental risks is known or suspected, phase II will also take effect independently of the PEC. The calculation of the PEC results from a simple algorithm that considers various conservative assumptions about market penetration, waste disposal and retention mechanisms.

In the second phase, the physical, chemical, and toxicological properties of the active ingredients are assessed. The so-called PNEC (Predicted No Effect Concentration) is determined from various degradation and toxicological tests. The basis for deriving the PNEC for a substance according to the European Commission (Technical Guideline Document 2003 for Risk Assessment) is the determined chronic toxicity (NOEC: no observed effective concentration) for the most sensitive organism in water bodies (Bergmann et al., 2011). Not every organism can be tested in this context. For this reason, tests are carried out on aquatic organisms with three stages, called trophic levels, of the food chain. Tests on plants (algae), invertebrates (daphnia) and vertebrates (fish) are most likely to identify the most sensitive organism. To take account of the remaining uncertainty, a safety factor is also included (EMA, 2006). Therefore, the mean effective concentration (EC_{50}) or the mean lethal concentration (LC_{50}) are divided by the corresponding safety factor to obtain the PNEC (Hernando et al., 2006).

The more and better data are available, the lower this safety factor can be set. If test results are available for fewer organisms, the uncertainty and thus the safety factor to be used increases. While a factor of 10 is considered sufficient for chronic values for three trophic levels, it increases to 50 for two trophic levels and to 100 for one trophic level. Acute toxicity values can also be used, but with a factor of 1000. An overview of the safety factors to be used according to the information provided by the European Commission are given by Bergmann et al. (2011).

The Swedish Medicines Agency has taken a step forward with the so-called "Stockholm Model". Gunnarsson and Wennmalm (2006) described the Swedish approach, which is based on three studies. To assess the environmental risk, the biodegradability, bioaccumulation potential and toxicity to aquatic organisms are assessed. The OECD test guidelines or similar degradation tests are used to determine the biodegradability. The three factors add up to an overall rating. A

substance with good degradability, low potential to bioaccumulate and low toxicity receives an overall rating of zero, a substance with poor degradability, high potential to bioaccumulate and very high toxicity receives an overall rating of nine. However, this model does not consider any metabolites that are possibly even more dangerous than the parent products.

2.2. Waste water pretreatment methods

Heavily loaded industrial waste water, such as that produced in the pharmaceutical industry, is often not completely biodegradable. According to Schroeder (2003) there are numerous special treatment methods with different degrees of effectiveness. The aim is to sufficiently eliminate trace substances while taking operating costs into account. Common waste water treatment processes are in most cases combined processes. Pretreatment methods include evaporation, extraction, stripping, absorption, etc., followed by incineration or biological treatment. Pinnekamp et al. (2003) examined many different methods for eliminating organic trace substances in a literature study. The project, funded by the Ministry of Environment and Transport of the State of Baden-Württemberg, brings together many published results on the various treatment methods. In the following, special emphasis should be placed on every type of oxidation process with subsequent biological wastewater treatment. Whether or not such pretreated wastewater can be subjected to biological treatment depends on several factors. The microorganisms in the biological treatment step must be able to effectively remove the contaminants and micropollutants. In addition, the pollutants should not be toxic in any form.

2.2.1. AOP processes

In contrast to physical processes, oxidative processes break down the trace substances chemically and thus convert them irreversibly. The chemical conversion takes place through reaction with an oxidizing agent such as ozone, hydrogen peroxide, but also through chlorination with hypochlorite or chlorine dioxide. It has been known since the 1970s that the use of chlorine as an oxidizing agent can lead to harmful and sometimes carcinogenic organic chlorine compounds, which is why this treatment was prohibited in the Drinking Water Ordinance of 1990 (Schmidt et al., 2010). The

oxidation rate, which is important for the design of such processes, depends on the concentration of the oxidizing agent as well as the concentration of the substance to be oxidized. In the presence of competing substances, complete mineralization to carbon dioxide and water requires considerable amounts of oxidizing agents and possibly long reaction times. The unused oxidizing agent, which remains in the treated wastewater, can itself represent a significant hazard potential, while incomplete degradation due to a lack of oxidizing agents or too short reaction times can lead to the formation of toxic substances. The individual processes sometimes require special reaction conditions, such as a certain pH value. In addition to the direct addition of oxidizing agents, an increased formation of more reactive OH radicals can increase the oxidizing effect. According to Andreozzi et al. (2004) all these processes are summarized under the term “Advanced Oxidation Process” (AOP). In all cases, the goal of this AOP is to increase the concentration of OH radicals. OH radicals are very effective, but at the same time very unselective oxidizing agents that can be generated from various reagents such as H_2O_2 / UV, O_3 / UV, H_2O_2, O_3 and Fe^{2+} / H_2O_2 (Fenton reagent). These processes have shown their fundamental applicability for the conversion and degradation of trace substances that are difficult to decompose. Strong and therefore relatively unselective oxidizing agents can oxidize pharmaceuticals and the precursors of endocrine disrupting residues in water and waste water and thus reduce their concentration. Ternes et al. (2003) successfully treated a few antibiotics, beta blockers, anti-inflammatory drugs, metabolites of lipid-lowering agents and the natural estrogen estrone.

In the past, several studies have been carried out on oxidative processes for the decomposition of organic substances. Rehman et al. (2006) successfully treated waste water streams from the textile, paper, leather, and metal processing industries in Pakistan with ozone. They were able to significantly reduce color and COD. Hoersch and Frimmel (2000) concentrated in their work on the combination of chemical oxidation and biological purification of industrial waste water. Chemical oxidation should continue until the treated waste water is suitable for continuous biological treatment. Hoersch (2004) also pointed out that the effects of waste water oxidation include the formation of non-biodegradable reaction products, the formation of toxic reaction products, and the removal of biodegradable substances. The goal of Hoersch's work is comparable to studies by Abegglen et al. (2010). There, in a large-scale pilot test at the Regensdorf sewage treatment plant (Switzerland), the efficiency of ozonation regarding the removal of organic trace substances was investigated. In a further step, ecotoxicological studies were carried out and the

formation of undesirable toxic reaction products was examined. Schmidt et al. (2010) determined possible transformation products and their toxicological assessment of waste water in the Ruhleben sewage treatment plant (Berlin). As early as 1993, Schmitt and Hempel (1993) pointed out that the biodegradability of wastewater can be improved by targeted pretreatment with ozone. They demonstrated this using the example of biodegradable chlorinated aromatics in leachate from industrial and landfill sites. In addition to determining possible oxidation products, the effects on degradability are also qualitatively assessed. Moebius et al. (1996) similarly investigated the effects of ozone on waste water from the paper industry. Without going into detail about individual components, the elimination was investigated regarding COD (up to 48%), color (up to 72%) and AOX (up to 86%). Straub et al. (2020) examined the disposal of waste water containing capecitabine from a Mexican production facility. Ikehata et al. (2006) provided a very comprehensive overview of the attempts at degradation of pharmaceuticals with ozonation and advanced oxidation processes.

2.2.2. Ozonation as waste water pretreatment method

In most studies, waste water treatment with ozone is only an additional purification step. For this reason, ozone has been tested as the 4th cleaning stage in municipal sewage treatment plants for several years. The aim of ozonation is to remove trace substances that are not sufficiently broken down in biological processes and therefore occur in the effluent. The results of numerous publications provide information about the degree of elimination, the ozone concentration, the influencing factors, and the energy used for ozonation or the costs of ozonation. The use of ozone in waste water treatment plants should not necessarily achieve complete mineralization (Hoigné, 1982). Complete mineralization to form carbon dioxide, water, nitrate, sulfate, etc. can only be achieved through an extended ozonation time. In practice, the ozonation process is stopped long before full mineralization is reached. The decisive factor for the success of the treatment is the destruction of the pharmaceutically active centers of a molecule. However, it is also important to ensure that the reaction products formed during ozonation do not have any negative effects on the environment, for example due to increased bacterial toxicity.

In contrast to many publications about ozonation, in this work the waste water from the original pharmaceutical production is examined exclusively. The expected concentrations of the target

compound in waste water are much higher. In addition, mono-fraction waste water with a limited number of compounds is used. The focus of this work is on the comparison of the current waste water disposal by evaporation and incineration with the alternative method of pretreatment with ozone and the subsequent biological wastewater treatment.

2.3. Ozonation

2.3.1. Properties of ozone

The Dutchman van Marum first described ozone scientifically in 1785. Van Marum discovered the typical smell of ozone in electrical discharges and reported this to Cavendish of the Royal Society of London (Hosselet, 1971). Such a smell can be noticed even after a thunderstorm and reminds of fresh hay. The name ozone is based on the ancient Greek word "ozein" (smell) and was first given by Schoenbein (Hosselet, 1971). In 1840, Schoenbein first recognized ozone as a chemical substance. Ozone is characterized by the distinctive smell that can be perceived by humans at very low concentrations. From a physiological point of view, ozone causes serious health concerns. Many short-term health effects and chronic lung diseases result most probably from exposure to ozone. The toxic effects are attributed to its ability to generate free radical reactions in the biological system (Mustafa, 1990).

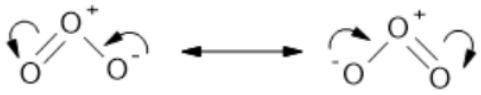


Fig. 2.3: Mesomeric boundary structures of ozone

One of the reasons why ozone is of great chemical interest is that due to its mesomeric boundary structures (Fig. 2.3) it can react electrophilically as well as nucleophilically (Constapel, 2005). Ozone is a very strong oxidizing agent, which is expressed in a high redox potential of 2.07 V (Mustafa, 1990). Only a few other substances show a similarly high redox potential (e.g. oxygen 2.42 V or OH radicals 2.8 V). For this reason, ozone is well suited to oxidizing complex organic molecules. The limitations of using ozone for such applications are the fact that ozone is very unstable and quickly breaks down into oxygen molecules. Storage or longer transport is not

possible. Therefore, the use of ozone is always in close proximity to the ozone generation. The stability of ozone and thus the amount that is available for oxidation depends on the temperature, the pH value, and the concentration of various ingredients (Abegglen, 2009).

2.3.2. Ozone generation

Ozone is a trivalent oxygen that is extremely unstable and easily breaks down into divalent and monovalent oxygen. In nature, ozone is mainly generated by high-energy absorption, on the one hand by high electrical voltage such as during a thunderstorm or by UV adsorption (e.g. in the stratosphere to form our ozone layer). These mechanisms are also used in industry to efficiently generate ozone. According to the theory of Chapman (1930), the photodissociation of oxygen molecules takes place at wavelengths < 242 nm.

According to information from Rüütel et al. (1999) there are various methods for generating ozone on a laboratory scale. Ozone can be generated by electrolysis of water, photochemically by UV light or with the additional use of TiO_2 as a catalyst, radiochemically by means of gamma radiation or by thermal dissociation. The technically most important process for generating ozone is the use of electrical discharges in a so-called ozonizer (Magara et al., 1995). This ozonizer consists of a gas space between two electrodes and a device to limit the intensity of the discharge. If a sufficiently high voltage is applied to the electrodes, discharges occur in the gas space between them. This type of discharge is known in the older literature as "silent discharge" (Hosselet, 1971 and Magara et al., 1995). When air or oxygen is passed through this space, ozone is created under the influence of the discharges that occur. When using air, a lower ozone yield due to the oxygen content and a 5-6 times higher volume flow can be expected. Depending on the concentration of nitrogen, carbon dioxide etc., but also on the supply air temperature and the moisture content, ozone formation rates of 2-16% can be achieved (Franco et al., 2008). The higher volume flow thus possibly leads to an increased stripping of trace substances (Noethe et al., 2007). Due to the short half-life, ozone formation and ozone depletion processes occur in an ozonizer. The ozone decomposition reactions increase due to a rise in temperature, higher ozone concentrations and the presence of moisture. For this reason, among other things, adequate dehumidification of the inflowing air or oxygen gas must be ensured and cooled during ozone formation. Such an ozonizer is basically constructed as shown in Fig. 2.4.

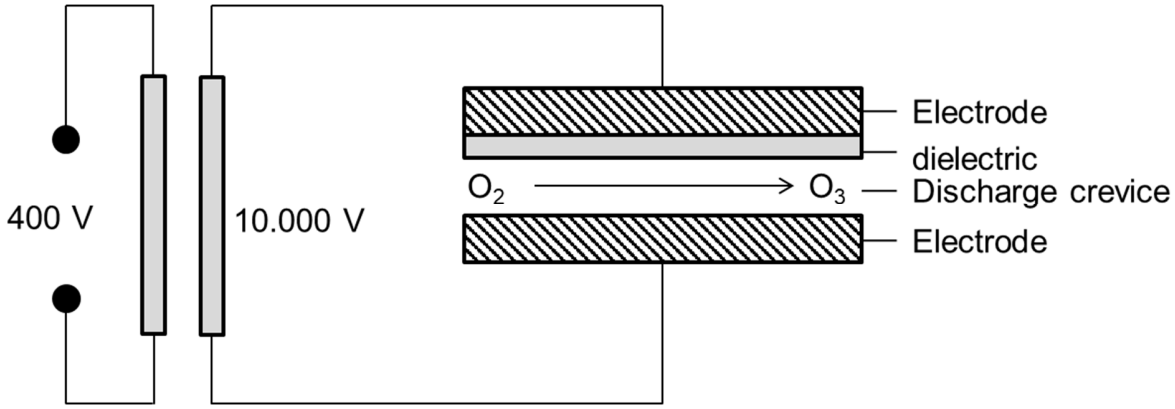


Fig. 2.4: Schematic image of an ozone generation according to the principle of the "silent discharge" (Rüütel et al., 1999)

2.3.3. Reactions of the ozone

Ozone is an unstable gas, which decomposes spontaneously. Because storage of ozone is not feasible, point of use should be very close to ozone generation. In addition to ozone decomposition due to elevated temperatures (Elovitz et al., 2000), ozone can also decay at higher pH values (Staehelin and Hoigné, 1982). Dissolved ozone is not stable in water and breaks down to form radicals. The kinetics depend on the characteristics of the water. There, ozone can react with organic and inorganic components. Degradation of organic and inorganic substances with ozone takes place via direct or indirect ozonation. In case of direct ozonation ozone molecules directly attack functional groups of the target compound to generate oxidized reaction products. This reaction is very selective with rather low reaction rate constants. The higher the electron density in organic compounds, the higher the reaction rate with ozone. According to Gottschalk et al. (2010), ozone reacts faster with certain aromatic and aliphatic substances, e.g. those that contain electron-donating groups such as hydroxyl or amino groups. The degradability depends on the molecular shape (cyclic or open chain), the spatial structure, the type and number of functional groups, the types of bonds and the polarity of the bonds.

In addition to the direct oxidation with ozone, there are also indirect reactions based on radicals, i.e. with molecules that lack a pair of electrons. They therefore react quickly and unspecific to compensate for this lack of electrons (Gottschalk et al., 2010). According to Hoigné (1982), O_3 decomposes before it reacts with dissolved substances and before it is stripped off. Decomposition

of ozone is catalyzed by OH- ions and accelerates with increasing pH. Due to their high reactivity, OH radicals only have a very short half-life.

To understand the formation of radicals the indirect reaction pathway is divided in three steps: initiation, radical chain propagation and termination (Staehelin and Hoigné, 1983). The first step is the ozone depletion triggered by OH ions. Substances that can convert OH radicals into superoxide radicals support this chain reaction. They are also called "promoters". Some organic and inorganic substances do not react with OH radicals to form the superoxide radical. By removing OH radicals, the chain reaction with such substances is interrupted. Such substances are called inhibitors (or scavengers). Due to their electrophilic properties, the OH radicals themselves react with the target compound at the positions with the highest electron density. The entire process of the direct and indirect ozone reaction is shown in Fig. 2.5. Since the reaction of ozone with organic substances is of greater interest for this study, I will explain various reaction pathways that are important for the target compounds examined.

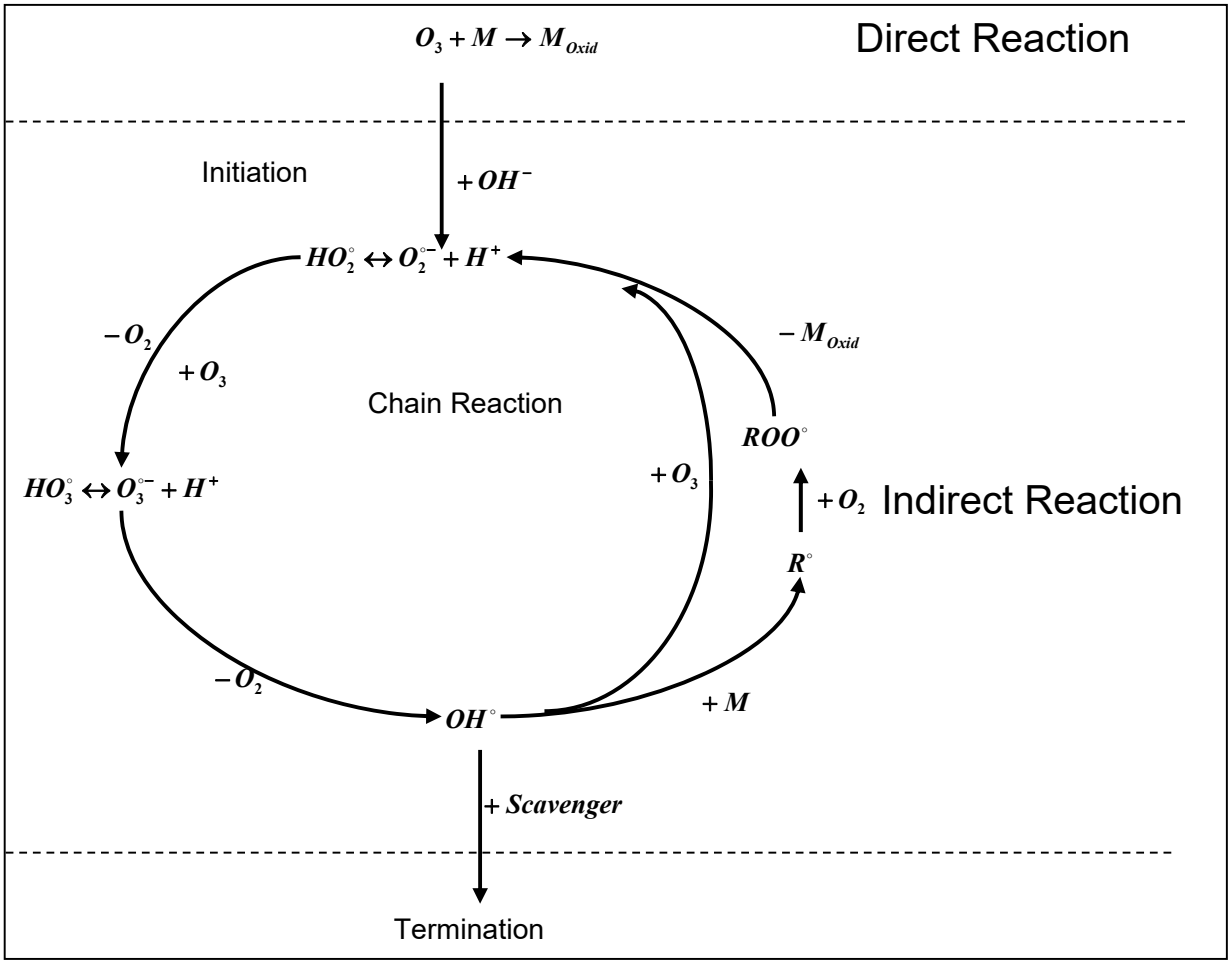


Fig. 2.5: Direct and indirect reactions according to Staehelin and Hoigné (1983) and Gottschalk et al. (2009)

Possible targets for ozone are unsaturated aliphatic compounds (i.e. double bonds). Such reactions take place according to the mechanism shown by Criegee (1975) in Fig. 2.6.

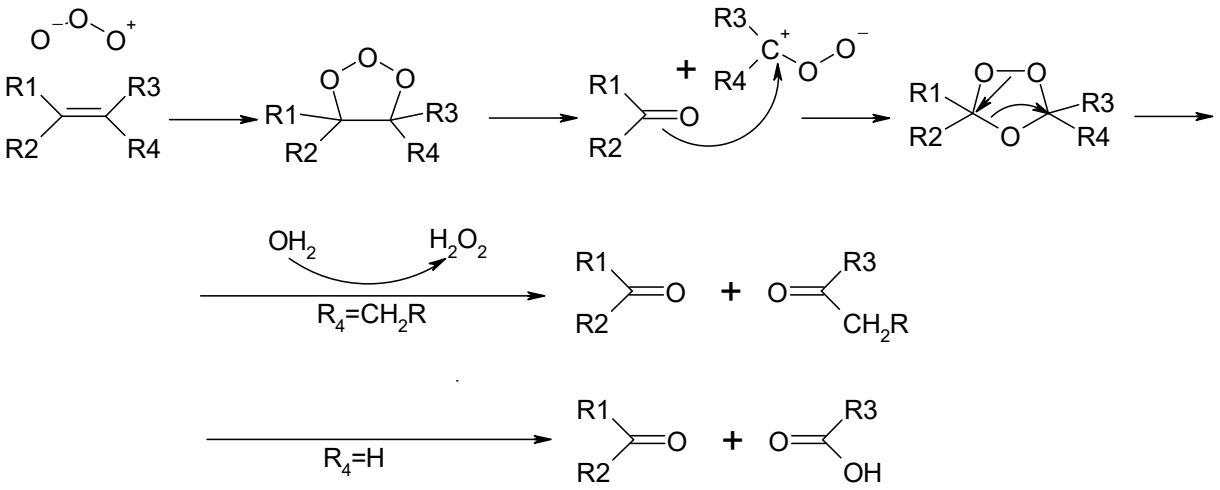


Fig. 2.6: Ozone reaction according to the Criegee mechanism (Criegee, 1975)

Due to its polar structure, ozone preferentially attacks unsaturated double bonds. The double bond cleaves with the temporary formation of the ozonide. In a further step the molecule breaks down into smaller molecules, which can contain aldehydes, ketones, or carboxylic acids (Beltrán, 2003). When two aldehydes are generated, a hydrogen peroxide radical, which is a precursor of the OH radical, can occur during the reaction. Ozone can also lead to hydroxylation on the aromatic ring (mainly in the ortho and para positions). According to Beltrán (2003), ozone can undergo three different reactions with organic substances in water: oxidation-reduction reactions, dipolar cycloaddition reactions and electrophilic substitution reactions. A possible fourth form of reaction is a nucleophilic addition. The reaction of ozone with aromatics also leads to the formation of ketones, aldehydes, dialdehydes and/or carboxylic acids according to the mechanism described by Benner (2009) using the Criegee mechanism via an ozonide stage.

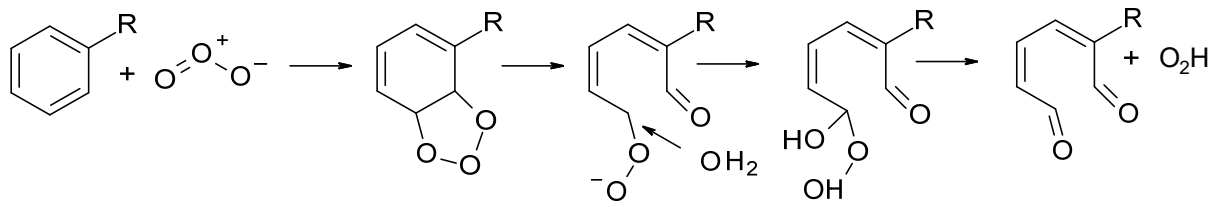


Fig. 2.7: Ozonation of an activated aromatic ring following the Criegee mechanism with formation of two aldehyde moieties (Benner, 2009)

For the ozonation of phenol, Mvula and von Sonntag (2003) investigated many more reaction products. Without describing all detected transformation products that can occur from direct and indirect reaction it is obvious that ozonation can lead to many different reaction paths and therefore, different transformation products.

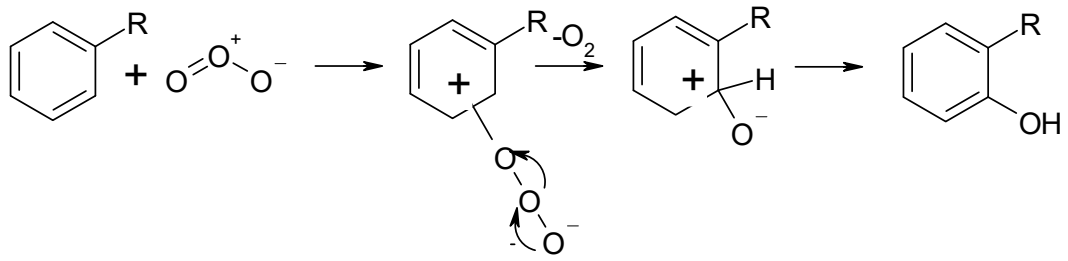


Fig. 2.8: Example for a possible reaction of an activated aromatic ring according to Mvula and von Sonntag (2003)

Another considerable reaction is the ozonation of alkylated amines. According to Brenner (2009), the reactivity depends on the number of substituents. The higher this number, the higher is the electron density and therefore the reactivity.

2.4. Technical model of the ozone reaction

2.4.1. Mass balances of ozone reaction

Ozonation tests were carried out in a batch reactor, prefilled with original waste water from pharmaceutical production. The reactor was operated with a constant ozone mass flow semi-batch-wise. The schematic installation of the experimental set-up and all incoming and outgoing mass flows is shown in Fig. 2.9. Only the most important influencing factors for the mass balances are displayed. Device-specific influencing factors such as reactor geometry, stirrer shape and speed, distribution of the gas phase are only described, but not examined in detail.

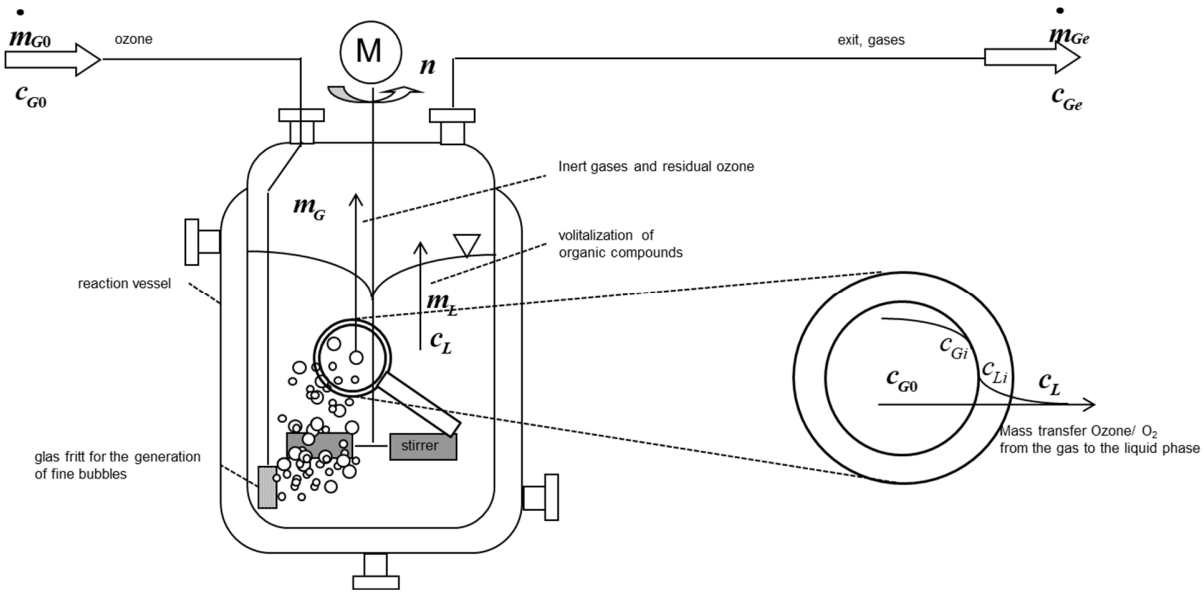


Fig. 2.9: Semi batch-reactor for ozonation experiments

For a semi batch reaction total mass balance can be described as following:

$$\frac{d}{dt} m_L + \frac{d}{dt} m_G = \dot{m}_{Ge} - \dot{m}_{G0}$$

$$\rho_L \frac{d}{dt} V_L + \rho_G \frac{d}{dt} V_G = \dot{m}_{Ge} - \dot{m}_{G0}$$

It is assumed that the gas volume above the liquid has no influence on the mass balance (d/dt V_G = 0). Furthermore, it is assumed that the samples taken from the reactor have a small volume and

are not considered in the mass balance (d/dt $V_L = 0$). Additionally, it is presumed that the density remains constant during ozonation.

In addition to the overall balance, the individual balances for ozone and for the target component can be delineated. The following equations describe the mass balances for ozone in the liquid phase:

$$V_L \frac{d}{dt} c_L = k_L a \cdot V_L \cdot E \cdot (c_L^* - c_L) - \sum_{i=1}^{n} r_{Li} V_{Li}$$

And in the gas phase:

$$V_G \frac{d}{dt} m_G c_G = Q_G \cdot c_{G0} - Q_G \cdot c_G - k_L a \cdot V_L \cdot E \cdot (c_L^* - c_L) - r_G m_G$$

According to Beltran-Heredia et al. (2001), an enhancement factor E should be considered to account for a better mass transfer than the one calculated. For the ozone mass in the liquid phase, the equation describes the supply of ozone through the transfer of ozone from the gas phase to the liquid phase. This mass flow is described by a driving concentration gradient between the saturation concentration c_L* and the concentration of ozone in the liquid phase. The totality of all devices and substance-specific influencing factors is summarized by the mass transfer coefficient k_L and the exchange area a. The depletion of ozone occurs mainly through a chemical reaction. Ozone can react directly with waste water constituents, but it can also decompose into OH radicals. These OH radicals can further react with components in the waste water. However, the ozone concentration in the liquid phase is limited to the maximum solubility in water so that no ozone accumulates in the liquid phase.

The mass balance for the gas phase can be simplified on the assumption that there is no reaction in the gas phase ($r_G = 0$). Furthermore, no significant change is to be expected due to the slight increase in temperature densities.

$$V_G \frac{dc_G}{dt} = Q_G (c_{G0} - c_{Ge}) - k_L a (c_L^* - c_L) \cdot V_L$$

Additionally, we insinuate that the concentration in the gaseous phase remains constant.

$$0 = Q_G (c_{G0} - c_{Ge}) - k_L a (c_L^* - c_L) \cdot V_L$$

$$c_L = c_L^* - \frac{Q_G}{k_L a \cdot V_L}(c_{G0} - c_{Ge})$$

2.4.2. Kinetics of ozone reactions

In addition to the mass balance for ozone, I will focus on the target components in waste water. In many cases the waste water examined consists of many waste water components, each of which has its own reaction kinetics. For this study, only waste water from the final purification of a pharmaceutical production was used. This means that there are only a limited number of different ingredients present, with the main compound being the most common in terms of quantity. When considering the overall treatment of waste water streams, it is initially assumed that adequate degradation not only requires the degradation of the target component, but also the transformation products generated. The focus, however, is on the target compound, which can decompose either through direct reactions with ozone or through indirect reactions with OH radicals (Gottschalk et al., 2010). Assuming that both the direct and indirect reactions of the target compound M take place during ozonation, the pseudo-first order reaction kinetics can be used for slowly reacting compounds in batch systems (Brenner, 2009).

$$r(M) = -\frac{d}{dt}c(M) = k_D c(M) c(O_3) + k_R c(M)\ \ c(OH^{\circ})$$

For further simplification it is presumed that the concentration of ozone and hydroxyl radicals remain constant during ozonation in the liquid phase.

$$r(M_i) = -\frac{d}{dt}c(M_i) = k_i \cdot c(M_i) \cdot c_L(O_3)$$

When ozone is added in a large excess compared to the concentration of the target compound, the ozone concentration is negligible and can be considered stable. Therefore, in this work we will use the apparent rate constant, which also includes the ozone concentration. Finally, the pseudo first order reaction for the target compound can be described by the following equation.

$$c(M_t) = c(M_{t=0}) \cdot \exp(-k_{ges} \cdot c(O_3) \cdot t)$$

2.5. Technical and ecotoxicological evaluation

The pretreatment of waste water by ozonation and subsequent biological treatment can be an alternative method to waste water incineration, the currently preferred method for non-biodegradable waste water streams. Since the economic comparison always depends on the current energy prices, the process comparison will only be made ecologically. In this work only the emission of CO_2 equivalents is considered. The method chosen here is based on the methodology of Remy et al. (2011) and Jiménez-Gonzáles (2001). For the ecological assessment, the direct CO_2 emissions in relation to the number of carbon atoms in the target compound are negligible. The content in the waste water is very low and the degree of degradation is most likely very high for both processes. Therefore, only greenhouse effects from indirect emissions are considered (e.g. electricity or cooling water). Since the inaccuracy in CO_2 calculations is relatively high, a very detailed investigation is not useful. For this reason, only those elements are considered that can be expected to make a significant contribution (see Fig. 2.10). For example, not all auxiliary and operating materials used are taken into account, as the share of the resulting CO_2 emissions does not significantly affect the overall ecological assessment.

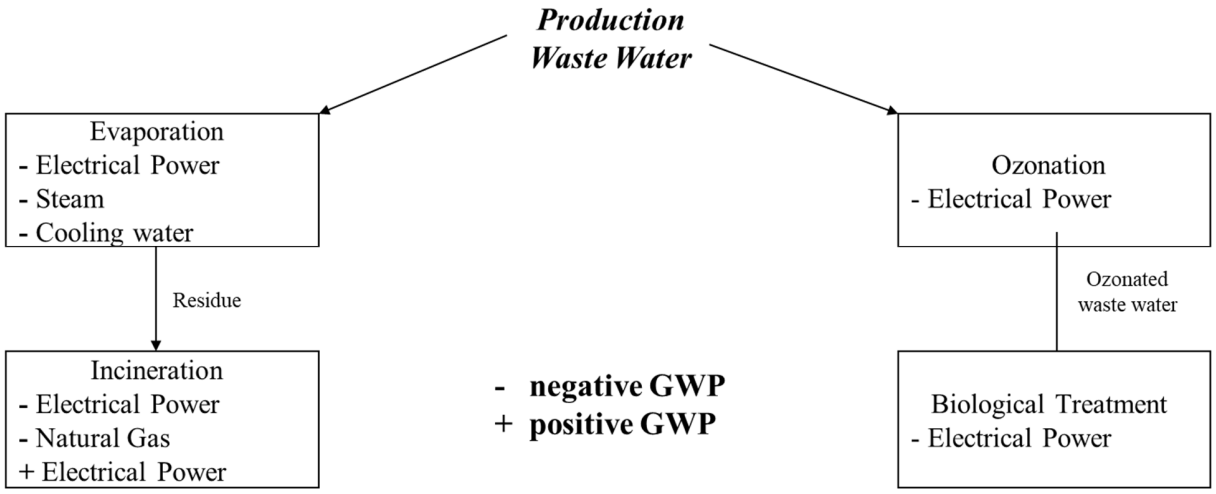


Fig. 2.10: Indirect emissions for evaporation/ incineration vs. ozonation/biological treatment and the impact on global warming potential (GWP)

A determination of the maximum residual concentration of the target compound in the environment is necessary both for disposal by incineration and by ozonation. In the case of a combustion process, a reduction of almost 100% is very likely. After ozonation of pharmaceutical

production waste water with subsequent biological treatment, the waste water directly drains off into receiving waters. To keep water pollution low, the PNEC or other target values are selected to determine the maximum concentration. Based on the concentration in the receiving waters, it is calculated backwards which concentration must be reached after ozonation. On the way from the outlet of the ozone reactor to its release into the receiving water, various dilution factors and degradation processes must be taken into consideration. At the Wuppertal site, the municipal sewage treatment plant in Buchenhofen is located downstream of the Rutenbeck industrial sewage treatment plant, as Bayer has not applied for direct discharge of the waste water into the river Wupper. Due to the official approval of the state government of Düsseldorf, the Rutenbeck sewage treatment plant has the status of an indirect discharger. The target component could thus be reduced in two stages. Since no data on the biological degradation of the target component were investigated in this work, only existing literature data are used for the calculation. If no literature data are available, a conservative approach is taken, assuming that the degree of elimination is 0%.

In the following, both the mass and energy balances are calculated for all process stages. Different CO_2 emissions were considered based on the primary energy sources used.

2.5.1. Waste water evaporation

An evaporation process in waste water treatment is not required to remove trace substances, but it does reduce operating costs. Assuming that the trace substances are non-volatile compounds, a large part of the water is evaporated in an on-site facility. In general, the condensate produced contains only pure water. It can be fed to the industrial sewage treatment plant. The evaporation step limits the amount to be disposed of in the incinerator to 10-20%. The volume reduction is of particular interest in the case of low-caloric liquid waste to keep the incineration costs low. In many cases, rotary evaporators or simple falling film evaporators are used as evaporation equipment. The entire waste water stream is heated from ambient temperature to boiling temperature and then evaporated. For the sake of simplicity, only the enthalpy of evaporation for water is considered. In most cases, the target compounds have a boiling point that is well above the evaporation temperature. After evaporation, tower cooling water is used for condensation and cooling.

2.5.2. Waste water incineration

The decomposition of target compounds and reaction by-products can be achieved by incinerating the residual waste water. After the water has evaporated and overheated, a temperature of 800-1200°C should be reached in the combustion zone. The temperatures are necessary to ensure complete decomposition and oxidation of all organic substances. When the oxidation is complete, all components are converted into the corresponding oxidation products. After combustion, the hot flue gases are cooled down and used to generate steam. The steam is then converted to electricity. Jiménez-González et al. (2001) provided information on the usable heat fraction. If all heat losses are considered, 75% of the calorific value of natural gas can be used to generate steam. When considering the overall efficiency of an incineration plant, it must be decided whether the heat can also be used effectively in a local or district heating network. According to Neuwahl et al. (2019) the efficiency of electricity generation is often below 25%, as most of the old systems are operated at temperatures below 400°C and pressures below 40 bars. Systems that work at temperatures above 420°C and pressures above 60 bars achieve a gross electrical efficiency of over 30%. For this work we assume electricity generation with an efficiency of 30%.

2.5.3. Biological treatment

The CO_2 emissions in biological waste water treatment plants are mainly caused by electricity, additives, and direct CO_2 emissions due to biological degradation. In general, the degradation of the target compound in waste water can be traced back to biodegradation and sorption on sludge. Data have been published for some of the compounds, but the literature values sometimes show significant deviations due to different treatment conditions and different composition of the biocenosis. Very often highly specialized industrial sewage treatment plants show a significantly better breakdown with different substances. The biochemical degradation is a result of microbial activity. Microorganisms use organic compounds as a source of carbon and energy. The use of a trace substance can lead to a transformation up to complete mineralization (CO_2 + H_2O + cell mass). An important factor to consider in this context is the age of the sludge. As the sludge ages, the microorganisms compete for more complex, less degradable substances, which generally include trace substances. In the presence of an easily degradable substrate and with strong

temporary substrate pollution, the degradation of trace substances can be impaired even when the sludge is old (Ternes et al., 2006). Some of the trace substances can also adsorb on the activated sludge and thus leave the system via the sludge path. The sorption behavior depends on the properties of the substance and the sludge. In addition to hydrophobicity, electrostatic interactions are also responsible for sorption on the activated sludge.

2.5.4. Waste water ozonation

The pharmaceutical waste water is ozonated in a 25 m³ batch reactor. Since the ozone supply was kept constant during ozonation, the electricity consumption was easy to calculate based on the ozonation time required. The ozonation time can be calculated based on the required final concentration of the target compound and the assessed rate constants. In theory, continuous processes are also possible, but since all tests in the laboratory system were investigated in a batch process and the large reactor is already in place, a continuous process was not considered.

2.6. Materials and methods

2.6.1. Production waste water

During production of active pharmaceutical ingredients, waste water occurs in most synthesis steps of the production process. The composition of the waste water is very different depending on the production process. Some of the waste water contains higher proportions of solvents, in many other cases the waste water only contains the target compound and reaction by-products. Each of these waste water streams is specified and examined at the start of production, with the appropriate disposal route being determined. Ideally, the wastewater can be treated in an industrial or municipal biological wastewater treatment plant. The suitability for this treatment route results from the biological degradability and the ecotoxicity for the aquatic environment in all receiving waters. For all other waste water flows, incineration with upstream evaporation is the current method of choice. Waste water incineration is very efficient, but also very expensive and resource intensive. Even if several waste water streams follow the same disposal route, the focus of this work is on a defined water composition that meets the following criteria:

- Sufficient amount of waste water
- Limited number of contaminants (target compound and by-products)
- Relatively high concentration of contaminants
- Low solvent content
- Disposal currently via incineration

The wastewater streams to be examined are exclusively waste water streams from the pharmaceutical production of Bayer Corp. at the Wuppertal site. Each of these waste water streams is currently being disposed of in approved waste incineration plants. In some cases, the waste water is evaporated beforehand to reduce disposal costs. In this work, the various waste water flows arise from the solid-liquid separation of suction filters or centrifuges. These “hotspots” are characterized by a high concentration of active pharmaceutical ingredients and their by-products, so that no unknown water matrix influences the analysis of degradation products and the reaction kinetics. Four different wastewater streams were tested and assessed in detail:

- Ciprofloxacin-containing waste water (see Chapter 3)
- Moxifloxacin-containing waste water (see Chapter 3)
- Rivaroxaban-containing waste water (see Chapter 4)
- DTPA-containing waste water (see Chapter 5)

Ozone tests are carried out on a 2-liter scale. The specific ozone reduction for various waste water components and various pH values is determined from these experiments. Samples are taken at predetermined times and examined for TOC and residual target compounds and reaction by-products as well as generated transformation products. To assess whether the biological degradation can then take place in the company's own sewage treatment plant, degradation tests are carried out with original activated sludge from this plant. In addition, ecotoxicological data are determined. To ensure adequate treatment with ozone, the required degree of elimination was calculated based on the maximum permissible concentration of the target component in the receiving water.

2.6.2. Laboratory equipment

A small laboratory plant for ozonation experiments is available at Bayer's waste water treatment plant in Wuppertal/Rutenbeck. The experimental design of ozonation was described in detail in Daoud et al. (2017; 2020). For all ozonation experiments, a stirred batch reactor of 156 mm in diameter and 265 mm in height was used (Fig. 2.11).

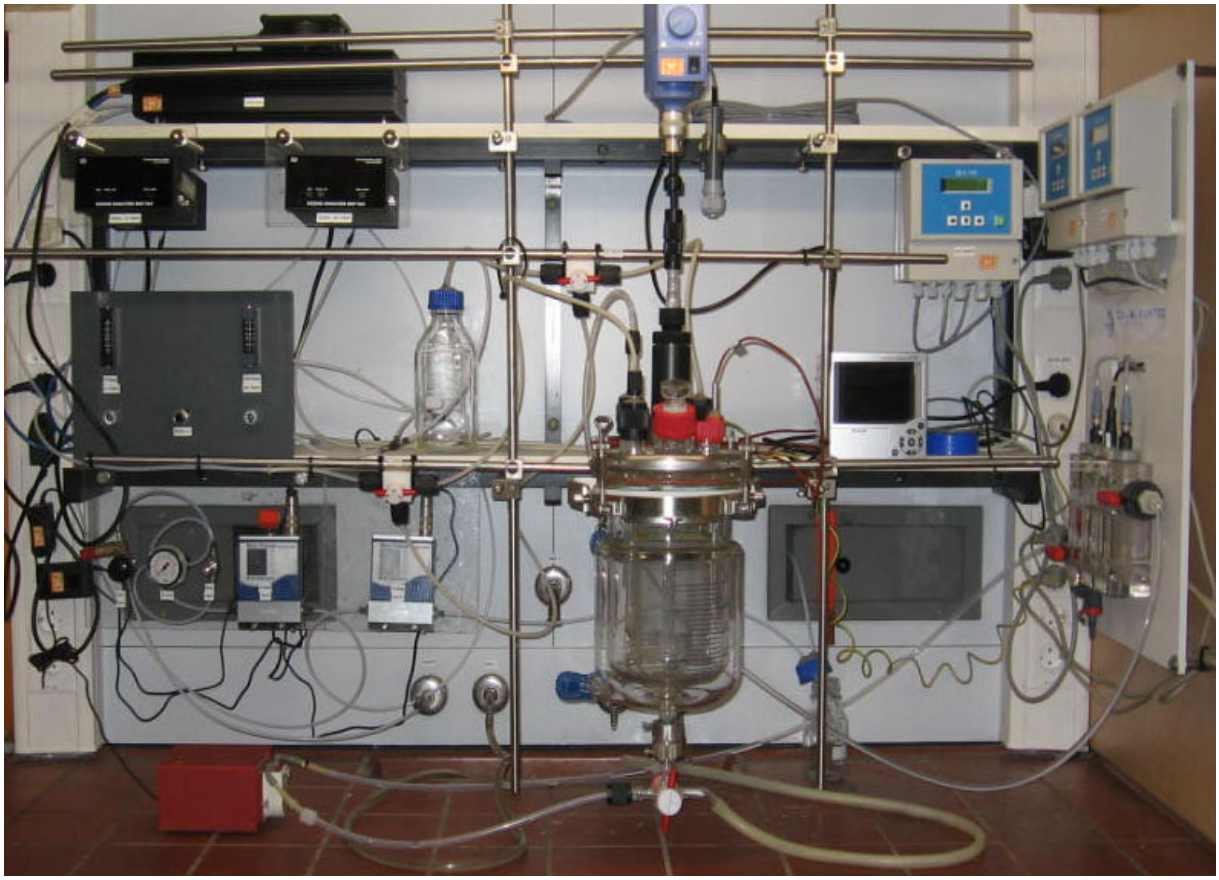


Fig. 2.11: Lab scale ozonation reactor (2L)

The reactor was filled with a predefined amount of waste water (max. 2 L) and operated in a semi-batch manner. Prior to ozonation, the pH of the waste water was adjusted to pH 3, pH 7 and pH 10 through addition of sulphuric acid (H_2SO_4) or sodium hydroxide (NaOH). A corona discharge ozone generator (BMT 803, BMT Messtechnik GmbH, Berlin, Germany) was used to produce ozone from pure oxygen. Ozone was introduced at the bottom of the reactor via a gas sparger. Ozonation was conducted with a constant flow rate (approx. 100-120 g O_3/m^3 and 1 L/min). During ozonation the concentration and flow rate of the gas were measured in the inlet and outlet; for mass

balancing, the dissolved ozone concentration was gauged as well. Off-gas from the reactor was collected in a liquid trap before release to ambient atmosphere. Additionally, the reactor was equipped with temperature and pH measurement devices. For determination of the TOC concentration, liquid samples of approx. 30 mL were manually withdrawn through the sampling port into small 40 mL flasks and analysed at defined time intervals. Thereof, 1.5 mL samples were retrieved for LC-HRMS analysis. Promptly after sampling, the flasks were flushed with nitrogen to remove residual ozone.

2.6.3. Technical scale ozonation plant

The large-scale ozonation plant of the Bayer sewage treatment plant in Wuppertal-Rutenbeck consists of an ozonation reactor with a total volume of 25 m³ (Fig. 2.12). The plant was built in 2004 to reduce excess sludge from the industrial sewage treatment plant. This measure reduced the amount of excess sludge by up to 90%. Due to new contractual constellations, the plant was only used to a limited extent for this purpose in 2009, so that free capacities were available for the ozonation of special wastewater.

According to Daoud et al. (2021) the reactor was operated semi-batchwise and filled with a known volume of process waste water (approx. 20 m³). Ozone was generated from pure oxygen as a feed gas using a corona discharge ozone generator (Kaufmann-Umwelttechnik GmbH, Wehr, Germany) and introduced into a pumped circulation loop using a jet loop injection. The inlet concentration and the flow rate of the gas were kept constant during ozonation. The ozonation reactor was not equipped with pH measuring devices, so that continuous pH measurement was not possible. For this reason, the process wastewater was initially adjusted to the defined pH. The TOC concentration was assessed at defined time intervals. Liquid samples of approximately 2 litres were taken manually through the sampling port. Thereof, 100 mL samples were retrieved for HPLC analysis.

Fig. 2.12: Technical scale ozonation reactor (25 m³)

Chapter 3: Ozone pretreatment of process waste water generated in course of fluoroquinolone production

Fares Daoud[a], David Pelzer[b], Sebastian Zuehlke[b], Michael Spiteller[b], Oliver Kayser[c]

[a] Bayer AG, Friedrich-Ebert-Str. 217-333, 42096 Wuppertal, Germany

[b] Institute of Environmental Research (INFU), Faculty of Chemistry and Chemical Biology, Technical University of Dortmund, Otto-Hahn Str. 6, 44227 Dortmund, Germany

[c] Department Technical Biochemistry, Faculty of Biochemical and Chemical Engineering, Technical University of Dortmund, Emil-Figge-Str. 66, 44227 Dortmund, Germany

FD conceived of the design of the study, carried out the ozonation experiments and provided the samples for quantification and identification of parent and transformation products. DP and SZ elaborated calibration curves with available standards and conducted LC-HRMS and MSn analysis. DP compiled part of the data in his master thesis. MS interpreted the mass spectra and the fragmentation patterns. All compiled data from ozonation trails and analytics were reviewed by OK. FD generated the draft of the manuscript. All authors read and approved the final manuscript.

Published in Chemosphere (2017), Volume 185, Pages 953-963

Abstract

During production of active pharmaceutical ingredients, process waste water is generated at several stages of manufacturing. Whenever possible, the resulting waste water will be processed by conventional waste water treatment plants. Currently, incineration of the process waste water is the method to eliminate compounds with high biological activity. Thus, ozone treatment followed by biological waste water treatment was tested as an alternative method. Two prominent representatives of the large group of fluoroquinolone antibiotics (ciprofloxacin and moxifloxacin) were investigated, focussing on waste water of the bulk production. Elimination of the target compounds and generation of their main transformation products were determined by liquid chromatography – high resolution mass spectrometry (LC-HRMS). The obtained results demonstrated, that the concentration of moxifloxacin and its metabolites can be effectively reduced (> 99.7%) prior entering the receiving water. On the contrary, the concentration of ciprofloxacin and its metabolites remained too high for safe discharge, necessitating application of prolonged ozonation for its further degradation. The required ozonation time can be estimated based on the determined kinetics. To assure a low biological activity the ecotoxicity of the ozonated waste water was investigated using three trophic levels. By means of multiple-stage mass spectrometry (MS^n) experiments several new transformation products of the fluoroquinolones were identified. Thus, previously published proposed structures could be corrected or confirmed.

3.1 Introduction

Antibiotics, such as fluoroquinolones, are essential drugs to cure human beings and animals that suffer from bacterial infections with severe consequences for their health. The first synthetic quinolone antibiotic ever developed was nalidixic acid (Lesher et al., 1962). While it was found to possess antibacterial property, its activity against Gram-negative microorganisms has been proved but was limited (Blondeau, 2004). Structurally, quinolones are characterized by the presence of an exocyclic oxygen and a carboxyl group at the naphthydridine nucleus (Appelbaum and Hunter, 2000). Improved activity against Gram-negative organisms was achieved through addition of piperazine, while the development of third generation quinolones also involved a fluorination. The newly established group of fluoroquinolones attained a broader spectrum of activity and showed enhanced effectiveness against Gram-positive bacteria (Appelbaum and Hunter, 2000). Fluoroquinolones belong to the family of gyrase inhibitors. They irreversibly inhibit microbial topoisomerase II (gyrase A subunit), an enzyme indispensable for the replication of DNA (Blondeau, 2004).

Bayer launched their first fluoroquinolone ciprofloxacin in 1981, followed by the second generation drug, moxifloxacin (Appelbaum and Hunter, 2000). Both antibiotics are composed of the quinolone-core carrying diverse moieties at defined positions. The common structural elements are the cyclopropyl substituent, the essential carboxylic group, the exocyclic oxygen and the fluorine atom, while specific substituents, the piperazine moiety for ciprofloxacin and the pyrrolo pyridine ring system in the case of moxifloxacin constitute their differentiating characteristics. The latter antibiotic additionally carries a methoxy group (Fig. 3.1). All fluoroquinolones are chemically synthesized active pharmazeutical ingredients.

In the course of production of fluoroquinolones, waste water is generated at several steps of the synthesis. This waste water has to be disposed of, but common WWTP's are not suitable because even low concentrations of fluoroquinolones can impair the effectiveness of biological waste water treatment (Dodd et al., 2006). While it is effective against bacterial infections in humans and animals, the biological activity of fluoroquinolones also causes a considerable challenge in biological waste water treatment. Therefore, in industrial countries, fluoroquinolone-containing waste water from production sites is not entering municipal or industrial WWTP's but is subjected to incineration or comparable treatment methods. As incineration of liquids characterized by a low

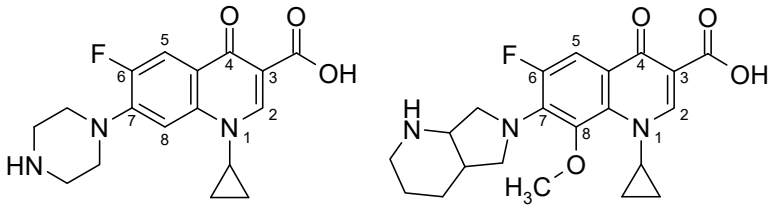
heat of combustion is very expensive and energy-consuming (Zhao et al., 2009), ozonation-based treatment was investigated in this study as an alternative. In the last twenty years, several attempts to remove the concentration of fluoroquinolones via ozonation have been made (Dodd et al., 2006), but all cited reports relied on analysis of either WWTP effluents or trace-level contaminated samples, both with high and low concentrations of fluoroquinolones. The investigated original process waste water discharged directly after the final separation step of the fluoroquinolone production, showed high concentrations close to the saturation point. Incineration has become an effective method for the elimination of process waste water containing significant levels of highly bioactive compound. The objective of this work was to evaluate the potential of ozonation for the effective removal of contaminants from process waste water, generated in course of industrial production of fluoroquinolones. The ozonation process shall assure an adequate reduction of the main contaminants and all newly formed transformation products with minor burden for the receiving waters. Therefore, knowledge about the transformation products and their achievable concentrations as well as the impact on the aquatic biota is required.

3.2. Materials and methods

3.2.1. Process waste water and active ingredients

Waste water from the last purification steps of ciprofloxacin and moxifloxacin production processes was used for ozonation experiments. In both cases, process waste water was collected from the final purification step, the crystallization for the removal of reaction by-products and subsequent solid-liquid separation. Contingent upon its solubility, the process waste water contains by-products and the target compound, but also small amounts of used solvents (e.g. ethanol). Higher amounts of solvents could have a significant impact on the ozone consumption. No other by-products from the previous synthesis steps are present. This reduces the number of transformation products from ozonation and interdependencies with other chemical compounds. Waste water from both syntheses was about pH 12. Ciprofloxacin containing waste water showed high TOC of > 20000 mg L^{-1}, whereas moxifloxacin containing waste water had TOC of approx. 7000 mg L^{-1}, substantiated by different amounts of solvents. For liquid chromatography – high resolution mass spectrometric evaluation (LC-HRMS), analytical standards of ciprofloxacin (1-

cyclopropyl-6-fluoro-7-piperazin-1-yl-1,4-dihydro-4-oxoquinolone-3-carboxylic acid) (Fig. 1), decarboxylated, defluorinated, ring-open (piperazine) ciprofloxacin as well as moxifloxacin (1-cyclopropyl-6-fluoro-7-piperazin-1-yl-1,4-dihydro-4-oxoquinolone-3-carboxylic acid) (Fig. 3.1) and decarboxylated moxifloxacin were provided by Bayer Corp., Leverkusen, Germany.



A **B**

Fig. 3.1. Structure of ciprofloxacin (A) and moxifloxacin (B)

3.2.2. Experimental setup

All experiments were conducted in a stirred batch reactor of 156 mm in diameter and 265 mm in height (see supplementary material). The reactor was operated in a semi-batch manner and filled with a known volume of waste water (max. 2 l) before ozonation. Ozone was made of pure oxygen, applied as feed gas, using a corona discharge ozone generator (BMT 803, BMT Messtechnik GmbH, Berlin, Germany) and introduced at the bottom of the reactor via a gas sparger. For all experiments inlet concentration and flow rate of ozone were held constant during ozonation (100-120 g m^{-3} and 1 L min^{-1}, respectively). Ozone concentration in the off-gas from the reactor was measured and its excess collected in a liquid trap before releasing to ambient atmosphere. During ozonation, the inlet, off-gas and dissolved ozone concentrations were continually measured, as were the temperature and pH. Prior to ozonation, the pH of the process water was adjusted to either pH 3, pH 7 and pH 10 through an addition of sulphuric acid (H_2SO_4) or sodium hydroxide (NaOH). pH had been maintained during ozonation by continuous addition of acid or caustic, but no buffer has been used. The maximum ozonation time was 300 min. TOC concentration was measured at defined time intervals. Therefore, liquid samples of approx. 30 ml were manually withdrawn through the sampling port into small 40 ml flasks. Thereof, 1.5 ml samples were retrieved for LC-HRMS analysis. Immediately after collection, the samples were flushed for approx. 30 s with nitrogen, in order to remove residual ozone.

3.2.3. Analytical measurements

pH and temperature were monitored by means of the PT100 compensated pH-electrode K100PR (Dr. A. Kuntze GmbH, Meerbusch, Germany). The mass flow was determined with the digital mass flow meter D-6300 (M+W Instruments GmbH, Leonhardsbuch, Germany). In gaseous streams, ozone concentration was measured by the ozone analyzers BMT 964 (BMT Messtechnik GmbH, Berlin, Germany), consisting of a double beam UV-photometer at a wavelength of 254 nm. The first ozone analyzer was located in the inlet gas stream, in line with the ozone generator; the second – in the reactor off-gas. Additionally, the concentration of the dissolved ozone was monitored inside the reactor by means of the potentiometric double electrode sensor Krypton K System (Dr. A. Kuntze GmbH, Meerbusch, Germany) with AuAu-600-OO-2-1-PG electrodes. All aforementioned data were collected with the Multi-Channel Recorder RSG30 (Endress+Hauser Messtechnik GmbH+Co. KG, Weil am Rhein, Germany) and saved on SD-card, followed by data export to Excel (Microsoft Windows). TOC was determined using the TOC-V_{CPN} analyzer (Shimadzu Deutschland GmbH, Duisburg, Germany) with the measurement range of 0.004-25.000 mg L^{-1}. ASI-V autosampler (Shimadzu Deutschland GmbH, Duisburg, Germany) is for feeding a water sample to TOC.

3.2.4. LC-HRMS measurements

Compounds were identified and quantified by LC-HRMS (LTQ-Orbitrap spectrometer, Thermo Scientific, Waltham, USA). Separation was achieved with a Surveyor-LC HPLC system (Thermo Fisher Scientific, Bremen, Germany) encompassing a quaternary, low-pressure mixing pump with vacuum degassing, an autosampler with a temperature-controlled tray (T = 8 °C), and a column oven (25 °C). Injection volume was 10 µl. For mass spectrometric detection, nitrogen was used as the sheath gas (6 arbitrary units) and helium served as the collision gas. Compound separation was performed on the Nucleodur Gravity C18 column (3 × 50 mm, 1.8 µm, Macherey-Nagel, Dueren, Germany) with the following solvent system: water (+ 0.5% formic acid) (A)/methanol (+ 0.1% formic acid) (B) (flow rate, 0.3 mL min^{-1}). Samples were analyzed using the following gradient program: 85% A isocratic for 3 min, linear gradient to 50% B within 9 min, to 100% B within 0.5 min and held for 11 min; the system returned to its initial conditions (85% A) within 0.5 min and

was equilibrated for 7 min. The spectrometer was operated in HESI positive mode (1 spectrum/s; mass range, 100-650) with the nominal mass resolving power of 60000 at m/z 400. Automatic gain control was used to provide high-accuracy mass measurements within 2 ppm deviation using one internal lock mass, m/z 391.284290; bis-(2-ethylhexyl)-phthalate. External calibration was performed at the concentration levels of 0.5, 2, 10, 50, 200, 1000, and 5000 ng mL^{-1} in water:methanol (v:v, 80:20). Calibration curves were generated using the reference standards available at Bayer Corp. Sum formulae were derived by comparison of measured and theoretical mass-to-charge ratios (m/z) within deviation < 2 ppm. To elucidate their structural formulae – the arrangement of atoms and chemical bonding, tandem and triple-stage mass spectrometry (MS-MS and MS^n) experiments were performed by Pelzer (2015). Collision induced dissociation (CID) was used for fragmentation, helium served as collision gas. Unknown components could be identified by comparison of fragmentation patterns with fragmentation of the reference standards and pure substances (see supplementary material).

3.2.5. Ecotoxicological parameters

Ozonated process water cannot be released to receiving water bodies without additional biological treatment. In order to evaluate its remaining toxicity, ecotoxicological parameters as well as the decrease in TOC content (Zahn-Wellens testing, DIN EN 9888) were measured. Therefore, ozonated waste water prior and after biological treatment has been tested. In order to simulate realistic conditions for biological treatment, tests have been conducted in an aerated reactor with originally activated sludge from the connected WWTP. The experiments were performed at a laboratory certified for ecotoxicological evaluation (Gobio GmbH, Aarbergen, Germany). In order to analyze the toxicity for aquatic biota, tests with three trophic levels have been conducted. For safe disposal without impact on the aquatic biota, the minimum dilution factor (G) for the drainage of process water into receiving waters had to be determined. Because bacteria, algae and invertebrates react differently on contaminants all trophic levels have to be taken into account, thereof the highest dilution factor has to be considered for safe disposal. The evaluation of dilution factors (G) for bacteria (GL), algae (GA) and invertebrates (GD) has been performed according to DIN EN 38412 L30, L33 and L34. Due to the fact that the Zahn-Wellens test has to be performed with a maximum TOC of 400 mg L^{-1}, the investigated process water was appropriately diluted.

3.3. Results and discussion

3.3.1. Ozonation

As the degree of fluoroquinolone degradation and the generation of their transformation products significantly depend on the pH, the ozonation experiments were performed under acidic, neutral and basic conditions (pH 3, 7 and 10). The ozone consumption was calculated according to Gottschalk et al. (2010). For ciprofloxacin, the highest ozone consumption rates were measured in neutral and basic reaction environments, while under acidic conditions the obtained values were 3-4 times lower (see supplementary material). The curves display over the 300 min ozonation time a straight line, which proves a constant reaction of ozone with available reactants. For moxifloxacin, the highest ozone consumption was registered under basic conditions (see supplementary material). The curves demonstrate under neutral and acidic conditions straight lines, but under basic conditions, the curve converges to a limiting curve which is an indication of a decelerating ozone reaction. In both cases, the obtained results can be attributed to the increased generation of hydroxyl radicals under basic conditions (Staehelin and Hoigné, 1982). These results prove that best ozonation results could be achieved under basic conditions. Based on these results further MS-experiments have been performed under basic conditions.

3.3.2. Mass spectrometric identification and quantitation

To check the variety of transformation products, samples after 300 min ozonation at all tested pH values were analyzed. Full degradation kinetics was studied in detail for conditions with the highest degradation rate of fluoroquinolones. For ciprofloxacin (initial concentration 3244 mg L^{-1}), the degradation was most pronounced under basic conditions (pH 10), reaching > 99.8%, with diminishing values recorded in alternative reaction environments (98.8% at pH 7 and 73.9% at pH 3). Degradation of moxifloxacin (initial concentration 5637 mg L^{-1}) was so fast, that detection limit (1ng mL^{-1}) was reached within defined ozonation time for all reaction conditions. Subsequent, in-depth analyzes were focused on ozonation under basic conditions.

Based on available reference standards, calibration curves were delineated for quantification of all detected components. Although reference samples were accessible for only a few relevant

compounds, concentration values of other transformation products were calculated assuming comparable ionization to that of parent antibiotic in full scan mass spectrometric detection. Relevant target compounds were quantified after distinct time of ozonation (0, 15, 30, 60, 120, 180, 300 min) and the obtained values were used for determination of degradation kinetics and calculation of rate constants. Verification of the structure of the transformation product was done by means of MS-MS and MS^3.

Summing up the content of Ciprofloxacin together with all other by-products and transformation products resulted in approx. 7200 mg L^{-1} at time 0 min. The total concentration kept constant for the first 120 min and then dropped down to 2600 mg L^{-1} at 300 min ozonation time. Transformation products with lower molecular weight were expected, but small transformation products below 100 g/mol have not been identified by the used analytics. Additionally, partial mineralization could have occurred which resulted in a carryover of components with low vapor pressure into the gaseous phase. Same was observed with the TOC which dropped from 28200 mg L^{-1} down to 18100 mg L^{-1} (see supplementary material).

The content of Moxifloxacin together with all other by-products and transformation products sums up to in approx. 5675 mg L^{-1} at time 0 min. The total concentration of moxifloxacin and all adjacent transformation products decreased rapidly. After 180 min all transformation products were below detection limit. Most probably all transformation products were degraded to small molecules (< 100 g mol^{-1}) and partly exited the reactor via offgas. During ozonation, the corresponding TOC showed a decrease to almost 50% (see supplementary material). Fig. 3.2 and 3.5 show mass spectra extracted from the total ion chromatograms of ciprofloxacin and moxifloxacin after 0 and 300 min of ozonation, as well as the sum formulae of compounds corresponding to the most prominent signals.

Ciprofloxacin

Prior to ozone treatment, the process waste water samples contained, in addition to ciprofloxacin (m/z 332.1405), its decarboxylated form (m/z 288.1506) and a chloroquinolone (vs. fluoroquinolone) derivative (m/z 348.1108) (Fig. 3.2). Post-ozonation, all main constituents of the original process water sample were almost completely degraded, while a great number of minor components could be determined (some are highlighted).

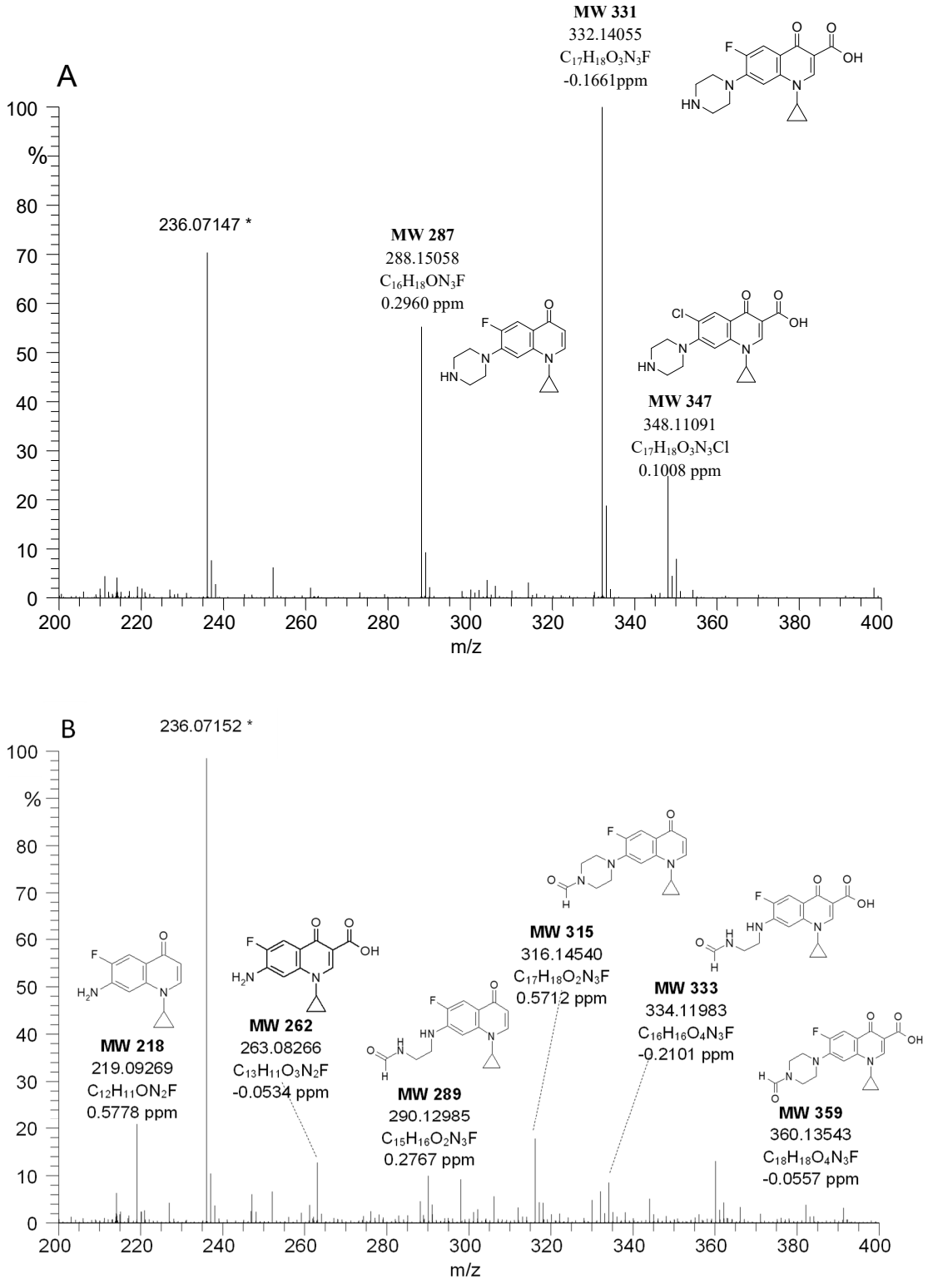


Fig. 3.2. Mass spectrum (extracted from total ion chromatogram) of ciprofloxacin process water before ozonation (**A**) and mass spectrum (extracted from total ion chromatogram) of ciprofloxacin process water after 300 min of ozonation (**B**) (*impurity from the LC-MS-system)

The exact masses, sum formulae and retention times of 22 transformation products were elucidated by LC-HRMS (see supplementary material).

Various authors had worked on potential transformation products in the last couple of years. To our knowledge, no data on direct ozone pre-treatment of process waste water have been published to date. However, results of several reports provide valuable insights concerning potential fluoroquinolone transformation products. Other investigations focused on degradation via photolysis (Burhenne et al.,1997; van Doorslaer et al., 2011; Calza et al., 2008; Tuerk et al., 2012; Burhenne et al., 1997; Kabasci, 2007), ozonation (de Witte et al., 2008; Heynderickx et al., 2011; Kabasci, 2007; Kovalova et al., 2013; Rodríguez et al., 2008), or other advanced oxidation processes for the generation of OH radicals (Tuerk, 2006; Wang et al., 2010; de Witte et al., 2009; Rodríguez et al., 2008). Degradation of fluoroquinolones has been recently reviewed by Sukul and Spiteller (2007).

Thus, most of the postulated structures (and equivalent molecular masses) have been identified in previous research reports, using various analytical methods. Three of the identified transformation products were available as reference substances within this study. These molecules were, therefore, unambiguously identified by their exact mass, fragmentation pattern and retention time, as compared to the standards. Their calibration curves were further established. Most of the other detected transformation products were identified based on their mass spectrometric fragmentation. Four compounds were reported for the first time, others compared with previously postulated structures. Fig. 3 shows the decrease in concentration of ciprofloxacin within 300 min, from 3244 to 61 mg L^{-1} (98.1%). Concurrent to the antibiotic depletion, levels of several intermediates increased during ozonation. Initial and final concentrations of occurred reaction by-products as well as transformation products have been quantified based on available reference standards. Within 300 min of ozonation time the chloroquinolone compound started at 1981 mg L^{-1} and degraded down to 7.3 mg mg L^{-1} (99.6%), the ethylene diamine compound started at 88 mg L^{-1}, increased up to 853 mg L^{-1} (120 min) and declined to 153 mg L^{-1}. The defluorinated compound started at 188 mg L^{-1} and decreased below detection limit. Most of the 22 transformation products were quantified at concentrations below 1 % of initial ciprofloxacin content and are, therefore, not shown in Fig. 3.3.

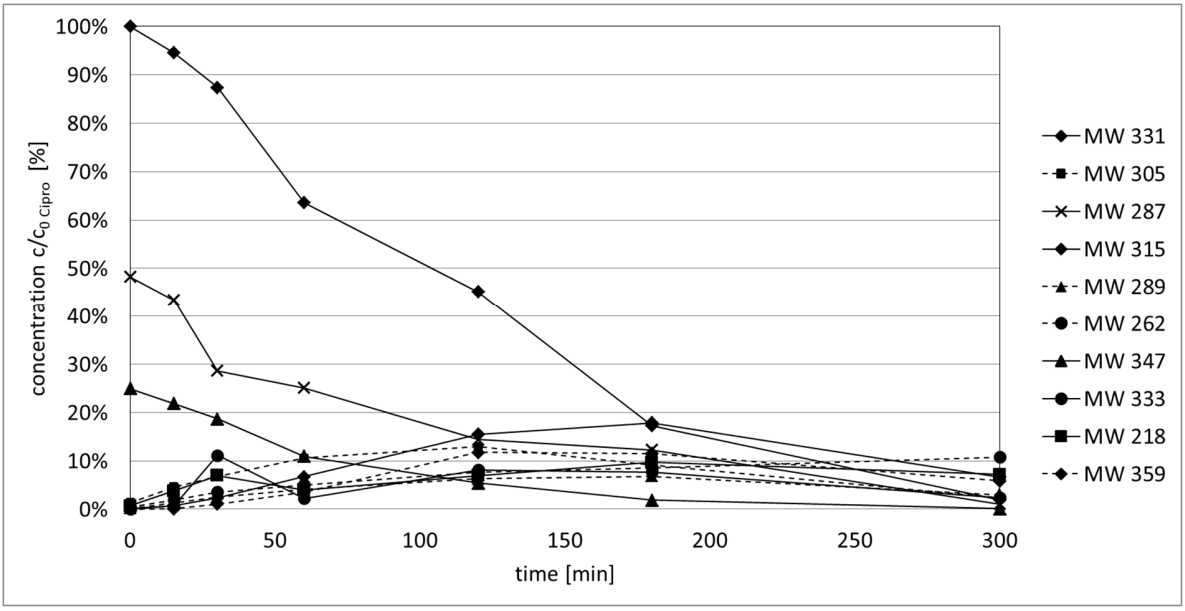


Fig. 3.3. Degradation of ciprofloxacin and formation of its most abundant transformation products ($c/c_0 > 5$ %) in course of waste water ozonation (relative to ciprofloxacin concentration at time 0 min)

Ciprofloxacin degradation occurred at various functional moieties of the molecule (Fig. 3.4). The piperazinyl substituent and the carboxyl group were most prone to disintegration. Additionally, defluorination at C-6 was observed, $C_{17}H_{19}N_3O_3$ (MW313), which is one of the known side-products of the synthesis of the antibiotic. Further, loss of the propyl moiety at N-1 leads to $C_{14}H_{14}FN_3O_3$ (MW291), a transformation product generated also by a combination of ozone and UV light (Tuerk et al., 2012). Three different products were generated from the parent molecule via oxidation of the piperazinyl substituent, either through its N-formylation ($C_{18}H_{18}FN_3O_4$, MW359), hydroxylation, ($C_{17}H_{18}FN_3O_4$, MW347) or addition of an exocyclic oxygen to the ring system, $C_{17}H_{16}FN_3O_4$ (MW345). In the two latter cases, localization of oxygen binding could not be determined by means of the applied analytical methods. MW359 was also induced by ozone/UV (Tuerk et al., 2012) or photolytic degradation (Cardoza et al., 2005). MW 347 was previously generated by photolytic reaction (Calza et al., 2008), ozonation (de Witte et al., 2008) or a combination of ozone and UV light (Tuerk et al., 2012). MW345 has been identified by photolytic degradation (Burhenne et al., 1997). While cleavage of the piperazine ring leads to the aldehyde metabolite, $C_{16}H_{16}FN_3O_4$ (MW333) its further oxidation generates a compound with two aldehyde groups in the ring system, $C_{17}H_{16}FN_3O_5$ (MW361). MW 333 has also been detected by photolytic reaction (Calza et al., 2008), ozonation (de Witte et al., 2008), photo-catalytic degradation (Wang et al., 2010) as well as ClO_2 induced oxidation (Paul et al., 2010). MW361 was identified by Wang

et al. (2010) and Paul et al. (2010). In the consecutive step, the piperazine moiety is further degraded to an ethylene diamine moiety, $C_{15}H_{16}FN_3O_3$ (MW305), a reference standard from Bayer Corp. Additionally MW305 is a transformation product that has been detected in the course of several different degradation processes (Burhenne et al., 1997; Calza et al., 2008; De Witte et al., 2008; An et al., 2010; De Witte et al., 2009; Thabaj et al., 2007; Cardoza et al., 2005; Paul et al., 2010). An et al. (2010) identified comparable transformation products of norfloxacin, levofloxacin and lemofloxacin.

Instead of the aforementioned generation of a double aldehyde within the piperazine moiety, further oxidation of the primary aldehyde to a carboxyl group is possible, $C_{16}H_{16}FN_3O_5$ (MW349). De Witte et al. (2008) identified also by ozonation a transformation product with the same chemical formula but proposed its alternative structure, an aldehyde group at a different location of the piperazine moiety. Exact location cannot be verified with currently available fragmentation pattern. Based on the structural formula of MW305, full cleavage of the piperazine moiety leads to a compound with an amino group at C-7, $C_{13}H_{11}FN_2O_3$ (MW 262). Also, here a broad variety of degradation processes lead to this transformation product (Burhenne et al., 1997; de Witte et al., 2008; Wang et al., 2010; An et al., 2010; Tuerk et al., 2012; de Witte et al., 2009; Cardoza et al., 2005; Paul et al., 2010). MW305 can be further oxidized to $C_{14}H_{11}FN_2O_4$ (MW 290), a transformation product previously identified by Paul et al. (2010) by photo-catalysis.

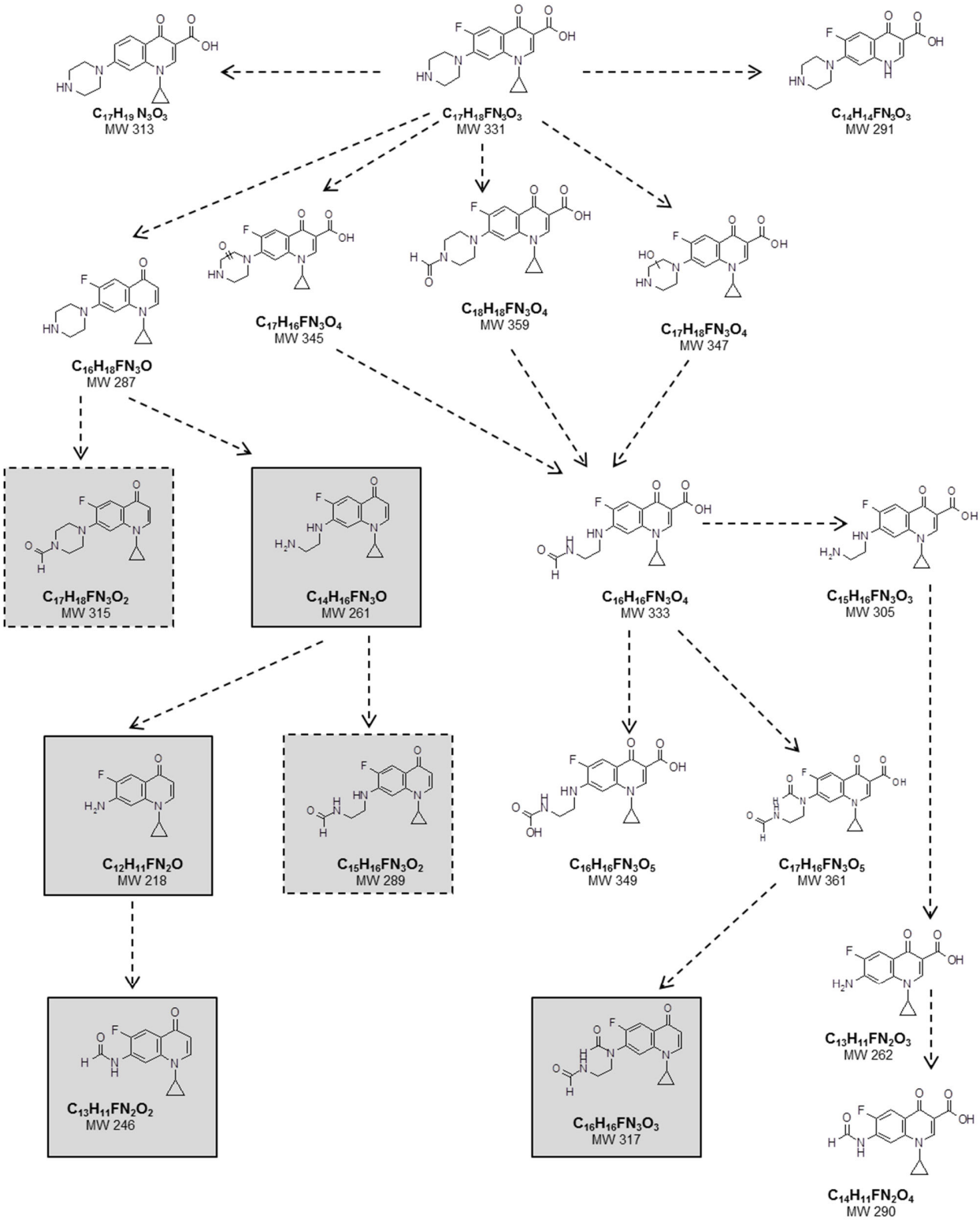


Fig. 3.4. Ozonation pathway of ciprofloxacin. Newly identified degradation products in grey boxes highlighted; highlighted compounds within dotted-line boxes have been published previously (de Witte et al., 2008; de Witte et al., 2009) as molecules defined by the same sum formulae but of different chemical structure

As indicated before, a decarboxylated compound, $C_{16}H_{18}FN_3O$ (MW287) was also identified. Its further degradation entails either a cleavage of the piperazine moiety or its oxidation. The latter leads to a molecule comparable to MW359, containing an N-formylated piperazine substituent but no carboxyl group, $C_{17}H_{18}FN_3O_2$ (MW315). Also, in this case, De Witte et al. (2009) identified by ozonation a transformation product with the equivalent chemical formula but of disparate structural formula. Again, fragmentation pattern does not offer valuable clues to the location of the aldehyde group. Cleavage of the piperazine moiety leads, in the decarboxylated form, to $C_{14}H_{16}FN_3O$ (MW261), a structure that has not been previously published. Based on MW261, either an oxidation or a full degradation of the ethylene diamine moiety can occur. The former leads to $C_{15}H_{16}FN_3O_2$ (MW289), yet another molecule differentially defined by De Witte et al. (2008) on the chemical structure - a molecule with a pyrrol moiety and two exocyclic oxygen atoms. In this case the fragmentation pattern (see supplementary material) supports the assumed chemical structure, because the C_2H_5N-fragment has only been discovered in conjunction with a carbon monoxide fragment. Full degradation of the piperazine moiety, on the other hand, resulted in a molecule with an amino group at C-7, $C_{12}H_{11}FN_2O$ (MW218) – a new transformation product that has not been published before. MW218, as well as MW289, can be further oxidized to two previously unknown molecules, $C_{13}H_{11}FN_2O_2$ (MW246) and $C_{16}H_{16}FN_3O_3$ (MW317). MW246 contains an aldehyde moiety at the C-7 amino group and MW317 carries two aldehyde groups at both nitrogen atoms of the ethylene diamine moiety.

Furthermore, the concentration of the chlorinated ciprofloxacin metabolite (MW347) declined in course of the ozonation from 1981.0 (in the original waste water sample) to 7.3 mg L^{-1} (99.6%), while the decarboxylated metabolite (MW287) content was reduced from 1565.0 to 33.0 mg L^{-1} (97.9%). With regard to the fact that decarboxylated ciprofloxacin compounds are products of its ozonation and were already present in the process water at time 0 min, the degree of degradation might have been even higher. Based on the exponential shape of the ciprofloxacin degradation curve pseudo-first-order rate constants can be determined from a plot of $\ln(c/c_0)$ versus time with rate constant k being the slope of the straight line (Prasse et al., 2012). For the degradation of Ciprofloxacin pseudo-first order reaction seemed to be the appropriate assumption indicated by a high linearity factor (R^2=0.934). Based on the concentration measured at different time intervals,

the apparent rate constant for a pseudo-first order reaction (see supplementary material) was 0.0115 min^{-1}.

Nevertheless 4 transformation products have only been identified with respect to their mass formula but MS^2 and MS^3 data were not appropriate to identify the chemical structure.

Moxifloxacin

In case of waste water samples derived from moxifloxacin (m/z 402.1825) production, the decarboxylated form of the antibiotic (m/z 288.1506) could also be detected (Fig. 3.5). It also shows the mass spectrum of the compound of interest after 300 min of ozonation. Similar to ciprofloxacin, ozone treatment of moxifloxacin resulted in detection of a high number of unknown degradation products. The exact masses, sum formulae and retention times of 13 transformation products were fully elucidated by LC-HRMS (see supplementary material).

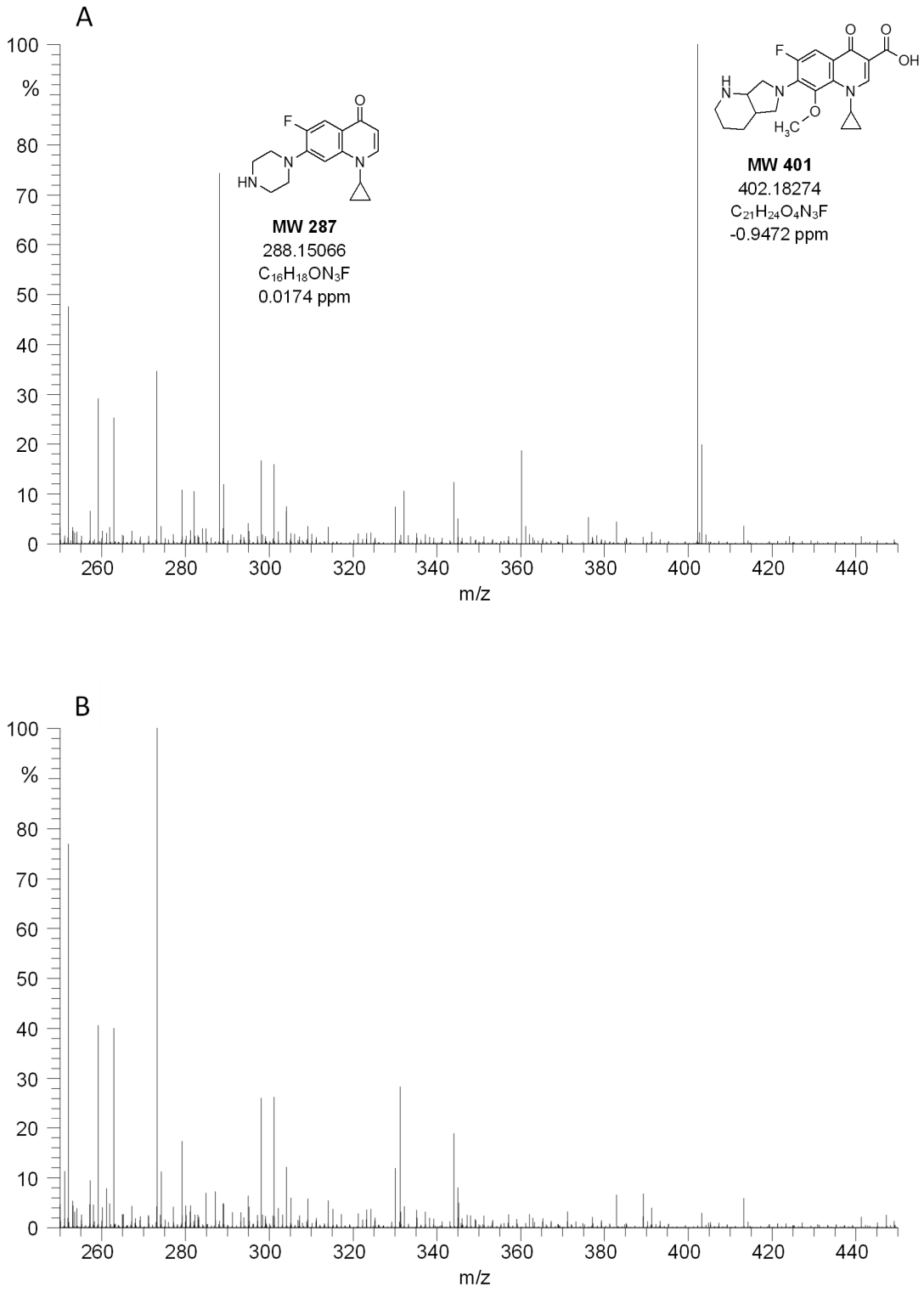


Fig. 3.5. Mass spectrum (extracted from total ion chromatogram) of moxifloxacin before ozonation (**A**) and mass spectrum (extracted from total ion chromatogram) of moxifloxacin after 300 min of ozonation (**B**)

None of the identified transformation products were available as reference substances. Thus, their identity was established through exact mass determination and comparative analyzes of their fragmentation patterns (as obtained in course of MS-MS and MS^3 experiments) and retention time values, relative to known related compounds. All transformation products detected have been identified for the first time.

As depicted in Fig. 3.6, the concentration of moxifloxacin decreased within 300 min of ozonation from 5637 to < 1 mg L^{-1} (> 99.9%). Levels of several transformation products rose and declined in course of the waste water ozone treatment. Thereof highest concentrations occurred for MW 292 with a peak of 178 mg L^{-1} at 15 min and for MW 307 with a maximum of 296 mg L^{-1} at 30 min ozonation time. After determination of degradation kinetics, based on measurements of individual product levels over time (rate constant for a pseudo-first order reaction established at 0.167 min^{-1}; see supplementary material), the ozonation pathway of moxifloxacin, as illustrated in Fig. 3.7, was elucidated. Moxifloxacin degradation occurs at different functional moieties of the molecule, as in case of ciprofloxacin.

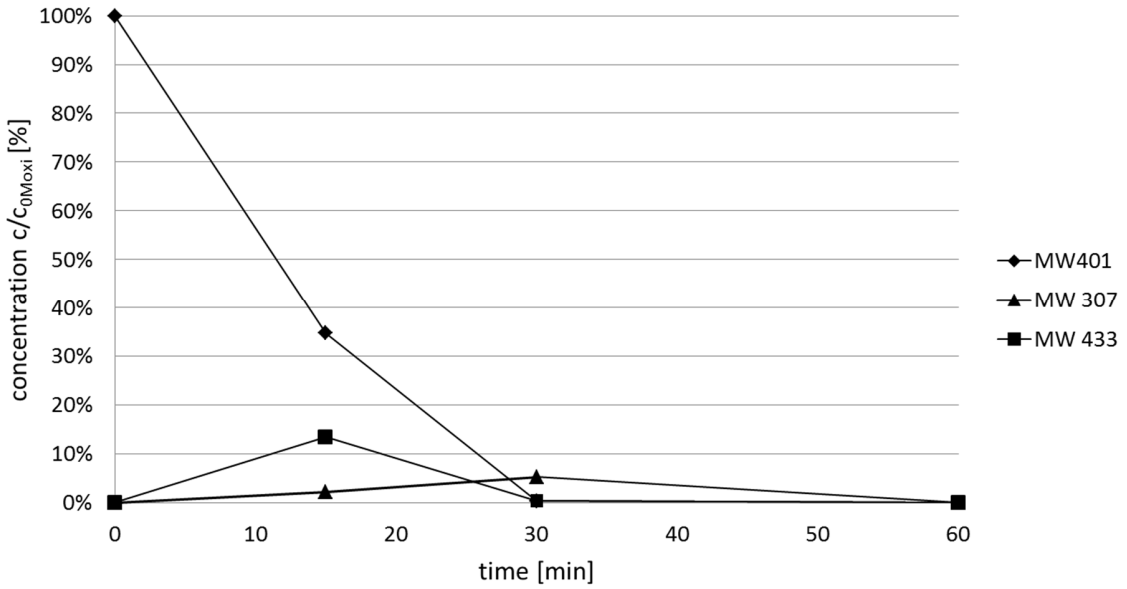


Fig. 3.6. Degradation of moxifloxacin and formation of its most abundant transformation products (c/c_0 > 5%) in course of waste water ozonation (relative to moxifloxacin concentration at time 0 min)

Loss of the carboxyl group at C-3 leads to $C_{20}H_{24}FN_3O_2$ (MW357). Another transformation product is generated by cleavage of the pyrrolo piperazine moiety at C-7 ($C_{19}H_{24}FN_3O_4$, MW377), while oxidative addition of two hydroxyl groups at the quinolone ring leads to $C_{21}H_{24}FN_3O_6$ (MW433).

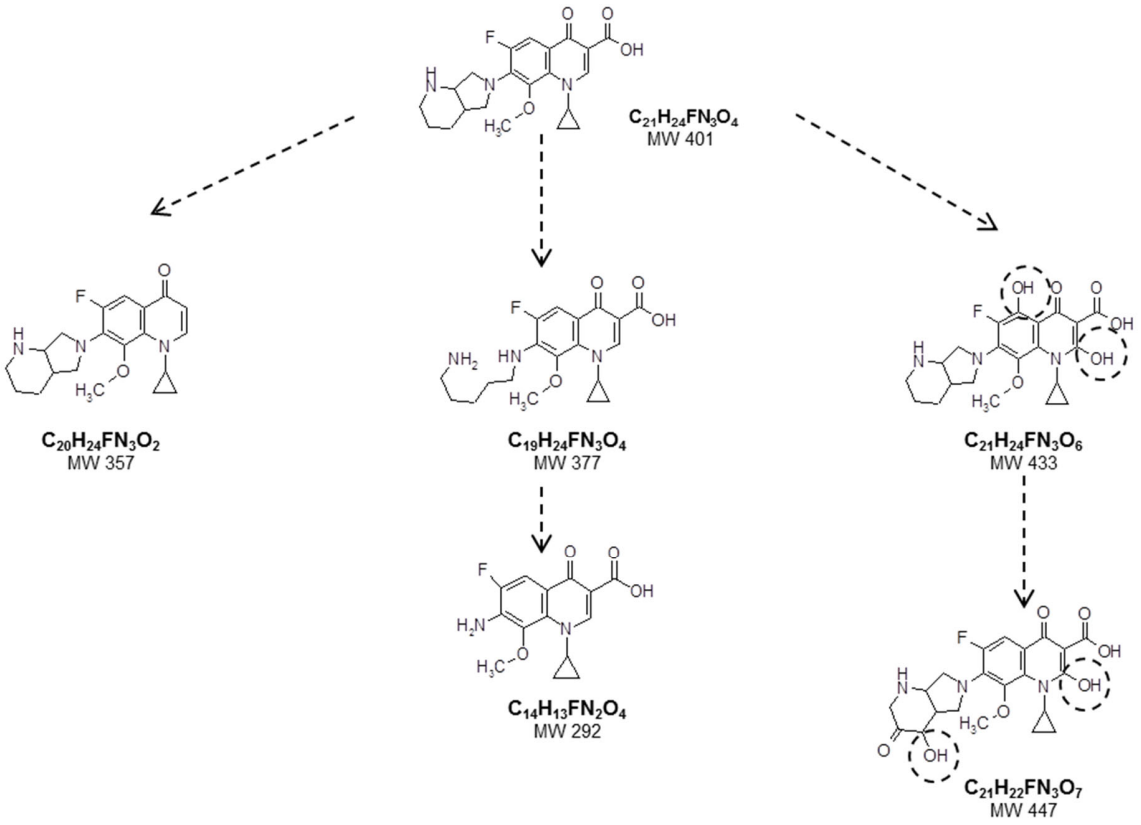


Fig. 3.7. Proposed ozonation pathway of moxifloxacin. All depicted compounds are newly identified degradation products and, to our knowledge, have not been published before. Position of one additional hydroxyl group (framed by dotted lines) may vary for MW433 and MW447

Further degradation of the pyrrolo piperazine moiety of MW377 can generate $C_{15}H_{13}FN_2O_4$ (MW304) and, in a consecutive step, oxidation leads to $C_{14}H_{12}FNO_7$ (MW325) with three additional hydroxyl groups at the quinolone ring.

Before mentioned transformation products MW292 and MW307 have been identified with respect to their molecular formula, but MS^2 and MS^3 data did not give appropriate evidence about the molecular structure. For MW292 the ozonation pathway shows at least the most probable structure.

3.4. Ecotoxicological investigations

Ciprofloxacin

Ozonated process water generated in course of ciprofloxacin production was characterized by the following EC_{50} dilution factors: $G_L = 40$ for bacteria, $G_A = 24$ for algae and $G_D = 48$ for daphnia. The Zahn-Wellens- test indicated TOC degradation of 34.6% after 7 days. Finally, the waste water was analyzed regarding its toxic impact on bacteria, algae and daphnia populations, after being submitted to biological degradation. The respective EC_{50} dilution factors were: $G_L = 18$, $G_A = 9$ and $G_D = 18$. The measured values indicated that, for safe disposal into receiving waters, the discharged ozonated water required dilution by a factor of 48, while the further biologically treated waste water had to be diluted at least 18 times. That means that toxicity to aquatic biota can be improved by treatment in industrial waste water treatment plants by the factor of approx. 2.5.

Moxifloxacin

The EC_{50} dilution factors for bacteria, algae and daphnia, characteristic of the investigated moxifloxacin production-derived ozonated process water, were: $G_L = 192$, $G_A = 128$ and $G_D = 32$, respectively. The Zahn-Wellens- test indicated TOC degradation of 86.7% after 7 days. The ozonated waste water was then subjected to biological degradation treatment and, once again, analyzed regarding its toxic impact on bacteria, algae and daphnia. The respective EC_{50} dilution factors were: $G_L = 12$, $G_A = 6$ and $G_D = 6$. Thus, for safe disposal into receiving waters, the discharged ozonated water had to be diluted 192 times, whereas the biologically treated waste water required dilution with the receiving water by a factor of at least 12, for safe discharge. Toxicity to aquatic biota can be improved by treatment in industrial waste water treatment plants by the factor of 16.

Due to residual biological activity of the investigated samples, appropriate dilution of the ozonated waste water was required before transferring to receiving water bodies. Assuming an average industrial WWTP effluent of approx. 3000 $m^3\ d^{-1}$, the established dilution factors were high enough to ascertain safe disposal of the ozonated moxifloxacin-containing process water. While ciprofloxacin was not sufficiently degraded under the process parameters described above, safe disposal of the waste water generated in course of its production could be achieved through prolonged ozonation. This hypothesis, however, requires further verification.

Moreover, further ecotoxicological data are needed to ultimately determine the toxicity of ciprofloxacin-containing process water. The residual toxic effects may be attributed to the fact that many metabolites of ciprofloxacin degradation (Fig. 3.4) still encompass the keto-acid moiety of the core structure which is an essential prerequisite for biological activity (Appelbaum and Hunter, 2000). In case of moxifloxacin, the concentration of transformation products is much lower.

3.5. Conclusions

The current study provides conclusive evidence that ozonation of ciprofloxacin- and moxifloxacin-containing process water can be effectively implemented as a treatment prior to biological degradation procedure. Both fluoroquinolones degraded to a high number of transformation products. The undertaken ozonation experiments resulted in degradation of 98.5% of ciprofloxacin and nearly 100% of moxifloxacin. In case of the former antibiotic, prolonged ozonation (beyond the applied 300 min) is assumed to lead to still higher degree of decomposition, while moxifloxacin content in the analyzed samples dropped below the limit of detection already after 60 min. In both cases, best results were achieved under basic conditions. Numerous degradation products were identified via LC-HRMS, MS-MS and MS^3. Four transformation products of ciprofloxacin (highlighted compounds in Fig. 3.4) and all five moxifloxacin transformation products (Fig. 3.7) are reported for the first time. Additionally, the structure of three already published products of ciprofloxacin could be enlightened.

All transformation products identified herein show an intact quinolone ring; most, still encompass functional moieties. Therefore, the ozonated process waste water still exhibits antibacterial properties (Sukul and Spiteller, 2007), but much less as indicated by the ecotoxicological data. Antibacterial properties can be further reduced with prolonged ozonation times. Nevertheless, the proposed ozone pre-treatment technology seems to be a valuable alternative for the removal of fluoroquinolones from their production waste water compared to the predominantly implemented incineration.

Acknowledgements

F.D. is grateful to the Bayer AG for providing a fully equipped laboratory reactor for ozonation trails. M.S. and S.Z. acknowledge the financial assistance of the Ministry of Innovation, Sciences, Research and Technology of the State of North Rhine-Westphalia, Germany, and the German Research Foundation (DFG) in purchasing a high-resolution mass spectrometer.

Chapter 4: Ozonation of rivaroxaban production waste water and comparison of generated transformation products with known in vivo and in vitro metabolites

Fares Daoud[a], Sebastian Zuehlke[b], Michael Spiteller[b], Oliver Kayser[c]

[a] Bayer AG, Friedrich-Ebert-Str. 217-333, 42096 Wuppertal, Germany

[b] Institute of Environmental Research (INFU), Faculty of Chemistry and Chemical Biology, Technical University of Dortmund, Otto-Hahn Str. 6, 44227 Dortmund, Germany

[c] Department Technical Biochemistry, Faculty of Biochemical and Chemical Engineering, Technical University of Dortmund, Emil-Figge-Str. 66, 44227 Dortmund, Germany

FD conceived of the design of the study, carried out the ozonation experiments and provided the samples for quantification and identification of parent and transformation products. Bastian Jansen and SZ elaborated calibration curves with available standards and conducted LC-HRMS and MSn analysis. Bastian Jansen compiled part of the data in his master thesis. MS interpreted the mass spectra and the fragmentation patterns. All compiled data from ozonation trails and analytics were reviewed by OK. FD generated the draft of the manuscript. All authors read and approved the final manuscript.

Published in Science for the Total Environment (2020), Volume 714, 136825

Corrigendum: Science for the Total Environment (2020), Volume 742, 140865

Abstract

During production of rivaroxaban, an orally administrated anticoagulant, process waste water is generated at several stages of manufacturing. Due to low biodegradability in conventional waste water treatment plants, it is currently incinerated. Thus, new pre-treatment methods for rivaroxaban-containing waste water could facilitate its subsequent biological processing. In this study, ozonation was investigated as the pre-treatment method, with special emphasis on the elimination of the target compound and the generation of its main transformation products. These were determined by high performance liquid chromatography – high resolution mass spectrometry (HPLC-HRMS). Our results show that the concentration of rivaroxaban in the ozonated waste water can be effectively reduced (below detection limit) under acidic conditions, whereas neutral or basic reaction environment proved less suitable. Four main transformation products were identified, and their concentrations determined. Rivaroxaban and its most prominent transformation product acetoxamide were elucidated in multiple-stage mass spectrometry (MS^n) experiments. Transformation products derived from the degradation of rivaroxaban were then compared to its natural (animal and human) metabolites as well as other known derivatives. In contrast to previously published rivaroxaban-derived metabolites, the presented ozonation experiments resulted in new transformation products.

4.1 Introduction

Rivaroxaban is a highly selective, orally active direct factor Xa inhibitor prescribed for the prevention of venous thromboembolism in adult patients after elective hip or knee arthroplasty (Perzborn et al., 2010). Bayer Corp. submitted the application for rivaroxaban to the European Medicines Agency (EMEA, 2008) in 2007 and received permission for its distribution in the European Union, under the brand name of Xarelto®, in 2008. FDA approval followed three years later (Hernandez and Zhang, 2017). Also, in 2011, rivaroxaban was approved by the European Commission for two new indications: prevention of stroke and systemic embolism in adult patients with non-valvular atrial fibrillation with one or more risk factors and treatment of deep vein thrombosis (Kasad, 2013).

Factor Xa plays a significant role in the blood coagulation cascade, which leads to thrombin activation and blood clotting (Rohde, 2008). It catalyses the conversion of prothrombin to thrombin through the prothrombinase complex (Roehrig et al., 2005), with one molecule of factor Xa leading to the generation of more than 1,000 thrombin molecules. Therefore, inhibition of this coagulation catalyst is of high interest for the prevention and treatment of thromboembolic disorders, which can result in heart attack, stroke or blockage in arteries and veins.

Before market entry of the novel anticoagulant rivaroxaban, low-molecular-weight heparins were the standard of care for thromboprophylaxis after major orthopaedic surgery. The main disadvantages of these medicines were the need for frequent coagulation monitoring and dose adjustment as well as subcutaneous administration (Rohde, 2008). On the contrary, rivaroxaban can be administered orally in a simple fixed-dose regimen (Yuan et al., 2014). After ingestion, rivaroxaban shows high oral bioavailability of 80 to 100 % (Perzborn et al., 2010) and rapid absorption; additionally, it is safe and well tolerated (Weinz et al., 2009). Nevertheless, remaining rivaroxaban and its metabolites, excreted via various pathways, will enter the sewerage and are, therefore, subject to waste water treatment in municipal waste water treatment plants.

Another source of discarded rivaroxaban is its production process, wherein waste water is generated at several stages of the synthesis. The waste water has to be disposed of, but common waste water treatment plants are not suitable for processing of rivaroxaban due to its low biodegradability and its ecotoxicology. Therefore, rivaroxaban-containing process waste water is currently subjected to incineration. Since the patent for rivaroxaban has not yet expired, Bayer

Corp. is the only manufacturer and there is currently no information on other wastewater disposal alternatives than the method of choice.

In this study, ozonation-based treatment was investigated as an alternative to incineration. Thus, original process waste water, discharged directly after the final separation step in the rivaroxaban production, was subjected to ozonation. The objective of this work was to identify transformation products of rivaroxaban generated in course of ozonation and to monitor the drug's degradation kinetics.

4.2. Material and methods

4.2.1. Process waste water and active ingredients

Rivaroxaban is chemically synthesized in production facilities for active pharmaceutical ingredients in a five-step synthetic process using 4-(4-nitrophenyl)-3-morpholinone as a starting material (EMEA, 2008). Process waste water was collected from the final purification step, crystallization for the removal of reaction by-products and subsequent solid-liquid separation. Contingent upon their solubility, process waste water contains by-products and the target compound, but also small amounts of solvents used in the synthesis (e.g., acetone). Rivaroxaban is only slightly soluble in organic solvents and practically insoluble in water (Perzborn et. al, 2010). The analysed rivaroxaban-containing waste water showed total organic carbon (TOC) content between 3,000 and 9,000 mg/L, total nitrogen (TN) between 13 and 17 mg/L and a pH of 7.7. To evaluate its remaining toxicity, ecotoxicological parameters were measured. The experiments were performed at a laboratory certified for ecotoxicological evaluation (Gobio GmbH, Aarbergen, Germany). In order to analyse the toxicity at three trophic levels, the experiments included bacteria (GL), algae (GA) and invertebrates (GD) (DIN EN 38412 L30, L33 and L34), resulting in determination of half-maximum effect (EC_{50}) values and the corresponding process water dilution factors. Prior to ozonation the dilution factors for Daphnia were GD 6000 (according DIN 38412-L30), for Algae GA 4225 (according DIN 38412-L33) and Luminous bacteria GL 155 (according DIN 38412-L34).

For liquid chromatography – high resolution mass spectrometric evaluation (LC-HRMS), analytical standards of rivaroxaban (5-chloro-N-({(5S)-2-oxo-3-[4-(3-oxo-4-

morpholinyl)phenyl]-1,3-oxzolidin-5-yl}methyl)-2-thiophene-carboxamide), and acetoxamide (N-({(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl)phenyl]-1,3-oxzolidin-5-yl}methyl)acetamide) were provided by Bayer Corp., Leverkusen, Germany (Fig. 4.1).

A B

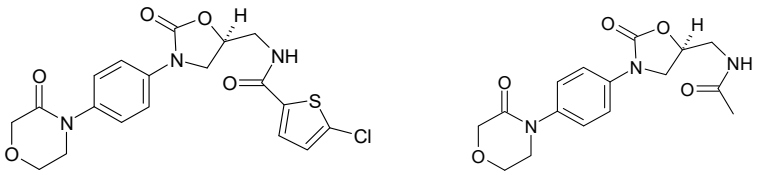


Fig.4.1. Chemical structures of rivaroxaban (A) and acetoxamide (B)

4.2.2. Experimental setup

Experimental design of ozonation was described in detail in Daoud et al. (2017). Shortly, all experiments were conducted in a stirred batch reactor of 156 mm in diameter and 265 mm in height (see supplementary material). The reactor was operated in a semi-batch manner and filled with a known volume of waste water (max. 2 L) before ozonation. The ozone gas was generated from pure oxygen, applied as feed gas, using a corona discharge ozone generator (BMT 803, BMT Messtechnik GmbH, Berlin, Germany) and introduced at the bottom of the reactor via a gas sparger. The inlet concentration and flow rate of ozone were held constant (approx. 100-120 g/m³ and 1 L/min, respectively). Ozone concentration in the off-gas from the reactor was measured and its excess collected in a liquid trap before release to ambient atmosphere. During ozonation, the inlet, off-gas and dissolved ozone concentrations were measured continuously, as were the temperature and pH. Prior to ozonation, the pH of the process water was adjusted to pH 3, pH 7 and pH 10 through addition of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH). The maximum time of ozonation was 300 min. TOC concentration was measured at defined time intervals. Therefore, liquid samples of approx. 30 mL were manually withdrawn through the sampling port into small 40 mL flasks. Thereof, 1.5 mL samples were retrieved for LC-HRMS analysis. Immediately after collection, the samples were flushed with nitrogen in order to remove residual ozone.

4.2.3. Analytical measurements

Temperature and pH were monitored by means of the PT100 compensated pH-electrode K100PR (Dr. A. Kuntze GmbH, Meerbusch, Germany; Daoud et al. 2017). The mass flow was determined with the digital mass flow meter D-6300 (M+W Instruments GmbH, Leonhardsbuch, Germany). In gaseous streams, ozone concentration was measured by the ozone analysers BMT 964 (BMT Messtechnik GmbH, Berlin, Germany), consisting of a double beam UV-photometer with the working wavelength of 254 nm. The first ozone analyser was located in the inlet gas stream, in line with the ozone generator; the second – in the reactor off-gas. Additionally, concentration of the dissolved ozone was monitored inside the reactor by means of the potentiometric double electrode sensor Krypton K System (Dr. A. Kuntze GmbH, Meerbusch, Germany) with AuAu-600-OO-2-1-PG electrodes, to detect its potential accumulation. All aforementioned measurement data were collected with the Multi-Channel Recorder RSG30 (Endress+Hauser Messtechnik GmbH+Co. KG, Weil am Rhein, Germany) and saved on an SD-card, followed by data export to Excel (Microsoft Windows). TOC was determined using the TOC-V_{CPN} analyser (Shimadzu Deutschland GmbH, Duisburg, Germany) within the range of 0.004-25.000 mg/L. Sampling was performed with the ASI-V autosampler (Shimadzu Deutschland GmbH, Duisburg, Germany).

4.2.4. LC-HRMS measurements

Rivaroxaban and its transformation products were identified by HPLC-HRMS (LTQ-Orbitrap spectrometer with heated electrospray ionization source, Thermo Fisher Scientific, Waltham, USA). Separation was achieved with the Surveyor-LC HPLC system (Agilent Technologies, Waldbronn, Germany) encompassing a binary pump, an autosampler and a temperature-controlled column oven (25 °C). Injection volume was 5 µL. For mass spectrometric detection, nitrogen was used as the sheath gas (55 arbitrary units) and helium served as the collision gas (8 arbitrary units). Compound separation was performed on the Nucleodur C18 gravity column (1.8 µm, 2 × 50 mm) (Macherey-Nagel, Dueren, Germany) with the following solvent system: A, H_2O (+ 0.1 % HCOOH) and B, acetonitrile (+ 0.1 % HCOOH) (flow rate 250 µL/min). Samples were analysed using a gradient program: 90 % A isocratic for 2 min, linear gradient to 100 % B within 12 min and held for 3.5 min. The system then returned to its initial setting (90 % A) and was equilibrated

for 6.5 min. The spectrometer was operated by heated electrospray ionization (HESI) in positive mode with an electro spray potential of 5kV. M/z range was 50 -600 with a resolution of 60.000 at m/z 400. Analysis was done in full scan.

To delineate the structural formulae of the analysed compounds, the arrangement of their atoms and chemical bonding, multiple-stage mass spectrometry (MS^n) experiments were performed. Collision induced dissociation (CID) with the collision energy of 35 eV was used for fragmentation, helium served as the collision gas. Linear external calibration was performed at concentrations ranging from 1 to 5,000 ng/mL for rivaroxaban and 1 to 4,000 ng/mL for acetoxamide. Previous extraction and clean up of the samples have not been conducted. Although exact quantitation was only possible for rivaroxaban and acetoxamide, the degradation kinetics of rivaroxaban's transformation products were calculated semi-quantitatively assuming similar ionization in mass spectrometry (all transformation products derived from acetoxamide were quantified based on the calibration curve of acetoxamide, all others with that of rivaroxaban). Sum formulae were determined through comparison of measured and theoretical mass-to-charge ratios (m/z).

The mass spectrometric identification of rivaroxaban and its possible metabolites in process waste water was based on available reference standards. In both the reference drug and the waste water samples, main signals characteristic of rivaroxaban were investigated post ozonation (see supplementary material).

Verification of the structure of rivaroxaban was accomplished based on the MS^2 and MS^3 spectra of the anticoagulant-containing waste water prior to ozonation (see supplementary material).

Concentration of rivaroxaban in the process waste water was quantified based on the delineated calibration curve (see supplementary material) at distinct times in course of ozonation (0, 15, 30, 60, 120, 180, 300 min), and the obtained values were used for the determination of degradation kinetics and the calculation of rate constants.

Processing and evaluation of all LC-MS data was performend with the Xcalibur Software Version 2.2.0.48 (Thermo Fisher Scientific, Waltham, USA). Chemical structures were illustrated with the software BIOVIA draw, Version 19.1 (Dassault Systèmes, Vélizy-Villacoublay, France). Graphs have been performed with Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA).

4.3. Results and discussion

4.3.1. Ozonation

As the degree of rivaroxaban degradation and the generation of its transformation products significantly depend on the pH, the ozonation trials were performed under acidic, neutral, and basic conditions (pH 3, 7 and 10). PH was set before start of ozonation and remained constant during ozonation. The highest ozone consumption rates, calculated according to Gottschalk et al. (2010), were measured in the basic reaction environment, while the lowest occurred under acidic conditions (see supplementary material). The graph curves, illustrating ozone consumption at the three pH values, became linear within a few minutes of the process, which points to a constant reaction of ozone with available reactants (with the initial few minutes used for accumulation in the two litres of reaction solution). According to the ozonation curves (see supplementary material) an immediate ozone demand (IOD) is highly probable for acidic conditions but cannot be verified with the experimental setup. The higher ozone consumption under basic conditions can be attributed to the increased generation of hydroxyl radicals (Staehelin and Hoigné, 1982).

Ozone consumption rates alone, however, do not fully correspond to the degree of degradation of the target compounds under different reaction conditions.

4.3.2. Concentration gradient of rivaroxaban

Full degradation kinetics of rivaroxaban in the course of ozonation was studied in detail for acidic, neutral and basic conditions. Therefore, original waste water from the last purification step - including crystallization and isolation- had been used. The initial concentration of rivaroxaban was 3 to 5 mg/L, half as much as the solubility of rivaroxaban in water (10 mg/L) according to Takács-Novák et al. (2017). The ozone-induced transformation was most pronounced under acidic conditions, exceeding 99 %. The degradation reached almost 95 % under neutral conditions but only 50 % in the alkaline environment (Fig.4.2).

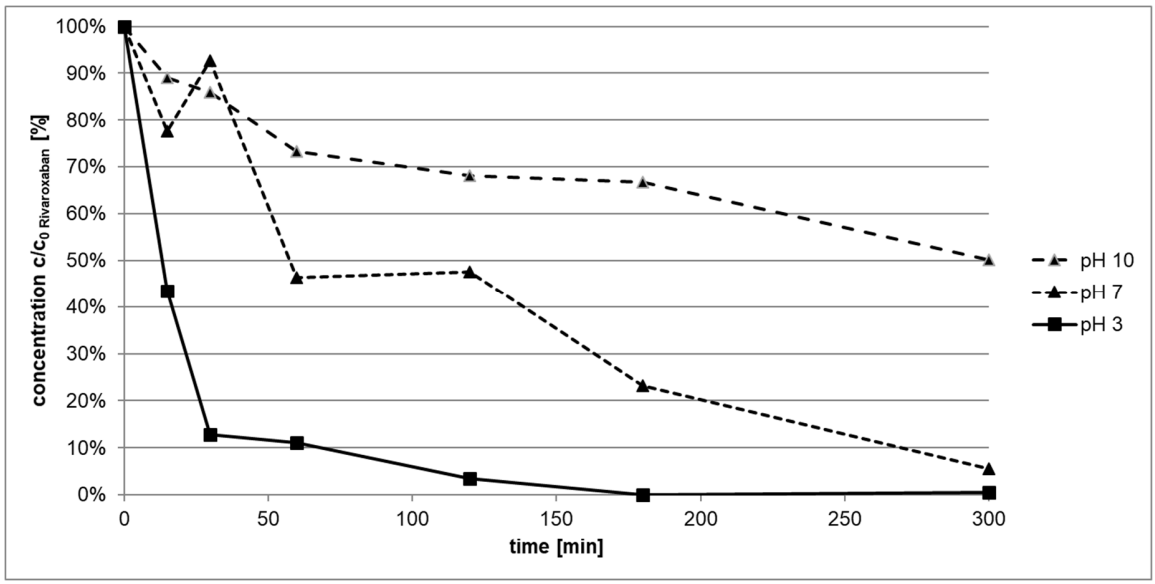


Fig.4.2. Degradation of rivaroxaban in course of waste water ozonation at pH 3, pH 7 and pH 10 (relative to rivaroxaban concentration at time 0 min, 3-5 mg/L)

According to Prasse et al. (2012), given an exponential shape of the rivaroxaban degradation curve, pseudo-first-order rate constants can be determined from a plot of $\ln(c/c_0)$ versus time with rate constant k being the slope of the straight line (Prasse et al., 2012). In case of rivaroxaban, pseudo-first order reaction seemed to be the appropriate assumption, as indicated by the high linearity factor. Based on the concentration values measured at different time intervals, the following apparent rate constants were determined: 0.019 min^{-1} under acidic conditions, 0.0039 min^{-1} at neutral pH and 0.0011 min^{-1} in the alkaline environment (see supplementary material).

4.3.3. O_2/O_3 dependency of rivaroxaban degradation

Ramisetti and Kuntamukkala (2014) determined the stability of rivaroxaban in basic and acidic environment. In their experiments, acid hydrolysis (1 N HCl) led to the generation of a degradation product with the m/z of 454 with the concomitant decline in rivaroxaban concentration of 6.5 %. Therefore, an additional test at low pH was conducted. In contrast to the previous study, it was maintained at pH 3 and sulfuric acid was used instead of hydrochloric acid. Rivaroxaban-containing waste water was exposed in the current setup and under acidic conditions to constant gassing with ozone or pure ambient air.

Rivaroxaban containing waste water aerated with ambient air showed no degradation of rivaroxaban throughout the process (300 min). In contrast, treatment with ozone led to a significant decrease of rivaroxaban concentration (below the detection limit) within a few minutes (Fig. 4.3). In both cases, initial concentration of rivaroxaban was 2.1-2.3 mg/L.

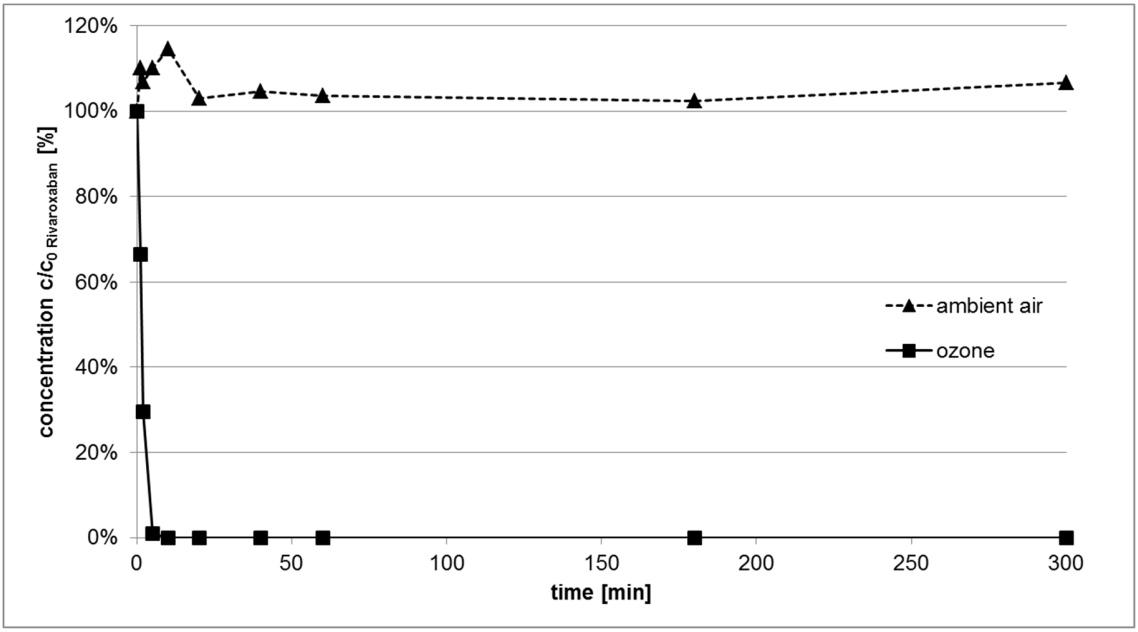


Fig. 4.3. Degradation of rivaroxaban at pH 3 in course of waste water ozonation vs. aeration with ambient air (relative to rivaroxaban concentration at time 0 min, 2.1-2.3 mg/L)

The results demonstrated that the hydrolysis of rivaroxaban due to acidic conditions was of marginal impact. Therefore, the clear impact of the presence of ozone on degradation outcomes in similar experiments under different pH conditions was assumed.

4.3.4. Concentration gradients of rivaroxaban transformation products

Concentration gradients of some prominent transformation products of the investigated drug were analysed during the ozonation (300 min at pH 3, pH 7 and pH 10). Fig. 4.4 illustrates the concentration of rivaroxaban and its main transformation products based on the initial concentration of the anticoagulant.

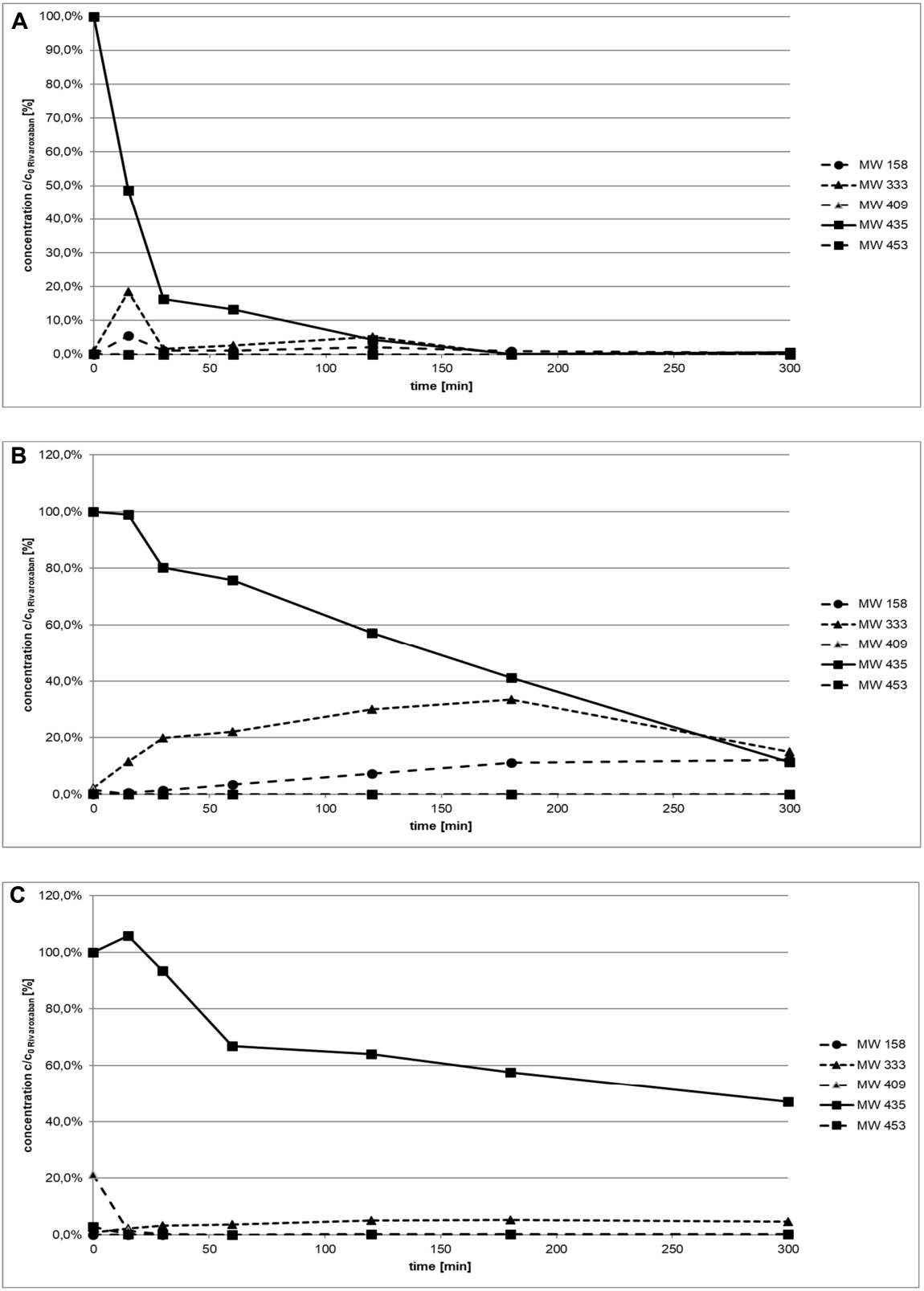


Fig. 4.4. Degradation of rivaroxaban and formation of its most abundant transformation products ($c/c_0 > 1$ %) in course of waste water ozonation at pH 3 (A), pH 7 (B) and pH10 (C) (relative to rivaroxaban concentration at time 0 min, 3-5 mg/L)

Fig. 4.4 A shows the concentration gradient of rivaroxaban and its ozonation products generated under acidic conditions. The content of rivaroxaban decreased rapidly and dropped below the detection limit after 180 min. A transformation product with the molecular weight (MW) of 333 g/mol reached its maximum concentration at 15 min (20 % of the initial rivaroxaban concentration), subsequently attained a low point at 30 min (< 5 %) and rose again to its second peak at 120 min, before finally being degraded below the detection limit. In parallel, an unknown transformation product characterized by MW of 158 g/mol showed a comparable trend with corresponding local maxima at 15, 30 and 120 min. Additionally, compounds MW409 and MW453 were displayed as they were generated in significantly higher concentrations under basic conditions (Fig. 4.4 C).

Under neutral conditions (Fig. 4.4 B), MW333 achieved a comparable maximum as at pH 3 (> 20 %), but much later in the course of ozonation (180 min). Afterwards, its concentration dropped below 10 %. Content of MW158 increased constantly throughout the ozone treatment process and attained its maximum at the end of ozonation (300 min). Again, the compounds MW409 and MW453 showed only marginal concentrations.

The last ozonation experiment was conducted under basic conditions. MW333 concentration increased up to 5 % at 180 min and afterwards decreased very slowly (Fig.4.4 C). Concentration of MW158 increased continuously at a very low level and finally closed at 0.5 % of the initial rivaroxaban concentration. In all experiments, the concentration gradient of MW158 was closely related to that of MW333. Therefore, it can be postulated that MW333 is a primary transformation product of rivaroxaban, whereas MW158 derives from MW333 as a secondary degradation product of the drug.

According to the submission documents for approval (EMEA, 2008) acetoxamide (MW 333) is an impurity generated in the course of the manufacturing process. MS^n spectra (see supplementary material) proved that the transformation product with MW333 could be identified as acetoxamide.

To corroborate this sequential reaction, an additional ozonation experiment was conducted with pure water containing acetoxamide (MW, 333 g/mol) (Fig. 4.5).

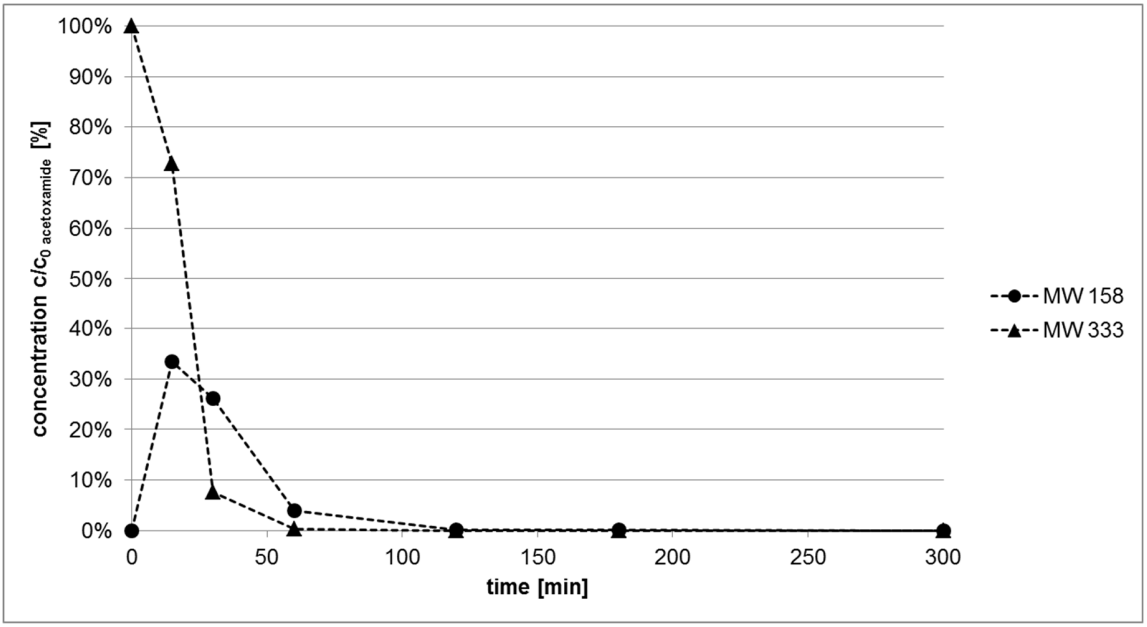


Fig. 4.5. Degradation of acetoxamide (MW333) and formation of its most abundant transformation products ($c/c_0 > 1$ %) in course of ozonation at pH 10 (relative to acetoxamide concentration at time 0)

In contrast to ozonation of rivaroxaban-containing waste water under acidic and neutral conditions, remarkable concentrations of MW453 and MW409 were detected at basic conditions. Both compounds had their maximum concentration at 0 min of ozonation and fell below detection limit in the course of the process. Thus, both compounds originated from by-products of the rivaroxaban synthesis and were generated based on basic conditions. They were not created during ozonation. This observation is in accordance with the results of Ramisetti and Kuntamukkala (2014).

Moreover, during all ozonation trials, the corresponding TOC content was measured and did not change (see supplementary material). The generated degradation products remained in solution and at such a molecular size that low vapour pressure and subsequent carryover into the off-gas path did not occur.

4.3.5. Mass spectrometric identification of rivaroxaban transformation products

Ozone-induced degradation of rivaroxaban led to the generation of several transformation products. The exact masses, sum formulae and retention times of all relevant compounds were

fully elucidated by LC-HRMS (see supplementary material). Four of them (MW333, MW409, MW453 and MW158; Fig. 4.6) were detected at remarkably high concentrations, while low amounts of the remaining derivates precluded their gradient delineation on the available concentration curves.

The most prominent transformation product was acetoxamide (MW333), a by-product of rivaroxaban synthesis. Crystallization and subsequent separation are the final purification steps in course of the drug manufacturing process. Reaction by-products are removed via filtration, and therefore occur in the process waste water. In contrast to other transformation products, a reference standard of acetoxamide was available; therefore, its exact quantification was feasible. The structural formula of the derivate was confirmed by its MS^2 and MS^3 spectra (see supplementary material). Acetoxamide is generated by cleavage of the 2-chlorothiophene moiety from the parent product, rivaroxaban (Fig. 4.6).

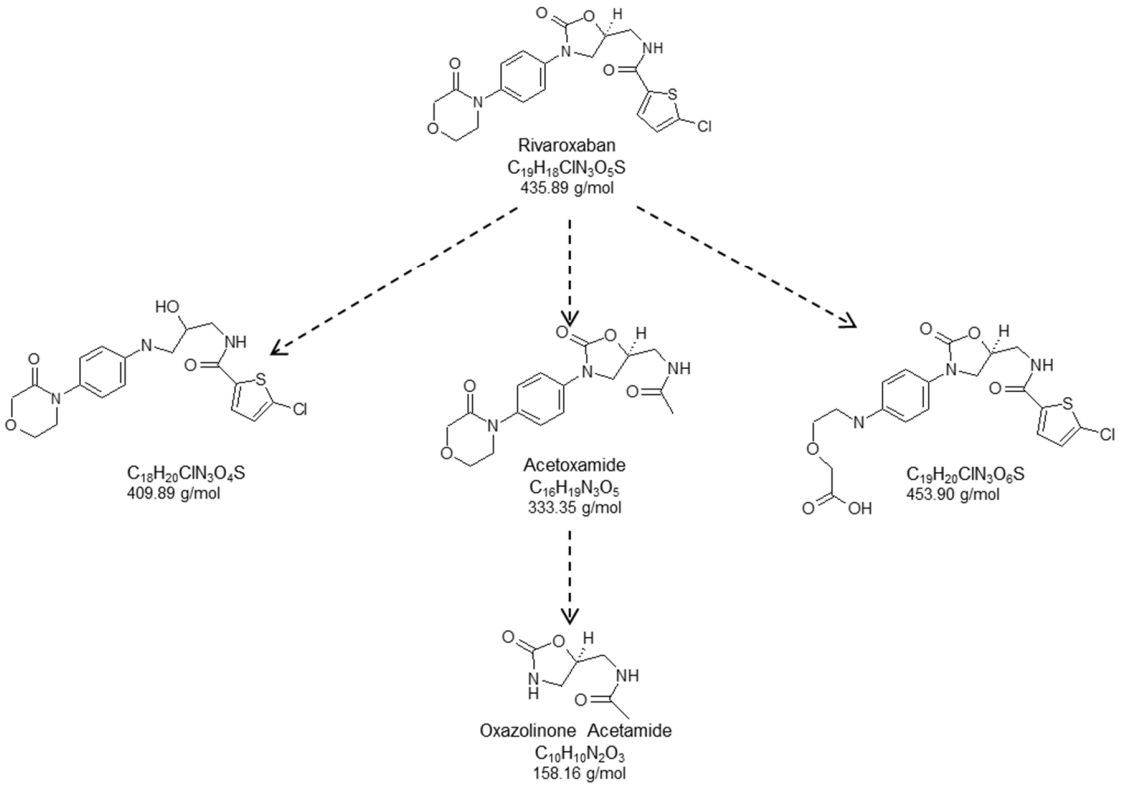


Fig. 4.6. Proposed ozonation pathway of rivaroxaban. Degradation products MW409 and MW453 have been published previously (Lang et al., 2009; Ramisetti and Kuntamukkala, 2014; Weinz et al., 2009; Wingert et al., 2016). Acetoxamide (MW333) and Oxazolinone acetamide (MW158) are new transformation products.

Various authors have investigated potential transformation products of rivaroxaban in the past few years. To our knowledge, no data on ozone pre-treatment of process waste water containing the compound have been published to date. However, results of some reports have contributed valuable insights concerning potential rivaroxaban transformation products. To provide support for clinical studies and application safety, several authors focussed on metabolites of the anticoagulant. Wingert et al. (2016) stated that the long-term use of medications might cause accumulation of chemical compounds in human bodies. Therefore, the knowledge about potential degradation products is of high interest for drug safety considerations. Weinz et al. (2009) determined the in vivo metabolisation and excretion of rivaroxaban in rats, dogs and humans. To that end, the [^{14}C]-labelled rivaroxaban was administered orally and intravenously (only rats) in different dosages. Rivaroxaban as well as its metabolites were rapidly excreted. In humans, 66 % of the dose was excreted renally (thereof 36 % in unchanged form) and 28 % in the faeces (7 % unchanged) (EMEA, 2008; Weinz et al., 2009). In total, Weinz et al. (2009) identified 18 metabolites, with 14 illustrated in the relevant metabolic pathway. Further, Lang et al. (2009) based their studies on the results of Weinz et al. (2009) and determined the same metabolites. Instead of in vivo experiments, however, the research team incubated [^{14}C]-rivaroxaban with liver microsomes and hepatocytes of rats and humans.

Moreover, Ramisetti and Kuntamukkala (2014) and Wingert et al. (2016) investigated the generation of rivaroxaban degradation products under chemical and physical stress conditions. In the former study, the drug was subjected to hydrolysis (acidic, alkaline and neutral), photolysis, thermolysis and oxidation. In total, three degradation products were determined. While Wingert et al. (2016) also exposed rivaroxaban to acid and alkaline hydrolysis, they additionally subjected it to UVC-radiation, ultimately identifying three degradation products.

According to Ramisetti and Kuntamukkala (2014) and Wingert et al. (2016), MW409 is a transformation product of rivaroxaban generated under basic hydrolytic stress conditions. The compound was detected after pH adjustment with NaOH (pH 10) at time 0 min. Its concentration in the tested waste water was almost 20 % of that of rivaroxaban prior to ozonation. Furthermore, acidic hydrolysis of the target drug with HCl was reported to result in a transformation product of MW of 453 g/mol (Ramisetti and Kuntamukkala, 2014). Also Wingert et al. (2016), Lang et al. (2009) and Weinz et al. (2009) identified this compound. We detected MW453 in course of ozonation under acidic conditions (pH 3), but only at a concentration of 0.2 % at time 0 min. The

exact mass values of both derivates (MW409 and MW453) were determined by LC-HRMS (see supplementary material), with their postulated chemical structures (Fig. 4.6) in accord with those published by Ramisetti and Kuntamukkala (2014).

Another major rivaroxaban transformation product was oxazolinone acetamide (MW158), generated by cleavage of 4-phenyl-3-morpholinone from acetoxamide (Fig. 4.6). Oxazolinone acetamide so far was not discovered in known publication regarding metabolites of rivaroxaban, therefore it is a new transformation product.

Additionally, a compound of MW 291 was detected in course of ozonation under acidic, neutral and basic conditions, but at a very low concentration (< 1 % of the initial rivaroxaban content). What is more, the fragmentation pattern of acetoxamide (MW333) showed a signal at m/z 292.12929 in the MS^2 analysis (S7, Fig. S7.6). Jansen (2015) stated that the occurrence of MW291 in the MS and MS^2 spectra of MW333 points to in-source-fragmentation – an undesired fragmentation that occurs in the ionisation source. This phenomenon cannot be confirmed based on our results.

Other potential rivaroxaban transformation products were detected in ozonated waste water, albeit at extremely low concentrations. Some thereof exhibited the same exact mass as metabolites of the anticoagulant identified in humans and animals (Lang et al., 2009; Weinz et al., 2009). Conversely, several previously postulated structures (and corresponding molecular masses) were not observed in our ozonation experiments. The full list of rivaroxaban-derived compounds can be found in supplementary material. Ultimately, one can say that there is little accordance between the determined metabolites from in vitro/in vivo experiments and the transformation products of ozonation. Only the previously published compounds with molecular masses MW409 and MW454 were confirmed. The compound that generated in the course of ozonation MW333 and MW158 are newly identified tranformation product. This indicates that degradation of rivaroxaban in human and animals proceeds differently to the degradation by ozonation.

4.3.6. Ecotoxicology

Before ozonation original process waste water from the rivaroxaban production was evaluated with regard to EC_{50} dilution factors: G_L = 155 for bacteria, G_A = 4225 for algae and G_D = 6000 for

daphnia. Ozonated process water generated in course of rivaroxaban production was characterized by the following EC_{50} dilution factors: G_L = 300 for bacteria, G_A = 1600 for algae and G_D = 48 for daphnia. Finally, the waste water was analysed, regarding its toxic impact on bacteria, algae and daphnia populations, after being submitted to biological degradation. The respective EC_{50} dilution factors were G_L = 82, G_A = 54 and G_D = 27. The ecotoxicological results are important for the entire evaluation of the ozone treatment process. At least with the regard to the ecotoxicity for daphnia and algae considerable improvement can be achieved by ozonation.

4.4. Conclusions

The current study provides conclusive evidence that ozonation is an effective means of degradation of rivaroxaban in process waste water and can, therefore, be an efficient method of its pre-treatment prior to biological treatment. Rivaroxaban was transformed into a limited number of products, with acetoxamide (MW333) and its derivative oxazolinone acetamide (MW158) proving most prominent and newly identified. Additionally, compounds characterized by the molecular weight values of 409 g/mol and 453 g/mol (MW409 and MW453) were unambiguously identified. Under acidic conditions (pH 3), the undertaken ozonation experiments resulted in degradation of nearly 100 % of rivaroxaban. The calculated rate constant under acidic conditions was 0.019 min^{-1}. Neutral and alkaline environments (pH 7 and 10) proved suboptimal for the desired reduction of the anticoagulant concentration within a short ozonation period. Ozone-induced rivaroxaban degradation profiles were traced via LC-HRMS, MS^2 and MS^3. Only four transformation products were detected at significant concentrations and therefore have been displayed in the postulated ozonation pathway (Fig. 4.6). All identified rivaroxaban derivatives were compared to the previously published ones (with the drug subjected to physicochemical stress conditions as well as human and animal metabolism). However, the majority of the known compounds could not be identified during ozonation. Process waste water was also subject to ecotoxicological evaluation for three different trophic levels before and after ozonation. The results show a significant improvement in ecotoxicology resulting in much less required dilution for safe discharge in receiving waters.

Chapter 5: Elimination of diethylenetriaminepentaacetic acid from effluents from pharmaceutical production by ozonation

Fares Daoud[a], Sebastian Zuehlke[b], Michael Spiteller[c], Oliver Kayser[d]

[a] Bayer AG, Friedrich-Ebert-Str. 217-333, 42096 Wuppertal, Germany

[b] Center for Mass Spectrometry, Faculty of Chemistry and Chemical Biology, Technical University of Dortmund, Otto-Hahn Str. 6, 44227 Dortmund, Germany

[c] Institute of Environmental Research (INFU), Faculty of Chemistry and Chemical Biology, Technical University of Dortmund, Otto-Hahn Str. 6, 44227 Dortmund, Germany

[d] Department Technical Biochemistry, Faculty of Biochemical and Chemical Engineering, Technical University of Dortmund, Emil-Figge-Str. 66, 44227 Dortmund, Germany

FD conceived of the design of the study, carried out the ozonation experiments and provided the samples for quantification and identification of parent and transformation products. SZ elaborated calibration curves with available standards and conducted LC-HRMS and MSn analysis. MS interpreted the mass spectra and the fragmentation patterns. All compiled data from ozonation trails and analytics were reviewed by OK. FD generated the draft of the manuscript. All authors read and approved the final manuscript.

Published in Ozone: Science & Engineering (2021), Page 1-13

Abstract

During production of diethylenetriaminepentaacetic acid (DTPA), process waste water is generated in several production stages. Process wastewater is usually disposed of via waste water treatment plants. However, due to low biodegradability of DTPA in conventional waste water treatment, incineration constitutes the current method of choice. The main disadvantage of incineration is high consumption of primary energy sources leading to substantial emission of carbon dioxide (CO_2). Thus, an alternative method of process waste water treatment was investigated, which consists of an initial application of ozone and a subsequent biological treatment. In 2009, preliminary laboratory experiments were conducted to evaluate the elimination of DTPA in process waste water. Based on the initial results, the responsible authorities granted approval for large-scale ozonation of DTPA-containing wastewater in 2011. Additional laboratory scale experiments were carried out to assess the elimination of the target compound and the generation of its main transformation products using liquid chromatography – high resolution mass spectrometry. Through application of the postulated method, the concentration of DTPA and its derivatives can be reduced to levels assuring safe discharge into the receiving water. Additionally, a comparison of CO_2 emissions showed that ozonation is an ecological alternative to incineration and, most likely, an economical as well, based on the local prices of primary energy sources.

5.1 Introduction

Diethylenetriaminepentaacetic acid (DTPA) is an organic complexing agent used in many industries. DTPA is an aminopolycarboxylic acid with five functional moieties. The most important chemical property of DTPA is the ability to form water-soluble complexes with multivalent metal ions in a wide pH range. Therefore, DTPA is used to reduce the concentration of free metal ions in aqueous systems, e.g., for water softening or for the elimination of earth alkali or heavy metals in the textile, soap, detergent and pulp and paper industries (Randt, Wittlinger and Merz 1993; Sillanpää, Kurniawan and Lo 1997; Rämö and Sillanpää 2001; AkzoNobel Product Stewardship Team 2011). The removal of metal ions is necessary because metal impurities often cause discoloration, rancidity, turbidity and disagreeable taste and odour (Means, Kucak and Crerar 1980).

However, there are also expedient applications for earth alkali and heavy metals in medical systems. One example is the field of diagnostic imaging. The paramagnetic properties of some elements can be used in magnetic resonance imaging (MRI) to resolve certain areas of the human body in more detail. One of these elements is the lanthanide gadolinium (Gd). The outer electron shell of gadolinium has seven unpaired electrons, which leads to its extreme paramagnetism. However, it is highly toxic in the ionic form, with the LD_{50} of 0.1 mmol/kg of body weight (Vogl et al. 2013), and therefore harmful when employed for MRI applications. Instead, gadolinium is used in a ligated form that is very stable and unable to permeate the blood-brain barrier. However, some Gd-based contrast agents were reported to cause nephrogenic systemic fibrosis, likely related to dechelation, in several patients. Further, considerably higher resistance to chelate disruption was observed in the so-called macrocyclic forms of gadolinium-based contrast media, as compared to their linear counterparts (Montagne, Toga, Zlokovic 2016). The complexing agent, DPTA forms such stable ligands with gadolinium, resulting in a non-toxic structure, without compromising its effectiveness in MRI applications.

In contrary to other industries the Bayer production process necessitates a higher purity of DTPA for medical applications, with fewer by-products, relative to that required for bulk chemical utilization. Therefore, raw DTPA must be purified using physical procedures without chemical modification. The production of pure DTPA involves its crystallization prior to separation, drying and filling. Gadolinium is added in a later production stage preceding formulation.

DTPA shows low microbial degradability (Means, Kucak and Crerar 1980) and, therefore, cannot be discharged to biological waste water treatment plants (WWTPs) without pre-treatment. Thus, DTPA-containing process waste water is currently subjected to incineration. The very stable gadolinium complex is excreted without metabolization and exits WWTPs without modification (Cyris et al. 2013). DTPA in water bodies is subject to a wide range of analytical test methods due to the broad usage of complexing agents in different industries. Several authors focussed on the degradation of complexing agents by ozonation (Stemmler, Glod and von Gunten 2001; Pinnekamp and Merkel 2008; Kreuzinger and Schaar 2011), others published research articles highlighting photolysis (Baeza et al. 2007) or biological degradation (Nispel, Baumann and Hardes 1990) as the techniques of choice. Only one report has provided a toxicological evaluation of ozonated waste water thus far (Cyris et al. 2013).

In 2009, the first ozonation experiments with DTPA-containing process waste water were conducted in Wuppertal, Germany. The test samples showed DTPA concentration between three and five g/L, which could be traced back to the solubility of DTPA in water. Under basic conditions, DTPA elimination rates of > 99% were achieved. After successful ozonation, the residual was tested for biological degradation applying the Zahn-Wellens test according to DIN EN ISO 9888 (DIN EN 9888 1999). Additionally, reduction of total organic carbon (TOC) was measured and all results were presented to the district council (Bezirksregierung Duesseldorf, Germany), which thereupon permitted large-scale pretreatment of DTPA-containing waste water with ozone. Based on an initial concentration of DTPA in waste water (≤ 5g/L), the minimum degree of elimination had to be > 99% and the maximum daily discharge of DTPA with the effluent was limited to 400 g. This prerequisite was calculated based on the maximum concentration of DTPA allowed in receiving water bodies (< 5 µg/L) according to the German drinking water ordinance (Pinnekamp and Merkel 2008) derived, in turn, from the health orientation value for complexing agents (10 µg/L) as per the German Federal Environment Agency (MUNLV 2009). The main aim of this work was to identify an alternative disposal route for DTPA-containing production waste water instead of incineration as the most common method. Other studies focused only on waste water with low DTPA concentrations and a variety of other contaminants, but not on production waste water with high DTPA concentrations. In addition, transformation products of DTPA that arise in the course of ozonation were detected and identified.

5.2. Material and methods

5.2.1. Process waste water and active ingredients

Original waste water from the DTPA production process was used for ozonation experiments. Process waste water was collected from the final purification step, crystallization for the removal of reaction by-products and subsequent solid-liquid separation. Contingent upon its solubility, process waste water contains the target compound and its by-products, but also small amounts of used solvents (e.g. ethanol). Total organic carbon (TOC) amount in the analysed DTPA-containing waste water was between 1.5 and 1.8 g/L; the pH was 9.4.

According to Daoud et al. (2020), ecotoxicological parameters were measured to evaluate the remaining toxicity of the process waste water. The experiments were performed at a laboratory certified for ecotoxicological evaluation (Gobio GmbH, Aarbergen, Germany) and involved testing three trophic levels , namely bacteria (GL), algae (GA) and invertebrates (GD) (according to DIN EN 38412 L30, L33 and L34), resulting in determination of half maximal effective concentration (EC_{50}) values and the corresponding process water dilution factors.

For reference and calibration in liquid chromatography – high resolution mass spectrometry (LC-HRMS), analytical standards of DTPA (diethylenetriaminepentaacetic acid; Figure 5.1) and secondary components, like EDTA (ethylenediaminetetraacetic acid), were available (provided by Bayer Corp., Leverkusen, Germany).

A B

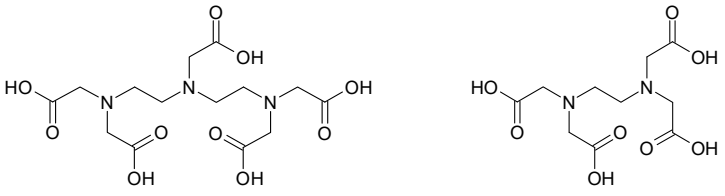


Fig. 5.1. Structures of DTPA, diethylenetriaminepentaacetic acid (A) and EDTA, ethylenediaminetetraacetic acid (B)

5.2.2. Experimental setup

The experimental design of ozonation was described in detail in Daoud et al. (2017). For all ozonation experiments, a stirred batch reactor of 156 mm in diameter and 265 mm in height was used (see supplementary material). The reactor was filled with a predefined amount of waste water (max. 2 L) and operated in a semi-batch manner. Prior to ozonation, the pH of the waste water was adjusted to pH 3, pH 7 and pH 10 through addition of sulphuric acid (H_2SO_4) or sodium hydroxide (NaOH). A corona discharge ozone generator (BMT 803, BMT Messtechnik GmbH, Berlin, Germany) was used to produce ozone from pure oxygen. Ozone was introduced at the bottom of the reactor via a gas sparger. Ozonation was conducted with a constant flow rate (concentration: approx. 100-120 g O_3/m^3; gas flow: 1 L/min). During ozonation the concentration and flow rate of the gas were measured in the inlet and outlet; for mass balancing, the dissolved ozone concentration was gauged as well. Off-gas from the reactor was collected in a liquid trap before release to ambient atmosphere. Additionally, the reactor was equipped with temperature and pH measurement devices. For TOC concentration determination, liquid samples of approx. 30 mL were manually withdrawn through the sampling port into small 40 mL flasks and analysed at defined time intervals. Thereof, 1.5 mL samples were retrieved for LC-HRMS analysis. Promptly after sampling, the flasks were flushed with nitrogen to remove residual ozone. The samples were stored in the dark at -20 °C.

The large-scale ozonation plant consisted of an ozonation reactor of 25 m^3 total volume. The reactor was operated in a semi-batch manner and filled with a known volume of process waste water (approx. 20 m^3). Ozone was generated from pure oxygen as a feed gas using a corona discharge ozone generator (Kaufmann-Umwelttechnik GmbH, Wehr, Germany) and introduced in a pumped circulation loop using a jet loop injection. The inlet concentration and flow rate of the gas were held constant during ozonation (concentration: approx. 100 g O_3/m^3; gas flow: 100 m^3/h). The ozonation reactors were not equipped with pH meters, thus continuous pH measurement was not possible. For this reason, process waste water was adjusted to the defined pH only initially. The concentration of TOC was assessed at defined time intervals. To that end, liquid samples of approximately 2 L were manually withdrawn through the sampling port. Thereof, 100 mL samples were retrieved for HPLC analysis.

5.2.3. Analytical measurements

As reported by Daoud et al. (2017 and 2020), temperature and pH were monitored by means of the PT100 compensated pH electrode K100PR (Dr. A. Kuntze GmbH, Meerbusch, Germany). The mass flow was determined with the digital mass flow meter D-6300 (M+W Instruments GmbH, Leonhardsbuch, Germany). In gaseous streams, ozone concentration was measured by the ozone analysers BMT 964 (BMT Messtechnik GmbH, Berlin, Germany), comprising a double beam UV-photometer with the working wavelength of 254 nm. The first ozone analyser was located in the inlet gas stream, in line with the ozone generator; the second – in the reactor off-gas. Additionally, concentration of the dissolved ozone was monitored inside the reactor by means of the potentiometric double electrode sensor Krypton K System (Dr. A. Kuntze GmbH, Meerbusch, Germany) with AuAu-600-OO-2-1-PG electrodes, to detect its potential accumulation. All aforementioned measurement data were collected with the Multi-Channel Recorder RSG30 (Endress+Hauser Messtechnik GmbH+Co. KG, Weil am Rhein, Germany) and saved on an SD card, followed by data export to Excel (Microsoft Windows). TOC was determined using the TOC-VCPN analyser (Shimadzu Deutschland GmbH, Duisburg, Germany) within the range of 0.004-25.000 mg/L. Sampling was performed with the ASI-V autosampler (Shimadzu Deutschland GmbH, Duisburg, Germany). In the large-scale reactor, the volume flow was measured using an RAMC-type rotameter (Yokogawa Deutschland GmbH, Ratingen, Germany). Ozone was measured only in the inlet gas stream using the ozone analyser BMT 964 (BMT Messtechnik GmbH, Berlin, Germany). Ozone concentration in the inlet gas stream and gas flow were controlled, visualized, and recorded on a supervisory process control system.

5.2.4. HPLC measurement for quantification of DTPA

Over the years, DTPA as well as EDTA have been analysed by a variety of techniques (Sillanpää and Sihvonen 1997), including titrimetric and spectrophotometric methods. These, however, have recently been substituted by far more sensitive chromatography-based approaches.

Sillanpää, Kokkonen and Sihvonen (1994) and Quitana and Reemtsma (2007) compared LC and GC methods. Both were certified by the International Standard Organisation (ISO) as well as the German Institute for Norms (DIN) as appropriate for the analysis of the chelating compounds of

interest (Quitana and Reemtsma 2007). GC techniques show higher sensitivity but require volatility of compounds. This can be achieved by conversion into methyl-, ethyl-, propyl- or butyl esters and makes sample preparation time-consuming and labour-intensive. As the process waste water contained relatively high concentrations of DTPA, LC-MS was the method of choice for this application.

The measurement of DTPA was performed by HPLC using a temperature-controlled column oven, a variable detector, and an electronic integrator. DTPA was identified and quantified by HPLC with UV detection (Agilent 1100, Boeblingen, Germany); the retention times were consistent with the reference standards available. The applied HPLC system included a quaternary, low pressure mixing pump with vacuum degassing, an autosampler with a temperature-controlled tray (T = 8°C), and a column oven (25 °C). The injection volume was 25 µL. The separation was performed using the Zorbax Eclipse XDB-C18 column (5 µm, 15cm × 4.6 mm) (Macherey-Nagel, Dueren, Germany) with the H2O (+ 0.25% tetrabutylammonium hydrogensulfate, 0.17% tetrabutylammonium hydroxide (40%), 0.004% sulforic acid (65%)) (A) / methanol (B) gradient. Samples were analysed by using a gradient program as follows: 95% A isocratic for 8 min, linear to 10% B within 7 min, linear to 100 % B within 6 min and held for 2 min (flow rate, 0.4 mL/min). The system returned to its initial conditions (95% A) within 0.01 min and was equilibrated for 7 min. UV detection was performed at 260 nm.

5.2.5. LC-HRMS measurement for identification and quantification of transformation products

DTPA and its transformation products were identified by HPLC-HRMS (LTQ-Orbitrap spectrometer with heated electrospray ionization source, Thermo Fisher Scientific, Waltham, USA). The separation was achieved with the Surveyor-LC HPLC system (Thermo Fisher Scientific, Waldbronn, Germany) encompassing a quaternary, low pressure mixing pump with vacuum degassing, an autosampler with a temperature-controlled tray (T = 5 °C), and a column oven (25 °C). The injection volume was 10 µL. For mass spectrometric detection, nitrogen was used as the sheath gas (5 arbitrary units) and helium served as the collision gas (8 arbitrary units). Compound separation was performed on the Nucleodur PolarTec column (1.8 µm, 2 × 50 mm)

(Macherey-Nagel, Dueren, Germany) with the following solvent system: H_2O (+ 0.1% HCOOH) (A) / acetonitrile (+ 0.1% HCOOH +10 mM ammonium acetate) (B) (flow rate, 0.3 mL/min). The samples were analysed using the following gradient program: 0% B isocratic for 1.5 min, linear to 10% B within 1.5 min, within 0.5 min to 50% B and held for 2 min. The system returned to its initial conditions (100% A) within 0.5 min and was equilibrated for 4.5 min. The spectrometer was operated in heated electrospray ionization (HESI) positive mode with the electrospray potential of 5 kV. The m/z range was 50-600 with n resolution of 60,000 at m/z 400. The analysis was done in full scan.

Mass transitions from 392.1 to 199.9 (collision energy of 26) and from 392.1 to 214.1 (collision energy of 24) were monitored at dwell times of 0.1 minute and peak widths of 0.7 each. The samples were diluted 1:100 (water) prior to injection and linear external calibration was performed at concentration levels of 1, 10, 25, 50, 100, 500, and 1,000 ng/mL in water:acetonitrile (v:v, 80:20). Calibration curves were generated using the reference standards available at Bayer Corp. Although exact quantitation was possible only for DTPA and EDTA, the degradation kinetics of DTPA's transformation products were calculated semi-quantitatively assuming similar ionisation in mass spectrometry. Sum formulae were determined through comparison of measured and theoretical mass-to-charge ratios (m/z).

To elucidate the structural formulae of the analysed compounds, the arrangement of their atoms and chemical bonding, multiple-stage mass spectrometry (MS^n) experiments were performed (see supplementary material). Collision-induced dissociation (CID) with the energy of 35 eV was used for fragmentation; helium served as the collision gas.

The available reference standards facilitated delineation of calibration curves for quantification of DTPA (range, 1-1,000 µg/mL) in the process waste water after ozonation at pH 10 (0, 120, 300 min).

Processing and evaluation of all LC-MS data was performed with the Xcalibur Software, Version 2.2.0.48 (Thermo Fisher Scientific, Waltham, USA). The chemical structures were illustrated with BIOVIA draw, Version 19.1 (Dassault Systèmes, Vélizy-Villacoublay, France). All graphs were obtained with Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA).

5.3. Results and discussion

5.3.1. Large-scale ozonation

In 2009, preliminary ozonation tests were performed, verifying the general possibility to degrade DTPA in waste water from the current manufacturing process. Consequently, first large-scale ozonation trials were conducted in November 2010, after receiving approval of the authorities, and achieved best degradation under basic conditions. Based on the above experience, we have replicated the large-scale ozonation experiments. A 25 m^3 reactor was filled with process waste water from the DTPA manufacturing facility. Prior to ozonation, the concentration of DTPA was measured by HPLC-UV. Three development runs (VR2 to VR4) were performed to define the optimal ozonation time to achieve more than 99% degradation of DTPA. The first batch (VR1) was used for adjustment of ozone flow and concentration. After successful validation, subsequent batches (VR5-VR12) were treated without measuring the kinetics (Figure 5.2).

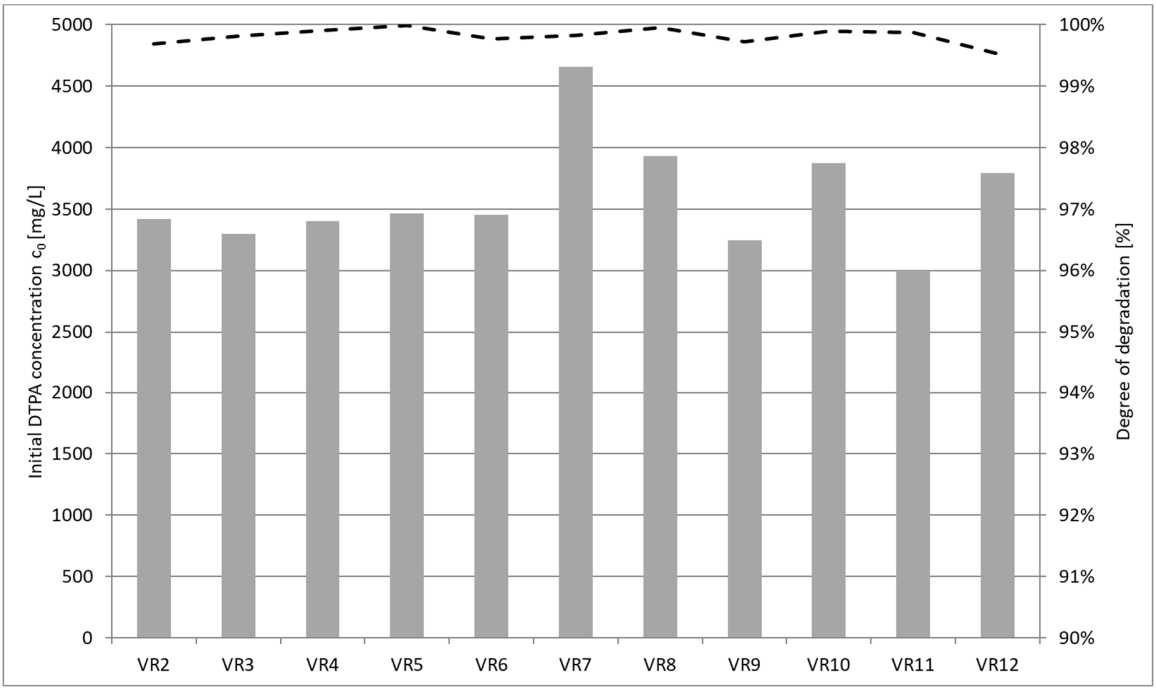


Fig. 5.2. Results of ozonation of DTPA-containing waste water in a large-scale reactor (20 m^3). Batches from VR2 to VR12; grey columns, initial DTPA concentration before ozonation; dashed line, degree of degradation after 480 min of ozonation

Based on the results of the development runs, the ozonation time was set between seven and eight hours, depending on the initial DTPA concentration. As shown in Figure 5.2, all validation batches achieved the required degree of elimination. According to Prasse et al. (2012), pseudo-first-order rate constants can be determined from a plot of -ln(c/c0) versus time based on the exponential shape of the concentration curve, with the rate constant k being the slope of the straight line. The average rate constant of 0.0128 ± 0.0014 1/min was determined by fitting the curves in Figure 5.3. The three curves showed high linearity, with the R^2 factors of 0.980-0.996.

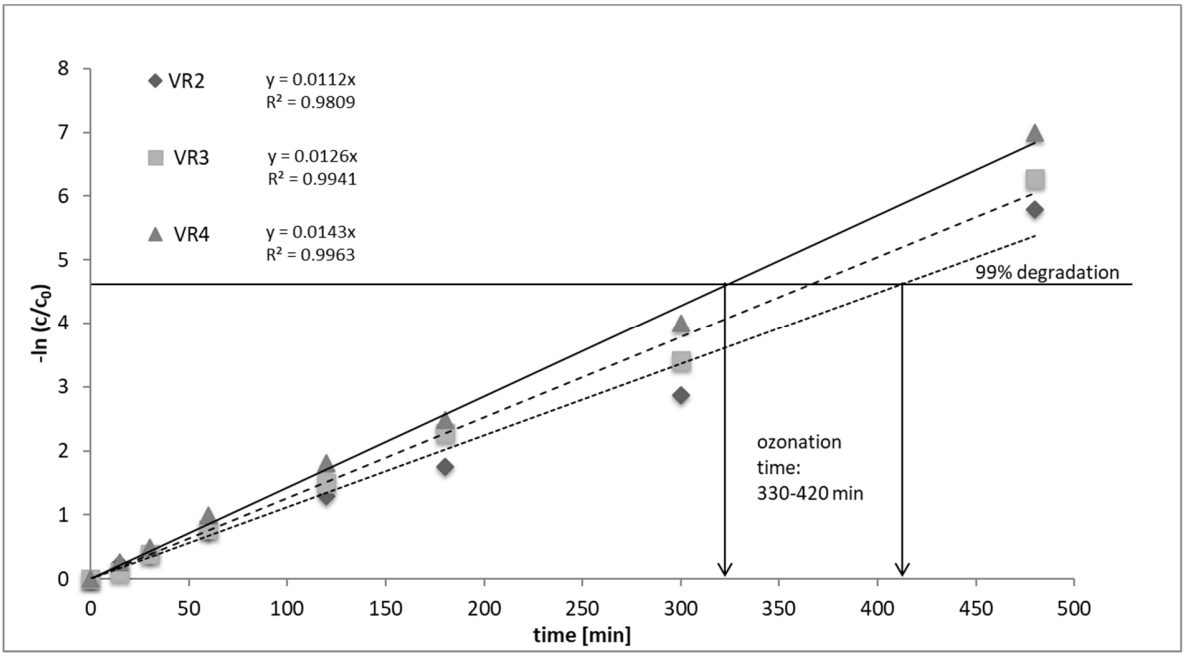


Fig. 5.3. Results of three large-scale ozonation batches. Logarithmic DTPA degree of elimination vs. time was plotted for validation batches VR2 to VR4. All ozonation batches were performed under basic conditions (pH = 10) and with a batch size of 20 m³

5.3.2. Lab-scale ozonation

2 L samples of waste water from batch VR3 were transferred to a laboratory reactor to get detailed information on the process under different conditions. Ozonation experiments were conducted under acidic, neutral, and basic conditions (pH 3, 7 and 10), maintained by continuous addition of sulphuric acid (H_2SO_4) or sodium hydroxide (NaOH). One additional experiment was performed with only the initial pH adjustment in order to simulate realistic applications in the large-scale process. Total mass balance of ozone was used to calculate its consumption according to

Gottschalk, Libra and Saupe (2010) (see supplementary material). As originally assumed, the highest ozone consumption rates were measured in basic and neutral reaction environments, while under acidic conditions the obtained values were much lower (Figure 5.4).

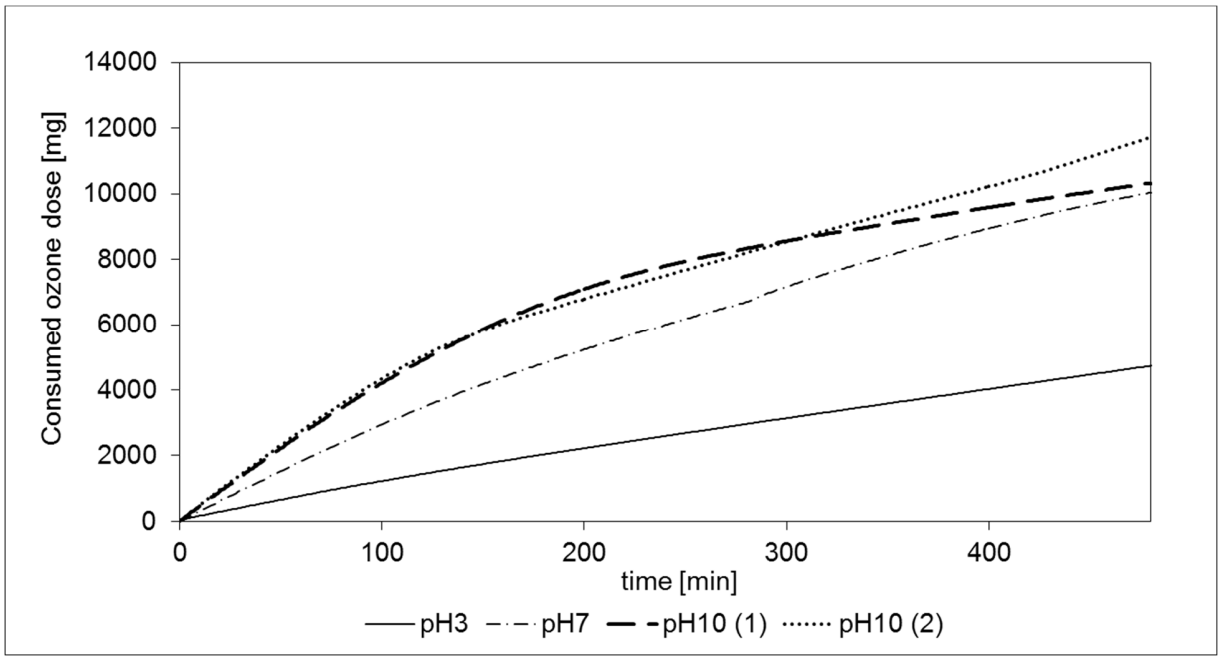


Fig. 5.4. Absorbed ozone vs. ozonation time. Results of four lab-scale ozonation batches processed at three different pH values; pH 3 held constant during ozonation, pH 7 held constant during ozonation, pH 10 held constant during ozonation (1), initially pH 10 reduced to pH 8.5 in the course of ozonation (2)

The laboratory reactor experiments showed that process waste water can be maintained under basic conditions (decrease of pH down to 8.5-9.0 over 8 h) without adjusting the pH during ozonation, given the initial pH value of 10. This will presumably also apply to the large-scale reactor process. These results are attributed to the increased generation of hydroxyl radicals under basic conditions, as postulated by Staehelin and Hoigné (1982). Hydroxyl radicals contribute to the degradation of organic compounds because they react very quickly, but less selectively, with the target compounds.

Additionally, concentration of total organic carbon (TOC) in the investigated waste water was determined at all sampling times. The TOC samples were taken only from the laboratory batch; all of them: process waste water from the validation batch VR3. Initial TOC levels in the DTPA-containing process waste water ranged between 1.57 and 1.78 g/L. Since the initial DTPA

concentration was 3.297 g/L, the TOC derived from carbon atoms in the DTPA molecule was calculated to be approx. 1.4 g/L. That means that between 81 and 90% of the determined TOC was caused by DTPA in the initial process waste water; only 10-20% of the organic carbon corresponded to by-products. All samples showed constant TOC decline (see supplementary material). Under acidic conditions, TOC levels reached 80% of the initial value after 480 min of ozonation, while in basic and neutral reaction environment, the final TOC content settled at 40%. The elimination of DTPA was close to 100%. That means that total mineralization took place for about 60% of the DTPA molecules under basic or neutral conditions, while 40% decomposed into smaller transformation products.

Ozonation experiments and associated TOC measurements delivered a strong indication for basic conditions to be the best for achieving substantive elimination of DTPA. Therefore, detailed analysis of the drug's degradation and the generation of its transformation products was performed only under basic conditions.

5.3.3. Mass spectrometric identification of DTPA

Mass spectrometric identification of DTPA in process waste water was based on available reference standards from Bayer Corp. For both, the reference standard and the waste water sample, two different adducts of DTPA ($[M+H]^+$ and $[M+Na]^+$) were investigated (Fig. 5.5 A).

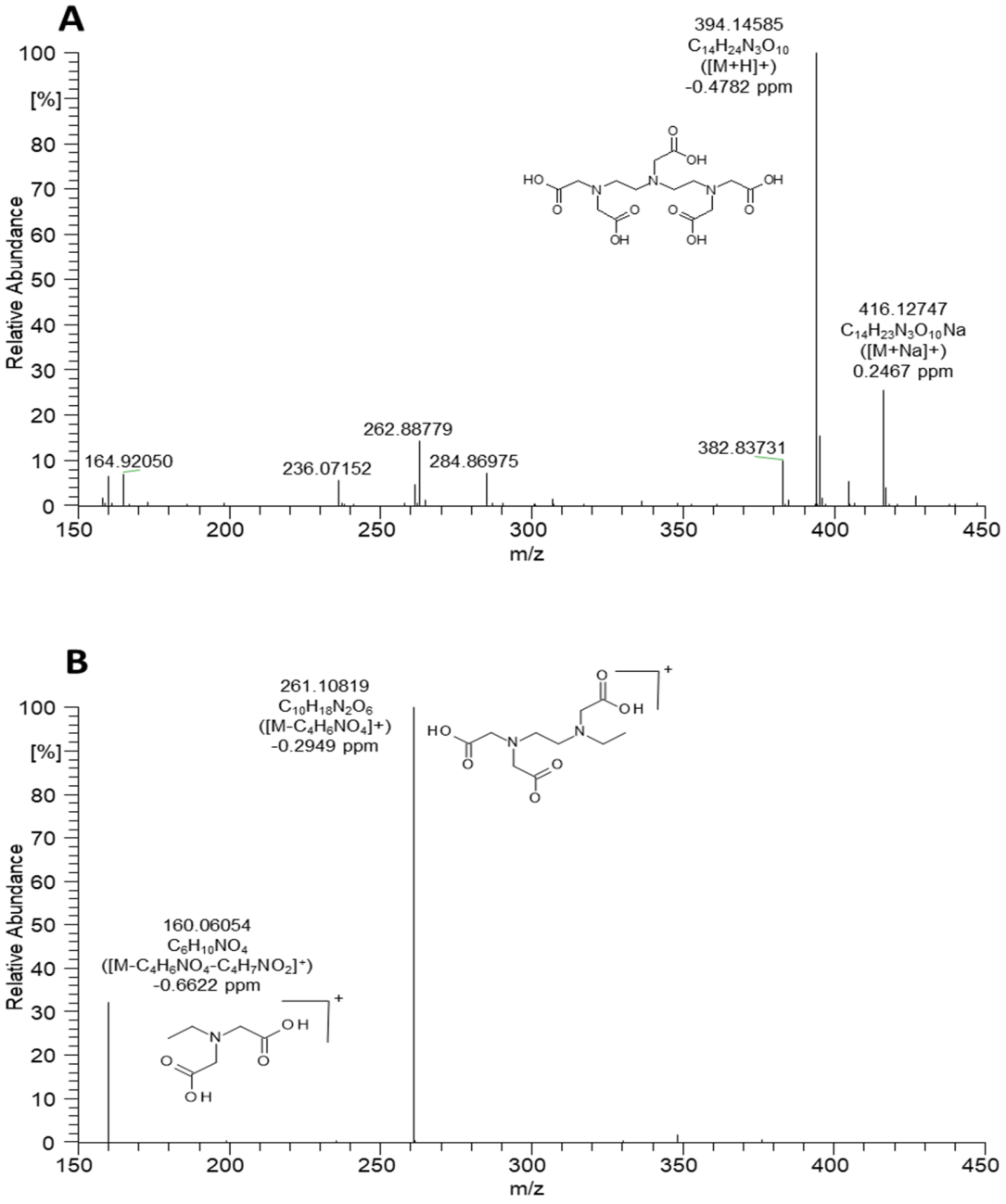


Fig. 5.5. Mass spectrum (A) and MS^2 spectrum (B) of DTPA process water before ozonation

Verification of the structure of DTPA was based on the MS^2 spectra of the drug-containing waste water prior to ozonation, resulting in the fragmentation pattern depicted in Fig. 5.5 B.

5.3.4. Mass spectrometric identification and quantitation of DTPA transformation products

Concentration gradients of some prominent transformation products of the investigated pharmaceutical compound were analysed during the ozonation (0, 120, 300 min at pH 10). Figure 6 illustrates the changes in concentration of DTPA and its main transformation products relative to the initial concentration of the main compound. The exact masses, chemical formulae, and retention times of six transformation products were fully elucidated by LC-HRMS (see supplementary material). Due to its high polarity, DTPA exited the HPLC column without recognizable adsorption to the solid phase and, therefore, with a short retention time of approx. 0.75 min. Potential transformation products eluted at comparable retention times for DTPA together with other impurities. Furthermore, as reference substances had only been available for DTPA and EDTA, quantification of the detected transformation products could not be directly calculated based on the calibration curves but only when assuming DTPA-comparable ionization. Therefore, mass spectra extracted from the total ion chromatograms were used mainly for qualitative analysis, not for quantification.

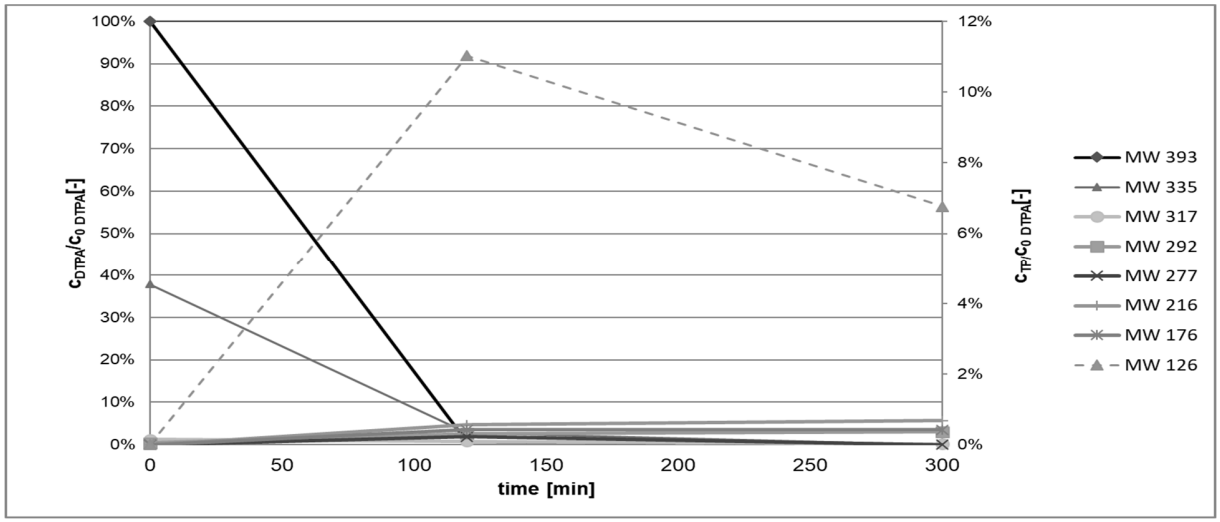


Fig. 5.6. Degradation of DTPA (primary axis) and formation of its most abundant transformation products (secondary axis) in course of waste water ozonation at pH 10 (relative to initial DTPA concentration at time 0 min)

Fig. 5.6 shows the concentration gradients of DTPA and its ozonation products generated under basic conditions. The content of DTPA decreased rapidly and dropped below 1% within 300 min. Several transformation products were identified. Only one, characterized by the molecular weight (MW) of 126 g/mol, reached a significant concentration during ozonation – 11% of the initial DTPA content at 120 min – and subsequently decreased down to 6.8%. A second prominent transformation product designated as MW335, was a by-product of DTPA synthesis. Crystallization and subsequent separation are the final purification steps in course of the drug manufacturing process. Reaction by-products are removed via filtration, and therefore occur in the process waste water. During ozonation, MW335 value fell from the initial 4.5% below the detection limit (at time 300 min.) All other compounds occurred at concentrations below 1%.

Additionally, the LC-HRMS analysis indicated a high number of transformation products as well as by-products of the DTPA manufacturing process. Fig. 7 shows the mass spectra extracted from total ion chromatograms recorded at different ozonation times (0 min and 300 min). In Fig. 5.7A, the most prominent peak is that of DTPA (m/z 394.14585) prior to ozonation. EDTA could be identified as a by-product of the chemical synthesis but was not detected in the scan diagram. After 300 min of ozonation (Fig. 5.7B), DTPA was not cognizable. Only MW126 (m/z 127.05021), as a potential transformation product, was identified according to its exact mass. The corresponding structural formula, as indicated in the mass spectrum, is a postulated structure based on the authors' assumptions and previously published information (see below).

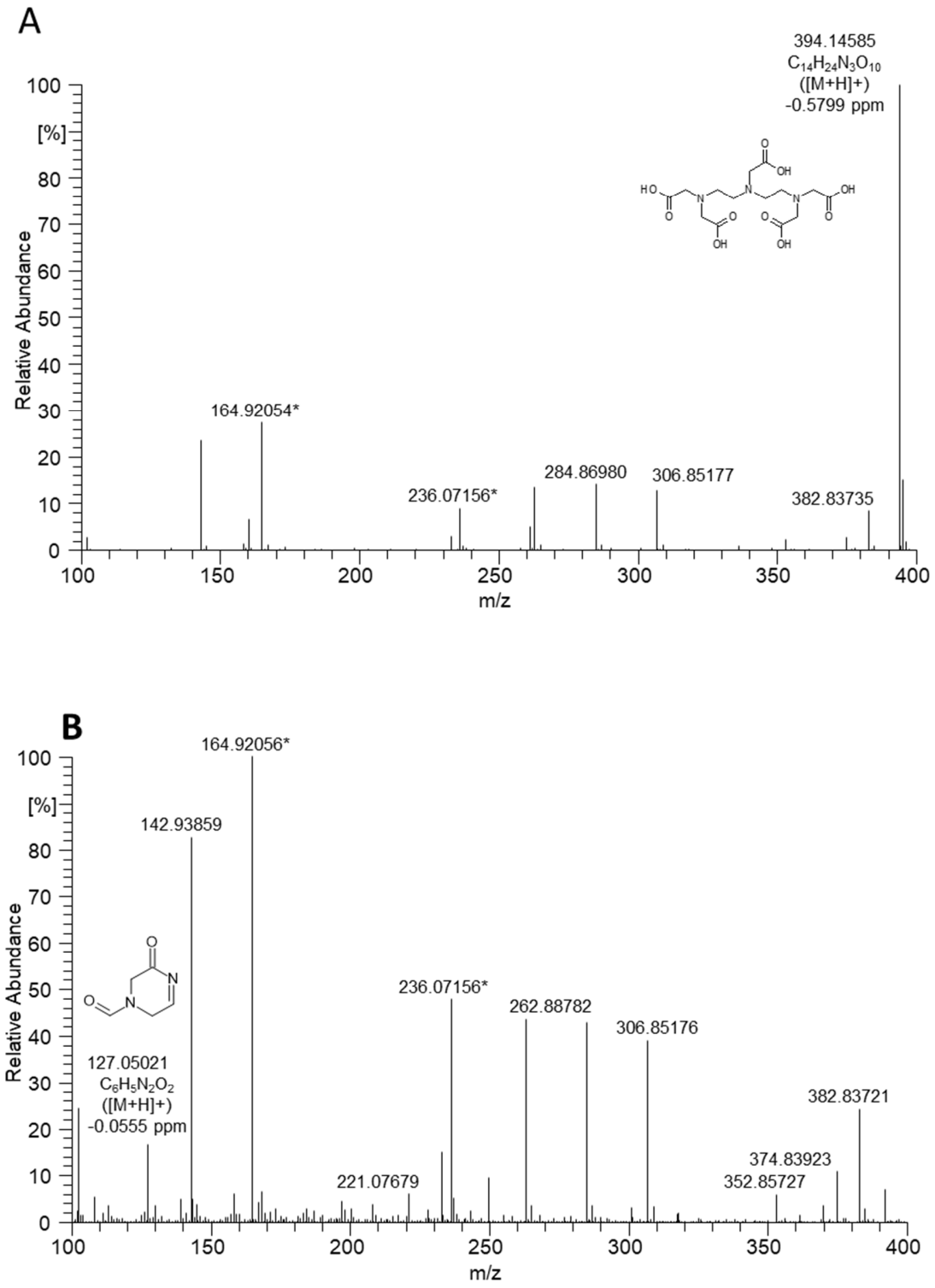


Fig. 5.7. Mass spectrum (extracted from total ion chromatogram) of DTPA process water before (A) and after 300 min of ozonation (B) (*impurity from the LC-MS system); mass spectrum limited to 0.7-0.9 min retention time

Several authors have worked on elucidation of potential DTPA transformation products in the past few years. Thus, most of the postulated chemical structures (and equivalent molecular masses) were identified in previous research reports. Ternes et al. (1996), Metsaerinne et al. (2004), Noertemann (2005) and Xian et al. (2018) characterised diethylenetriaminetetraacetic acid (DTTA; MW335) as a degradation product of DTPA, generated by biodegradation, photochemical degradation or electrochemical incineration. It derives from the parent compound via the loss of one carboxymethyl group. DT4A was successfully identified in this research study. According to Metsaerinne et al. (2004), DT3A is generated from DT4A through decoupling of a second carboxymethyl group. However, our own experiments suggested an additional intermediate (MW317). As reported by Ternes et al. (1996), cyclisation of DT4A leads to ethylamine-2-ketopiperazine triacetate (E-KPTA), $C_{12}H_{19}N_3O_7$ (MW of 317). E-KPTA is a heterocyclic aromatic organic compound with two nitrogen atoms and exocyclic oxygen. Contrarily, our MS/MS data indicated a different transformation product of the same molecular mass. We identified $C_{12}H_{19}N_3O_7$ as a degradation product of DT4A generated via the loss of a hydroxyl group. This transformation product is in accordance with the subsequent derivative, DT3A (MW277).

The cleavage of an acetate group from DT4A leads to diethylenetriaminetriaacetic acid (DT3A; MW277), a compound previously identified by Metsaerinne et al. (2004) and Xian et al. (2018). Additionally, Ternes et al. (1996) and Noertemann (2005) identified ethyleneamineketopiperazinetriacetic acid (KPTA), a heterocyclic compound, as a transformation product of DTPA generated by cyclisation and separation of water. Our ozonation experiments did not confirm the formation of KPTA.

With EDTA as the parent molecule, a second reaction pathway can be delineated. The loss of an acetic acid moiety leads to ethylenediaminetriacetic acid (ED3A, MW234) according to Metsaerinne et al. (2004). In contrast, ED3A is also a potential degradation product of DT3A, which is generated by the removal of an ethylamine moiety (Ternes et al., 1996). ED3A then transforms into ketopiperzindiacetate (KPDA; MW216) by intramolecular cyclisation. In recent research, ED3A has not been identified at significant concentrations; therefore, it is not displayed in the proposed degradation path (Fig. 5.8). Instead, EDTA is postulated to decompose directly to KPDA. Alternatively, cyclisation of DT3A can also occur. Further, upon cleavage of an acetaldehyde group and an ethylamine group from DT3A, N,N-EDDA (MW176) is formed; it is

a transformation product formerly mentioned by Metsaerinne et al. (2004). The most prominent product of the postulated degradation pathway is MW126 ($C_5H_6N_2O_2$). Based on the MS/MS data, the proposed structure contains a piperazine ring with an exocyclic oxygen and an aldehyde moiety. MW126 derives from KPDA by cleavage of a carboxymethyl group and a hydroxyl group. Additionally, Ternes et al. (1996) stated that ethylenediamine carboxylic acids can generate heterocyclic piperazine derivates that are very stable. This observation is in accordance with the results of our research.

Fig. 5.8 illustrates the postulated degradation of DTPA, depicting several transformation products, with the drug and EDTA as parent molecules. The respective molecules contain five and four acetate moieties that can be either removed completely or detach their hydroxy groups.

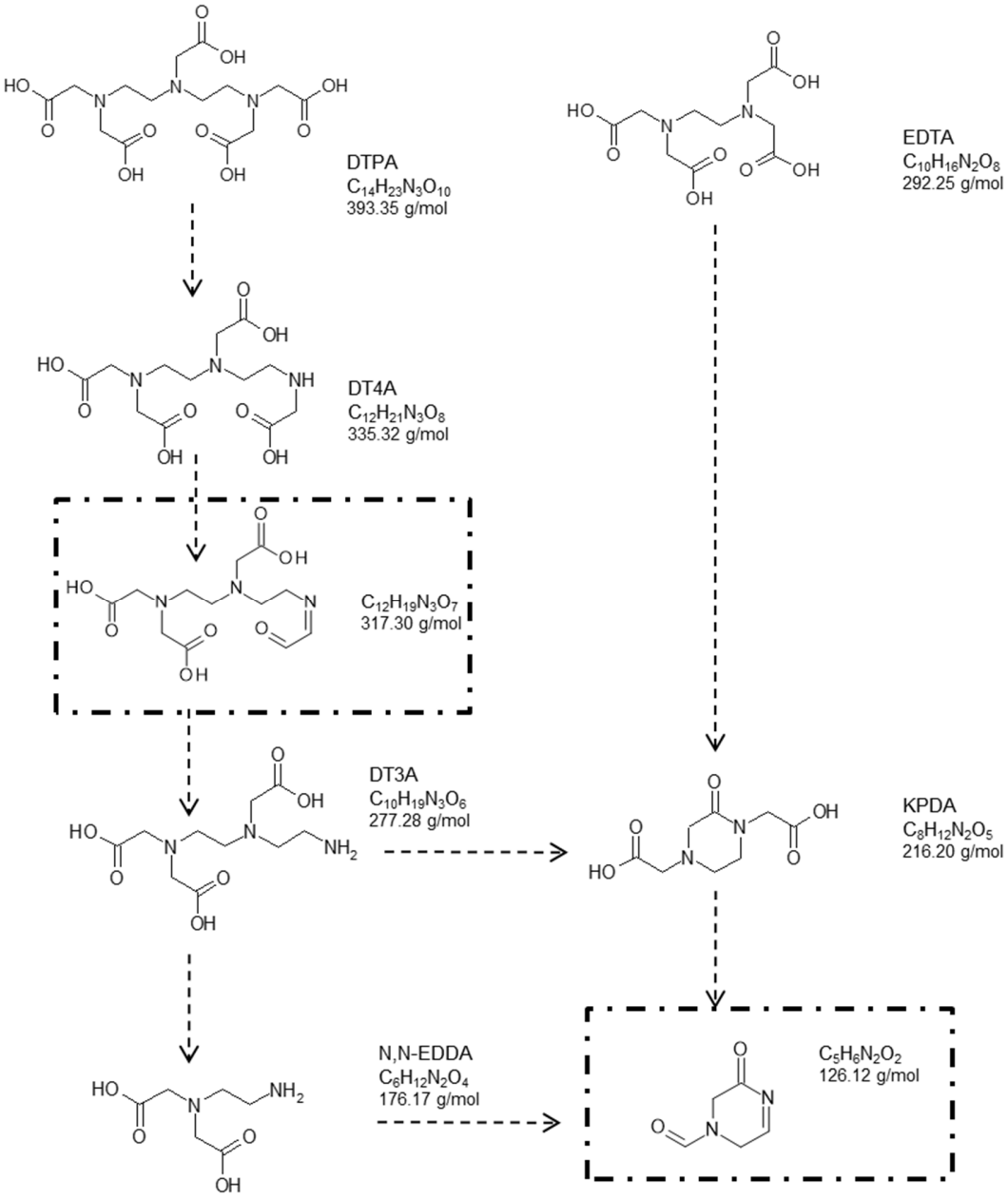


Fig. 5.8. Proposed ozonation pathway of DTPA and EDTA. Newly identified degradation products are enclosed in the dash-dotted line boxes; all other transformation products have been published previously

5.3.5. Ecotoxicology

Prior to ozonation, half maximal effective concentration (EC_{50}) dilution factors for original process waste water from DTPA production were determined. The relevant values were $G_L = 7$ for bacteria,

G_A = 4,320 for algae and G_D = 173 for daphnia. Equivalent dilution factors were assessed after ozonation: G_L = 10, G_A = 10 and G_D = 7. After biological degradation of the ozonated production waste water, the respective EC_{50} dilution factors were G_L = 3, G_A = 2 and G_D = 3. Whilst the impact of DTPA degradation by ozonation on bacteria was negligible, the effect on algae and daphnia was significant.

5.3.6. Ecological evaluation

Previously conducted trials proved that direct ozonation of process water can effectively remove DTPA prior to conventional waste water treatment. Because of the low biological degradability of the target compound, however, process waste water was subject to incineration over the past few years, without consideration of the ecological burden caused by emissions of the greenhouse gas, CO_2. A simple calculation model was used to compare incineration and ozonation with regard to the CO_2 emissions. The incineration process usually starts with process water evaporation to reduce the amount of waste water. Therefore, evaporation down to 20% residue was assumed, utilizing steam as the heat transfer medium and cooling water for condensation. Subsequently, the residual process waste water has to be incinerated with natural gas. Steam, cooling water and natural gas induce CO_2 release, whereas the generation of electrical power in course of incineration can be calculated as a negative CO_2 discharge and subtracted from the overall emission value. On the other hand, ozone is generated from ambient air or pure oxygen using electrical power. The ozone demand can be calculated based on the initial DTPA concentration and the determined rate constants, considering the final concentration of DTPA that meets the criteria of the relevant provisions specified by the district council (minimum degree of elimination > 99%; maximum daily discharge 400 g).

The ozonated process water is subject to discharge in industrial waste water treatment plants (WWTPs). Operation of WWTPs also requires electrical power as well as various operating supplies. Loss of DTPA due to adsorption to the activated sludge or due to biological degradation was not considered. In contrast, CO_2 emissions based on the carbon content of DTPA were taken into account in the underlying calculation but are of marginal impact. Calculated utility demands were converted to CO_2 emissions according to Leimkuehler (2010). Summing up all the contributing emissions, incineration generates a total of approx. 300 kg CO_2/t process waste water,

whereas ozonation emits only approx. 100 kg CO_2/t process waste water (see supplementary material). For incineration, the most significant greenhouse contributors are the use of steam (85%) and natural gas (13%). In case of ozonation, CO_2 emissions are predominantly induced through utilization of electrical power. The balance can be even better if we assume that only renewable energies are used in the generation of electricity, rather than the assumed mix of primary energy sources.

5.4. Conclusions

The current study provides conclusive evidence that ozonation can be effectively used for pre-treatment of DTPA-containing process waste water prior to its biological processing. Both laboratory and large-scale ozonation tests clearly demonstrated the effectiveness of the postulated approach, achieved the relevant requirements (minimum degree of elimination > 99%; maximum daily discharge 400 g) and indicated an active ingredient degradation of > 99%. Based on the results of preliminary laboratory trials, German authorities approved large-scale ozonation of DTPA-containing waste water. Basic reaction conditions (pH 10) proved to be the most favourable, with nearly 60% of all organic components completely decomposed, as indicated in the TOC elimination tests. Numerous transformation products of the chelating agent of interest were identified via LC-HRMS and the most probable structures were postulated. Two of them were reported for the first time with respect to DTPA degradation. Since the LC-HRMS data were not consistent enough for quantification at all tested times tested, they were used only for structural elucidation of the detected metabolites. Quantification of DTPA during ozonation was achieved through employment of HPLC with UV detection. The ozonated waste water was additionally examined for biological degradation. The results of the chemical and biological experiments demonstrated that the concentrations of DTPA and its derivatives were reduced to levels assuring safe discharge into the receiving water. Moreover, a rough estimate of CO_2 emissions showed that ozonation is ecologically beneficial and emits only 30% of the greenhouse gas compared to incineration.

Chapter 6: Discussion and outlook

6.1 Introduction

Ozonation as an alternative waste water treatment method can be established if ecological and economic requirements are met. For this reason, four different active pharmaceutical ingredients were investigated in previous chapters. Waste water from original production of these pharmaceuticals was subject to ozonation experiments. The ozonated waste water, which showed the best results regarding the set pH, was further determined. Potential transformation products were identified by means of mass spectrometry. In addition, ecotoxicological data were evaluated. In the following chapter I will holistically determine which API containing wastewater can be considered for pre-treatment with ozone. The protection of the receiving water and the reduced consumption of primary energy sources are paramount for this decision. Therefore, the concentration of the target compounds should not exceed the calculated PNEC in receiving waters. On the other hand, the CO_2 emissions linked to ozonation and subsequent biological treatment should be less than for waste water incineration.

6.2. Comparison of results

To assess whether ozonation is feasible as a pre-treatment method, all information from previous studies must be collected and compared. First, the target concentration in the receiving waters must be defined. The PNEC can be found in literature for some of the target compounds. In other cases, only target values for groups of substances have been available. To determine the degree of degradation, the PNEC or the target values for the receiving water has been used. The required concentration of the target compound after ozonation is calculated backwards by ascertaining the PNEC or target values in the receiving waters. Therefore, the dilution factor in the industrial sewage treatment plant Rutenbeck, the municipal sewage treatment plant Buchenhofen and finally in the river Wupper as well as degradation by biological treatment and sorption on sludge have been considered. Since the biological degradation was not evaluated experimentally in this work, the calculations had to be based on available degradation rates from the scientific literature. Nevertheless, the test conditions can differ significantly from the actual conditions in the existing sewage treatment plants. Therefore, the author chose to only consider the biodegradation or

sorption available. When data were not available, degradation was not considered in the calculation (Tab. 6.1).

Target Compound	PNEC or target values	Biological degradation	Sorption to sludge
Ciprofloxacin	PNEC: **0.06 µg/L** (Tell et al., 2019)	**0%** (Al-Ahmad et al., 1999) 20-35% (Dorival-Garcia et al, 2013)	**67%** (Puettmann et al., 2008) 88-92% (Xifra, 2000; Golet et al., 2003) in general for fluoroquinolones
Moxifloxacin	PNEC: **0.13 µg/L** (Tell et al., 2019)	**20-35%** (Dorival-Garcia et al, 2013)	**40-60%** (Dorival-Garcia et al, 2013)
Rivaroxaban	Target value for drug residues: **0.1 µg/L** (Pinnekamp et al., 2008)	**0%** (no reference available)	**0%** (no reference available)
DTPA	Target value for chelating agents: **5 µg/L** (Pinnekamp et al., 2008) Target value for X-ray contrast media: **1 µg/L** (Pinnekamp et al., 2008) Lower value has been used	**0%** (Metsärinne et al., 2004)	**0%** (no reference available)

Tab. 6.1 PNEC and target values, biological degradation rates and sorption rates for different pharmaceuticals

The determined rate constants indicate the efficiency of ozonation based on the degree of degradation for the target compound. The higher the rate constant the faster the requested concentration will be achieved. Comparing the four target compounds, Moxifloxacin can be significantly faster degraded than all other compounds (see Fig. 6.1).

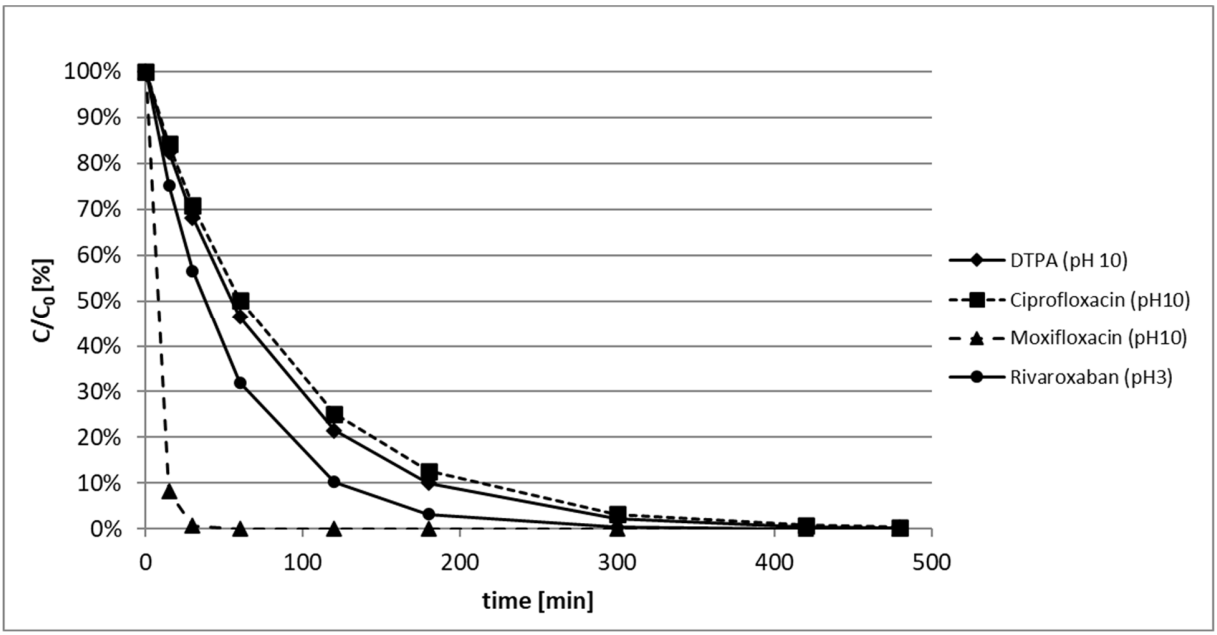


Fig. 6.1 Degradation of all target compounds over time

Even the structurally comparable Ciprofloxacin compound has a much lower rate constant. Apparently, the pyrrolo pyrimidine moiety of moxifloxacin can be much easier degraded than the piperazine moiety of ciprofloxacin. Ciprofloxacin decomposes worst, DTPA slightly better. Rivaroxaban breaks down faster than DTPA. In contrast to all other target compounds, best degradation has been performed for rivaroxaban under acidic conditions where all other compounds have been decomposed best at basic conditions. Based on the rate constants and the predetermined target concentration the ozonation time has been calculated for all target compounds.

Target Compound	**Rate constants**	**Required concentration after ozonation**	**Ozonation time**
Ciprofloxacin	**0.0115 min^{-1}**	**0.058 %** (calculated based on PNEC)	**712 min**
Moxifloxacin	**0.167 min^{-1}**	**0.072 %** (calculated based on PNEC)	**52 min**
Rivaroxaban	**0.0128 min^{-1}**	**1 %** (reduced to 1%, even if calculated value was much higher due to low initial concentration)	**242 min**
DTPA	**0.019 min^{-1}**	**0.3 %** (calculated based on target values)	**454 min**

Tab. 6.2 Rate constants, concentration after ozonation and ozonation time for all target compounds

Other important data are based on the TOC reduction during ozonation. It happens that TOC remains constant during ozonation, but the target compound has been completely degraded. In these cases, smaller transformation products occur that are still dissolved in the waste water. Contrariwise, only part of the target compound degrades, but the TOC decreases (Fig. 6.2). In this case, smaller degradation products have been created and left the ozonation reactor via off gas path. According to the TOC curves Ciprofloxacin, Moxifloxacin and DTPA degrade to a certain extent to volatile compounds whereas all degradation products of rivaroxaban remain in solution during ozonation. The TOC decline indicates that also secondary and tertiary degradation takes place during ozonation (Tab. 6.2).

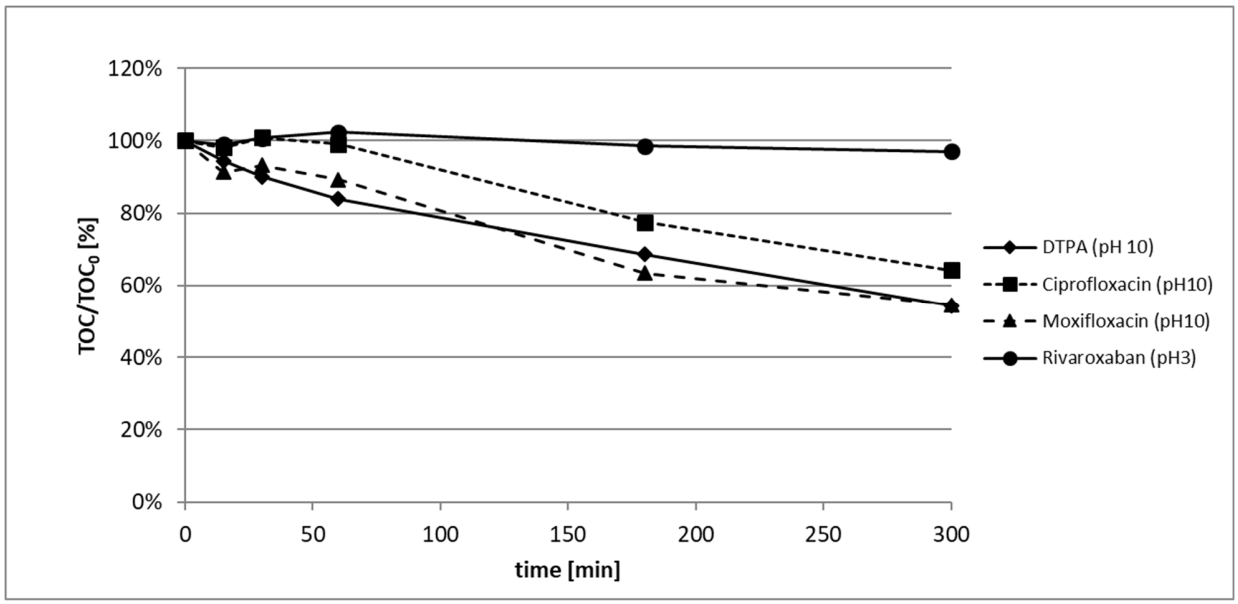


Fig. 6.2 TOC over time for all ozonated waste water streams

Even if the PNEC for the target compounds were not reached, the ecotoxicity of all remaining transformation products for aquatic organisms in receiving waters could be too high. Therefore, the ecotoxicity data for three different trophic levels for ozonated waste water were examined. The calculated dilution factors were compared with the maximum dilution factors that must be adhered to according to the AbwVO, 2004. Taking all trophic levels into account the ozonated rivaroxaban containing waste water cannot be discharged into receiving water at a safe level. The dilution factors of all trophic levels are higher than the requested dilution factors. For ciprofloxacin, only the dilution factor for daphnia exceeds the maximum factor. Extended ozonation time might destruct problematic transformation products, such that a safe discharge of the ozonated waste water is possible. In case of DTPA and moxifloxacin, dilution factors for all trophic levels have been met (Fig.6.3).

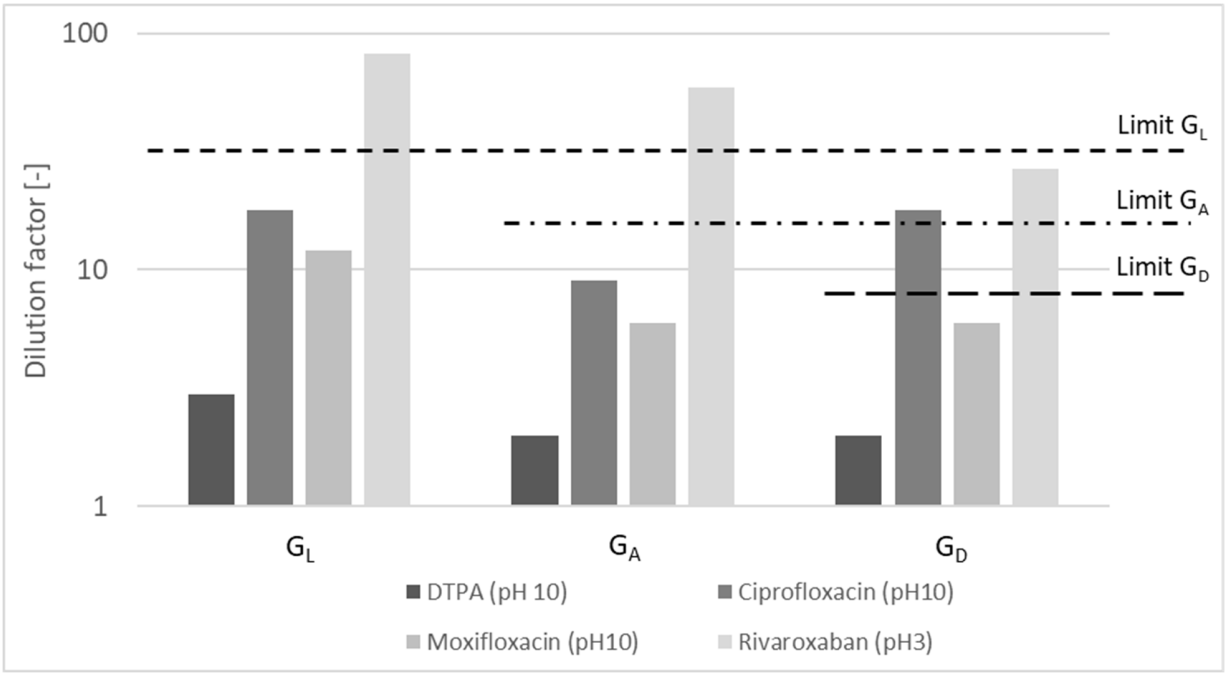


Fig. 6.3 Dilution factors for all trophic levels of all target compounds

After checking the technical feasibility, an ecological assessment was also carried out. Therefore, the ozonation process was compared to the incineration process with precursory waste water evaporation. Both processes were compared based on CO_2 emissions, although incineration has a much higher degradation efficiency. Since the ozonation conditions were the same for all target compounds, the ozonation time is the main driver in the calculation. All target compounds had much lower CO_2 emissions than incineration. From an ecological point of view, ozonation is a valuable alternative to the incineration of non-biodegradable waste water (Figure 6.4). When comparing the CO_2 emissions of all target compounds, the best results were obtained for moxifloxacin followed by rivaroxaban.

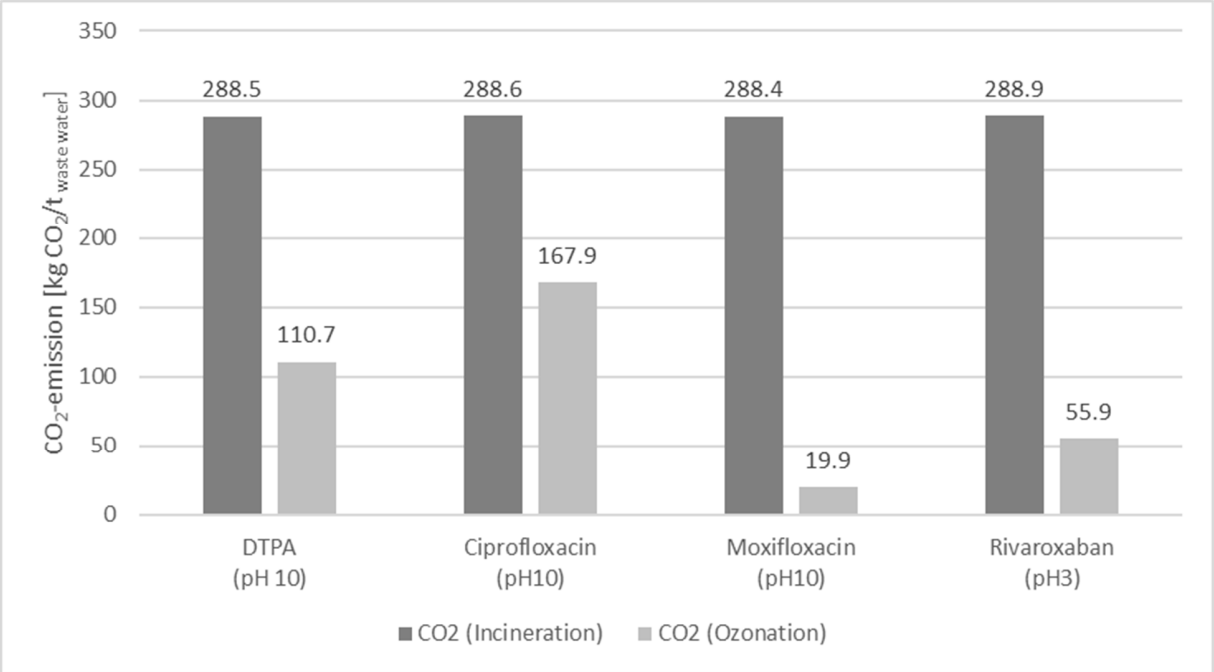


Fig. 6.4 Comparison of CO_2 emissions for incineration and ozonation process for all target compounds

6.3. Discussion and outlook

The current work provides conclusive evidence that ozonation can be used effectively for the pretreatment of process waste water from pharmaceutical production. Four different active pharmaceutical ingredients were tested (ciprofloxacin, moxifloxacin, rivaroxaban, DTPA). In all cases, the original process waste water from the last purification step was used. All laboratory experiments clearly showed that ozone effectively breaks down all examined pharmaceutical compounds. Due to different rate constants for all pharmaceutical compounds, different ozonation times are required to reach the target concentration. At least for waste water containing DTPA, the German authorities have approved large-scale ozonation based on the results of preliminary laboratory tests. The work defined the best reaction conditions by varying the pH value, which had been kept constant during ozonation. In most cases, basic reaction conditions (pH 10) proved to be the most favorable, only rivaroxaban achieved the best results under acidic conditions. Numerous transformation products were identified using LC-HRMS and the most likely structures were postulated. All ozonated waste water was also tested regarding their ecotoxicity. The results

of both experiments showed the possibility for some drugs to safely drain into the receiving water. In addition, a rough estimate of CO_2 emissions has shown that ozonation is environmentally beneficial and emits far fewer greenhouse gases compared to incineration. Even though not all target concentrations and ecological data have been achieved, the results of all ozonation pathways are very promising. In most cases, an extended ozonation time can lead to the required target concentrations in the receiving waters. The test plan, which consists of ozonation experiments and subsequent biological degradation tests, is useful to evaluate the feasibility of ozonation as a pre-treatment method. Since the decomposition of the target compounds itself does not provide complete evidence for the method, additional parameters must be assessed that also take the transformation products into account. With the presented experiments and the analytical setup, any waste water that meets the requirements can be examined for ozonation pretreatment. In summary, it can be stated that using ozone as a pretreatment method can avoid elaborate waste water incineration. With the pretreatment method ecological and economic advantages can be achieved through the lower operating costs and lower CO_2 emissions.

References

Chapter 2

Abegglen, C., Escher, B., Hollender, J., Koepke, S., Ort, C., Peter, A., Siegrist, H., von Gunten, U., Zimmermann, S. G., Koch, M., Niederhauser, P., Schärer, M., Braun, C., Gälli, R., Junghans, M., Brocker, S. & Rensch, D. (2009). Ozonung von gereinigtem Abwasser. Schlussbericht Pilotversuch Regensdorf, Publikation zum Projekt «Strategie MicroPoll». Eawag Dübendorf.

Abegglen, C., Escher, B., Hollender, J., Siegrist, H., von Gunten, U., Zimmermann, S. G., Häner, A., Ort, C. & Schärer, M. (2010). Ozonung von gereinigtem Abwasser zur Elimination von organischen Spurenstoffen: Grosstechnischer Pilotversuch Regensdorf (Schweiz). KA: Korrespondenz Abwasser Abfall, 57(2), 155-160. Gesellschaft zur Förderung der Abwassertechnik.

AbwV. (2004). Verordnung über Anforderungen an das Einleiten von Abwasser in Gewässer. Anhang 1 Häusliches und kommunales Abwasser. Bundesministerium der Justiz. Available at https://www.gesetze-im-internet.de/abwv/anhang_1.html (last accessed 30 July 2021).

Andreozzi, R., Campanella, L., Fraysse, B., Garric, J., Gonnella, A., Lo Giudice, R., Marotta, R., Pinto, G. & Pollio, A. (2004). Effects of advanced oxidation processes (AOPs) on the toxicity of a mixture of pharmaceuticals. Water Science and Technology, 50(5), 23-28. IWA Publishing.

Beltran-Heredia, J., Torregrosa, J., Dominguez, J. R., & Peres, J. A. (2001). Kinetics of the reaction between ozone and phenolic acids present in agro-industrial wastewaters. Water Research, 35(4), 1077-1085. Elsevier.

Beltran, F. J. (2003). Ozone reaction kinetics for water and wastewater systems. Boca Raton, Fla. CRC Press.

Benner, J. (2009). Ozone reactivity in wastewater treatment plant effluent and reverse osmosis concentrate ozonation of beta blockers: kinetic studies, identification of oxidation products and pathways. (Doctoral dissertation, Universität Koblenz-Landau Fachbereich 3: Mathematik/Naturwissenschaften)

Bergmann, A., Fohrmann, R., & Weber, F. A. (2011). Zusammenstellung von Monitoringdaten zu Umweltkonzentrationen von Arzneimitteln. Umweltbundesamt Dessau-Rosslau.

Bound, J. P., & Voulvoulis, N. (2005). Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. Environmental health perspectives, 113(12), 1705-1711. National Institute of Environmental Health Sciences.

Buffle, M. O., Schumacher, J., Salhi, E., Jekel, M., & Von Gunten, U. (2006). Measurement of the initial phase of ozone decomposition in water and wastewater by means of a continuous quench-flow system: application to disinfection and pharmaceutical oxidation. Water Research, 40(9), 1884-1894. Elsevier.

Chapman, S. (1930). XXXV. On ozone and atomic oxygen in the upper atmosphere. The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science, 10(64), 369-383. Taylor & Francis.

Constapel, M. (2005). Untersuchungen zum Abbau ausgewählter Reaktivfarbstoffhydrolysate mit Ozon: Charakterisierung der Produkte durch LC-MS-Analyse (Doctoral dissertation, Universität Wuppertal, Fakultät für Mathematik und Naturwissenschaften).

Criegee, R. (1975). Mechanismus der ozonolyse. Angewandte Chemie, 87(21), 765-771. Wiley Online Library.

Daoud, F., Pelzer, D., Zuehlke, S., Spiteller, M., Kayser, O, 2017. Ozone pretreatment of process waste water generated in course of fluoroquinolone production. Chemosphere 185, 953–963. Elsevier.

Daoud, F., Zuehlke, S., Spiteller, M., Kayser, O, 2020. Ozonation of rivaroxaban production waste water and comparison of generated transformation products with known in vivo and in vitro metabolites. Sci. Total Environ. 714, 136825. Elsevier.

Elovitz, M. S., von Gunten, U., & Kaiser, H. P. (2000). Hydroxyl radical/ozone ratios during ozonation processes. II. The effect of temperature, pH, alkalinity, and DOM properties. Ozone: science & engineering, 22(2), 123-150. Taylor & Francis.

EMA, E. (2006). Guideline on the environmental risk assessment of medicinal products for human use. EMEA/CHMP/SWP/4447/00. Committee for Medicinal Products for Human Use

(CHMP), London, UK. Available at https://www.ema.europa.eu/en/environmental-risk-assessment-medicinal-products-human-use (last accessed 30 July 2021).

Fahlenkamp, H., Nöthe, T., Nowotny, N., & Launer, M. (2008). Untersuchungen zum Eintrag und zur Elimination von gefährlichen Stoffen in kommunalen Kläranlagen. Phase 3 (Projekt-Nr.: IV-9-0421720030). Abschlussbericht zum Forschungsvorhaben, Ministerium für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz (MUNLV) des Landes NRW.

Fent, K. (2008). Effects of pharmaceuticals on aquatic organisms. In pharmaceuticals in the environment (pp. 175-203). Wiesbaden. Springer Berlin Heidelberg.

Franco, D. V., Jardim, W. F., Boodts, J. F., & Da Silva, L. M. (2008). Electrochemical ozone production as an environmentally friendly technology for water treatment. CLEAN–Soil, Air, Water, 36(1), 34-44. Wiley Online Library.

Githinji, L. J., Musey, M. K., & Ankumah, R. O. (2011). Evaluation of the fate of ciprofloxacin and amoxicillin in domestic wastewater. Water, Air, & Soil Pollution, 219(1-4), 191-201. Springer.

Goetz, K., & Keil, F. (2007). Medikamentenentsorgung in privaten Haushalten: Ein Faktor bei der Gewässerbelastung mit Arzneimittelwirkstoffen?. Umweltwissenschaften und Schadstoff-Forschung, 19(3), 180-188. SpringerOpen.

Gottschalk, C., Libra, J.A., Saupe, A., 2010. Ozonation of water and waste water. 2nd Ed. Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim.

Gunnarsson, B., & Wennmalm, Å. (2006). Environmental risk assessment and environmental classification of drugs. Environment and pharmaceuticals. Apoteket AB (The National Corporation of Swedish Pharmacies), Stockholm County Council and Stockholm University, 117-25.

Heberer, T. (2002). Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicology letters, 131(1-2), 5-17. Elsevier.

Heberer, T. (2006). Verhalten von Arzneimittelrückständen bei der Abwasserreinigung. In Heil-Lasten, 175-205. Springer, Berlin, Heidelberg.

Hernando, M. D., Mezcua, M., Fernández-Alba, A. R., & Barceló, D. (2006). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta, 69(2), 334-342. Elsevier.

H. Jones, O. A., Voulvoulis, N., & Lester, J. N. (2005). Human pharmaceuticals in wastewater treatment processes. Critical reviews in environmental science and technology, 35(4), 401-427. Taylor & Francis.

Hoersch, P. (2004). Einfluss der chemischen Oxidation auf das toxische Potenzial und das biologische Abbauverhalten von Industrieabwässern. Band 39. Schriftenreihe des Lehrstuhls für Wasserchemie und der DVGW-Forschungsstelle am Engler-Bunte-Institut der Universität Karlsruhe (TH).

Hoersch, P., & Frimmel, F. H. (2000). Oxidation of an industrial waste water by ozone and UV/H2O2. VOM WASSER, 95, 119-130. VCH VERLAGSGESELLSCHAFT

Hoigne, J. (1982). Mechanisms, rates and selectivities of oxidations of organic compounds initiated by ozonation of water. Handbook of Ozone Technology and Applications., 1.

Hosselet, L. M. L. F. (1971). Ozonbildung mittels elektrischer Entladungen. Technische Hogeschool Eindhoven.

Huber, M. M., Goebel, A., Joss, A., Hermann, N., Loeffler, D., McArdell, C. S., Ried, A., Siegrist, H., Ternes, T. & von Gunten, U. (2005). Oxidation of pharmaceuticals during ozonation of municipal wastewater effluents: a pilot study. Environmental science & technology, 39(11), 4290-4299. ACS Publications.

Ikehata, K., Jodeiri Naghashkar, N., & Gamal El-Din, M. (2006). Degradation of aqueous pharmaceuticals by ozonation and advanced oxidation processes: a review. Ozone: Science and Engineering, 28(6), 353-414. Taylor & Francis.

Jiménez-González, C., Overcash, M. R., & Curzons, A. (2001). Waste treatment modules–a partial life cycle inventory. Journal of Chemical Technology & Biotechnology, 76(7), 707-716. Taylor & Francis.

Kabasci, S., Börgers, A., Vitz, H., Kiffmeyer, T., Tuerk, J., & Becker, B. (2007). Oxidative Behandlung von Krankenhausabwasser-Teilströmen zur Beseitigung von persistenten,

hochwirksamen Pharmazeutika. Abschlussbericht zum AiF-geförderten Forschungsvorhaben, (14396).

Kuemmerer, K. (Ed.). (2008). Pharmaceuticals in the environment: sources, fate, effects and risks. Springer Science & Business Media.

Kuemmerer, K., Schuster, A., Längin, A., Happel, O., Thoma, A., Schneider, K., Hassauer, M., Gartiser, S. & Hafner, C. (2011). Identifizierung und Bewertung ausgewählter Arzneimittel und ihrer Metaboliten (Ab-und Umbauprodukte) im Wasserkreislauf. German Environment Agency. Dessau-Roßlau. Available at https://www. umweltbundesamt. de/en/publikationen/ identifizierung-bewertung-ausgewaehlter (last accessed 30 July 2021).

Laengin, A., Schuster, A., & Kuemmerer, K. (2008). Chemicals in the environment–the need for a clear nomenclature: parent compounds, metabolites, transformation products and their elimination. CLEAN–Soil, Air, Water, 36(4), 349-350. Wiley Online Library.

Larsson, D. J., de Pedro, C., & Paxeus, N. (2007). Effluent from drug manufactures contains extremely high levels of pharmaceuticals. Journal of hazardous materials, 148(3), 751-755. Elsevier.

Magara, Y., Itoh, M., & Morioka, T. (1995). Application of ozone to water treatment and power consumption of ozone generating systems. Progress in Nuclear Energy, 29, 175-182. Elsevier.

Moebius, C. H., Cordier, D., Helble, A., Kaulbach, R., & Cordes-Tolle, M. (1996). Elimination persistenter Verbindungen in biologisch gereinigtem Abwasser mit Ozon und weitergehender biologischer Reinigung. Gas-und Wasserfach. Wasser, Abwasser, 137(12), 646-651.

Mustafa, M. G. (1990). Biochemical basis of ozone toxicity. Free Radical Biology and Medicine, 9(3), 245-265. Elsevier.

Mvula, E., & von Sonntag, C. (2003). Ozonolysis of phenols in aqueous solution. Organic & Biomolecular Chemistry, 1(10), 1749-1756. Royal Society of Chemistry.

Neuwahl, F., Cusano, G., Benavides, J. G., Holbrook, S., & Roudier, S. (2019). Best available techniques (BAT) reference document for waste incineration. Industrial Emissions Directive 2010/75/EU (Integrated Pollution Prevention and Control), EUR 29971 EN, Publications Office of the European Union, Luxembourg, 2019.

Noethe, T., Hartmann, D., Von Sonntag, J., Von Sonntag, C., & Fahlenkamp, H. (2007). Elimination of the musk fragrances galaxolide and tonalide from wastewater by ozonation and concomitant stripping. Water science and technology, 55(12), 287-292. IWA Publishing.

Pinnekamp, J., Merkel, W., Tacke, D., Herbst, H., Köster, S., Beier, S., Bergmann, A. & Mälzer, H. J. (2008). Senkung des Anteils organischer Spurenstoffe in der Ruhr durch zusätzliche Behandlungsstufen auf kommunalen Kläranlagen-Güte-und Kostenbetrachtungen. Abschlussbericht zum Forschungsvorhaben des Ministeriums für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz NRW. MUNLV.

Pinnekamp, J., Metzger, J. W., Kuch, B., Schneider, C., & Schrader, C. (2003). Untersuchung und Optimierung der Abwasserreinigung zur Eliminierung von organischen Spurenstoffen unter verfahrenstechnischen und ökonomischen Aspekten. Literaturstudie gefördert durch das Ministerium für Umwelt und Verkehr Baden-Württemberg. Available at http://www.iswa.uni-stuttgart.de/awt/files/Schlussbericht_M1.pdf . (last accessed 30 June 2010).

Rehman, M. S. U., Ahmad, N., Sarwar, M., & Hussain, W. (2006). Pretreatment of complex industrial wastewater by ozonation. Journal of Water and Environment Technology, 4(1), 51-59. Japan Society on Water Environment.

Remy, C., Lesjean, B., & Hartmann, A. (2011). Die Methodik der Ökobilanz zur ganzheitlichen Erfassung des Energieverbrauchs in der Abwasserreinigung. KA Abwasser Abfall, 58(6), 572.

Roegener, W. 2010 Medikamente aus der Kläranlage, Süddeutsche Zeitung (09.09.2010). Available at https://www.sueddeutsche.de/wissen/umweltverschmutzung-medikamente-aus-der-klaeranlage-1.997961 (last accessed 30 July 2021).

Rüütel, P. I., Griffiths, J., Xiong, F., & Barratt, P. (1999). Ozone-technical aspects of its generation and use. Knowledge paper, (3).

Schmidt, T. C., Lutze, H., & Merkel, W. (2010). Möglichkeiten und Grenzen oxidativer Verfahren in der Wasseraufbereitung. Vom Wasser, 108(2), 41.

Schmitt, M., & Hempel, D. C. (1993). Verbesserung der biologischen Abbaubarkeit durch Vorbehandlung mit Ozon. Korrespondenz Abwasser, 40, 1469-1469. GESELLSCHAFT ZUR FORDERUNG DER ABWASSERTECHNIK EV (GFA)

Schroeder, H. F. (2003). Abwasserreinigungsverfahren zur verbesserten Elimination pharmazeutischer und endokrin wirksamer Reststoffe. Spurenstoffe in Gewässern; Pharmazeutische Reststoffe und endokrin wirksame Substanzen. T. Track, G. Kreysa (Hrsg.), 153-172. Wiley Online Library.

Staehelin, J., & Hoigne, J. (1982). Decomposition of ozone in water: rate of initiation by hydroxide ions and hydrogen peroxide. Environmental Science & Technology, 16(10), 676-681. ACS Publications.

Staehelin, J., & Hoigné, J. (1983). Reaktionsmechanismus und kinetik des ozonzerfalls in wasser in gegenwart organischer stoffe. Vom Wasser, 61, 337-348.

Straub, J. O., Acklin, C., Klemmer, J., Kurlbaum, M. A., Li, B., Zurbruegg, R., & Haener, A. (2020). Assessment, pretreatment and treatment of pharmaceutical production wastewaters in the Roche Group. CHIMIA International Journal for Chemistry, 74(3), 161-167. Swiss Chemical Society.

Ternes, T. A., Stüber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M., & Teiser, B. (2003). Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater?. Water research, 37(8), 1976-1982. Elsevier.

Ternes, T., Siegrist, H., & Joss, A. (2006). Vorkommen und Herkunft von Arzneistoffen in Fließgewässern. In Heil-Lasten (pp. 89-103). Springer, Berlin, Heidelberg.

World Health Organization. Regional Office for South-East Asia. (2010). Water safety plans. WHO Regional Office for South-East Asia. Available at https://apps.who.int/iris/handle/10665/206528 (last accessed 30 July 2021).

Chapter 3

Akmehmet Balcioğlu, I., Oetker, M., 2003. Treatment of pharmaceutical waste water containing antibiotics by O_3 and O_3/H_2O_2 processes. Chemosphere 50 (1), 85-95. Elsevier.

An, T., Yang, H., Song, W., Li, G., Luo, H., Cooper, W.J., 2010. Mechanistic considerations for the advanced oxidation treatment of fluoroquinolone pharmaceutical compounds using TiO_2

heterogeneous catalysis. The Journal of Physical Chemistry A 114, 2569-2575. Publ. ACS Publications.

Andreozzi, R., Campanella, L., Fraysse, B., Garric, J., Gonella, A., Lo Giudice, R., Marotta, R., Pinto, G., Pollio, A., 2004. Effects of advanced oxidation processes (AOPs) on the toxicity of a mixture of pharmaceuticals. Water Science and Technology 50 (5), 23-28. IWA Publishing.

Appelbaum, P.C., Hunter, P.A., 2000. The fluoroquinolone antibacterials: past, present and future perspectives. International Journal of Antimicrobial Agents 16, 5-15. Elsevier.

Blondeau, J.M., 2004. Fluoroquinolones: mechanism of action, classification, and development of resistance. Survey of Ophthalmology 49 (2), S73-S78. Elsevier.

Burhenne, J., Ludwig, M., Spiteller, M., 1997. Photolytic degradation of fluoroquinolone carboxylic acids in aqueous solution: Part 1. Environmental Science and Pollution Research International 4 (2), 10-15. Springer.

Burhenne, J., Ludwig, M. Spiteller, M., 1997. Photolytic degradation of fluoroquinolone carboxylic acids in aqueous solution: Part 2. Environmental Science and Pollution Research International 4 (2), 61-67. Springer.

Calza, P., Medana, C., Carbone, F., Giancotti, V., Baiocchi, C., 2008. Characterization of intermediate compounds formed upon photoinduced degradation of quinolones by high-performance liquid chromatography/high-resolution multiple-stage mass spectrometry. Rapid Communications in Mass Spectrometry 22 (10), 1533-1552. Wiley Online Library.

Cardoza, L.A., Knapp, C.W., Larive, C.K., Belden, J.B., Lydy, M., Graham, D.W., 2005. Factors affecting the fate of ciprofloxacin in aquatic field systems. Water, Air and Soil Pollution161 (1-4), 383-398. Springer.

De Witte, B., Dewulf, J., Demeestere, K., van de Vyvere, V., de Wispelaere, P. van Langenhove, H., 2008. Ozonation of ciprofloxacin in water: HRMS identification of reaction products and pathways. Environmental Science and Technology 42 (13), 4889-4895. ACS Publications.

De Witte, B., Dewulf, J., Demeestere, K., van Langenhove, H., 2009. Ozonation and advanced oxidation by the peroxone process of ciprofloxacin in water. Journal of Hazardous Materials 161 (2-3), 701-708. Elsevier.

Dodd, M.C., Buffle, M.O., von Gunten, U.,2006. Oxidation of antibacterial molecules by aqueous ozone: moiety-specific reaction kinetics and application to ozone-based wastewater treatment. Environmental Science and Technology 40 (6), 1969-1977. ACS Publications.

Gottschalk, C., Libra, J.A., Saupe, A., 2010. Ozonation of water and waste water. 2nd Ed. Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim.

Heynderickx, P.M., Demeestere, K., Dewulf, J., de Witte, B., van Langenhove, H., 2011. Formal bimolecular kinetic model for the ozonation of ciprofloxacin in the liquid phase. Journal of Advanced Oxidation Technologies 14 (1) 71-80. De Gruyter.

Kabasci, S., 2007. Oxidative treatment of partial streams of hospital effluents to eliminate persistent, highly active pharmaceuticals, Part 2: scale-up of the process, installation and optimization of a pilot plant (Oxidative Behandlung von Krankenhausabwasser-Teilstroemen zur Beseitigung von persistenten, hochwirksamen Pharmazeutika, Teil 2: Scale-up des Verfahrens, Aufbau und Optimierung einer Demonstrationsanlage). Fraunhofer Institut fuer Umwelt-, Sicherheits- und Energietechnik UMSICHT.

Kovalova, L., Siegrist, H., von Gunten, U., Eugster, J., Hagenbuch, M., Wittmer, A., Moser, R., McArdell, C.S., 2013. Elimination of micropollutants during post-treatment of hospital wastewater with powdered activated carbon, ozone, and UV. Environmental Science and Technology 47 (14),7899-7908. ACS Publications.

Lesher, G.Y., Froelich, E.J., Gruett, M.D., Bailey, J.H., Brundage, R.P., 1962. 1-8-Naphthyridine derivates. A new class of chemotherapeutic agents. Journal of Medicinal Chemistry, 5(5), 1063-1065. ACS Publications.

Paul, T., Dodd, M.C., Strathmann, T.J., 2010. Photolytic and photocatalytic decomposition of aqueous ciprofloxacin: transformation products and residual antibacterial activity. Water Research 44 (10), 3121-3132. Elsevier.

Pelzer, D., 2015. Identification and purification of ozonation products of 6-fluoroquinolones (Identifizierung und Aufreinigung der Ozonisierungsprodukte von 6-Fluorchinolonen). Master thesis, Institute of Environmental Research (INFU), Department of Chemistry and Chemical Biology, Technical University of Dortmund, Germany.

Prasse, C., Wagner, M., Schulz, R., Ternes, T.A.,2012. Oxidation of the antiviral drug acyclovir and its biodegradation product carboxy-acyclovir with ozone: kinetics and identification of oxidation products. Environ. Sci. Technol. 46, 2169−2178. ACS Publications.

Rodríguez, A., Rosal, R., Perdigón-Melón, J. A., Mezcua, M., Agueera, A., Hernando, M. D., Letón, P., Fernández-Alba, A.R., García-Calvo, E., 2008. Ozone-based technologies in water and wastewater treatment. The handbook of environmental chemistry 5 (S/2), 127-175. Springer.

Staehlin, J., Hoigné, J., 1982. Decomposition of ozone in water: rate of initiation by hydroxide ions and hydroxide peroxide. Environmental Science and Technology 16, 676-682. ACS Publications.

Sukul, P., Spiteller, M., 2007. Fluorquinolone antibiotics in the environment. Reviews of Environmental Contamination and Toxicology 191, 131-162. Springer.

Thabaj, K., Kulkarni S., Chimatadar S., Nandibewoor S., 2007. Oxidative transformation of ciprofloxacin by alkaline permanganate : a kinetic and mechanistic study. Polyhedron 26 (17), 4877-4885. Elsevier.

Tuerk, J., 2006. Presentation: Degradation of pharmaceuticals in partial streams of hospital effluents using AOP (Praesentation: Abbau von Pharmazeutika in Krankenhausabwasser-Teilstroemen mittels AOP). 4. Krankenhausumweltag NRW Essen, den 01.09.2008. Bereich Umweltmedizin–Analytik, Institut fuer Energie-und Umwelttechnik e.V. (IUTA). Duisburg.

Tuerk, J., Boergers, A., vom Eyser, C., Richard, J., Dopp, E., Bester, K., 2012. Evaluation and prevention against toxic oxidation by-products in oxidative water treatment of fluoroquinolones (Untersuchungen zur Bewertung und Vermeidung von toxischen Oxidationsnebenprodukten bei der oxidativen Abwasserbehandlung von Fluorquinolonen). Mitt Umweltchem Oekotox18. Jahrg. 2012/Nr.2.

Van Doorslaer, X., Demeestere, K., Heynderickx, P.M., van Langenhove, H., Dewulf, J., 2011. UV-A and UV-C induced photolytic and photocatalytic degradation of aqueous ciprofloxacin and moxifloxacin: reaction kinetics and role of adsorption. Applied Catalysis B: Environmental 101, 540-547. Elsevier.

Wang, P., He, Y.-L., Huang, C.-H., 2010. Oxidation of fluoroquinolone antibiotics and structurally related amines by chlorine dioxide: reaction kinetics, product and pathway evaluation. Water Research 44 (20), 5989-5998. Elsevier.

Zhao, W., van der Voet, E., Huppes, G., Zhang, Y., 2009. Comparative life cycle assessments of incineration and non-incineration treatments for medical waste. The International journal of life cycle assessment, 14(2), 114-121. Springer.

Chapter 4

European Medicines Agency. CHMP assessment report forXarelto EMEA/543519/2008. 2008. Available at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_ report/human/000944/ WC500057122.pdf (last accessed 30 July 2021).

Gottschalk, C., Libra, J.A., Saupe, A., 2010. Ozonation of water and waste water. 2nd Ed. Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim.

Hernandez, I., Zhang, Y., 2017. Comparing stroke and bleeding with rivaroxaban and dabigatran in atrial fibrillation: Analysis of the US Medicare Part D Data. American Journal of Cardiovascular Drugs, 17(1), 37-47. Springer.

Janssen, B., 2015. Ozonation of production waste water from the manufacturing of rivaroxaban and sorafenib (Ozonierung von Produktionsabwaessern aus der Herstellung von Rivaroxaban und Sorafenib). Master thesis, Institute of Environmental Research (INFU), Department of Chemistry and Chemical Biology, Technical University of Dortmund, Germany.

Kasad, P.A., 2013. Photolytic-thermal degradation study and method development of rivaroxaban by RP-HPLC. International Journal of PharmTech Research, 5(3), 1-5.

Lang, D., Freudenberger, C., Weinz, C., 2009. In vitro metabolism of rivaroxaban, an oral, direct factor Xa inhibitor, in liver microsomes and hepatocytes of rats, dogs, and humans. Drug Metabolism and Disposition. 37(5), 1046-1055. ASPET.

Perzborn, E., Roehrig, S., Straub, A., Kubitza, D., Mueck, W., Laux, V., 2010. Rivaroxaban: a new oral factor Xa inhibitor. Arteriosclerosis, thrombosis, and vascular biology, 30(3), 376-381. American Heart Association.

Prasse, C., Wagner, M., Schulz, R., Ternes, T.A.,2012. Oxidation of the antiviral drug acyclovir and its biodegradation product carboxy-acyclovir with ozone: kinetics and identification of oxidation products. Environ. Sci. Technol. 46, 2169−2178. ACS Publications.

Ramisetti, N.R., Kuntamukkala, R., 2014. Development and validation of a stability indicating LC-PDA-MS/MS method for separation, identification and characterization of process related and stress degradation products of rivaroxaban. RSC Advances, 4(44), 23155-23167. Royal Society of Chemistry.

Roehrig, S., Straub, A., Pohlmann, J., Lampe, T., Pernerstorfer, J., Schlemmer, K.H., Reinemer, P., Perzborn, E., 2005. Discovery of the novel antithrombotic agent 5-Chloro-*N*-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide (Bay 59-7939): an oral, direct factor Xa inhibitor. Journal of medicinal chemistry, 48(19), 5900-5908. ACS Publications.

Rohde, G., 2008. Determination of rivaroxaban – a novel, oral, direct Factor Xa inhibitor – in human plasma by high-performance liquid chromatography-tandem mass spectrometry. Journal of Chromatography B, 872(1-2), 43-50. Elsevier.

Staehlin, J., Hoigné, J., 1982. Decomposition of ozone in water: rate of initiation by hydroxide ions and hydroxide peroxide. Environmental Science and Technology 16, 676-682. ACS Publications.

Takács-Novák, K., Urac, M., Horváth, P., Völgyi, G., Anderson, B.D., Avdeef, A., 2017. Equilibrium solubility measurement of compounds with low dissolution rate by Higuchi's facilitated dissolution method. A validation study. European Journal of Pharmaceutical Sciences, 106, 133-141. Elsevier.

Weinz, C., Schwarz, T., Kubitza, D., Mueck, W., Lang, D., 2009. Metabolism and excretion of rivaroxaban, an oral, direct factor Xa inhibitor, in rats, dogs, and humans. Drug Metabolism and Disposition, 37(5), 1056-1064. ASPET.

Wingert, N.R., dos Santos, N.O., Nunes, M.A.G., Gomes, P., Müller, E.I., Flores, É.M.M., Steppe, M., 2016. Characterization of three main degradation products from novel oral anticoagulant rivaroxaban under stress conditions by UPLC-Q-TOF-MS/MS. Journal of pharmaceutical and biomedical analysis, 123, 10-15. Elsevier.

Yuan, J., Liu, K., Li, L., Yuan, Y., Liu, X., Li, Y., 2014. A novel synthesis of the oxazolidinone antithrombotic agent rivaroxaban. Molecules, 19(9), 14999-15004. Multidisciplinary Digital Publishing Institute.

Chapter 5

AkzoNobel Product Stewardship Team, 2011. Product Stewardship Summary – Chelates: DTPA. Rev.0-19 August 2011. Available

Baeza, C., Oviedo, C., Zaror, C., Rodríguez, J., Freer, J., 2007. Degradation of EDTA in a total chlorine free cellulose pulp bleaching effluent by UV/H_2O_2 treatment. Journal of the Chilean Chemical Society, 52(1), 1069-1072. Sociedad Chilena de Química.

Cyris, M., Knolle, W., Richard, J., Dopp, E., von Sonntag, C., Schmidt, T.C., 2013. Reaction of gadolinium chelates with ozone and hydroxyl radicals. Environmental science & technology, 47(17), 9942-9949. ACS Publications.

Daoud, F., Pelzer, D., Zuehlke, S., Spiteller, M., Kayser, O, 2017. Ozone pretreatment of process waste water generated in course of fluoroquinolone production. Chemosphere 185, 953–963. Elsevier.

Daoud, F., Zuehlke, S., Spiteller, M., Kayser, O, 2020. Ozonation of rivaroxaban production waste water and comparison of generated transformation products with known in vivo and in vitro metabolites. Science of The Total Environment, 714, 136825. Elsevier.

Gottschalk, C., Libra, J.A., Saupe, A., 2010. Ozonation of water and waste water. 2nd Ed. Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim.

Kreuzinger, N., Schaar, H., 2011. KomOzon. Technische Umsetzung und Implementierung einer Ozonungsstufe für nach dem Stand der Technik gereinigtes kommunales Abwasser.

Heranführung an den Stand der Technik. lebensministerium.at Available at https://www.umweltfoerderung.at/fileadmin/user_upload/media/umweltfoerderung/Dokumente_Betriebe/Wasser_Betriebe/Studien_Wasserwirtschaft/Ozonierung_von_gereinigtem_Abwasser.pdf (last accessed 27 April 2020).

Leimkuehler, H.J., 2010. Managing CO_2 Emissions in the Chemical Industry. Publ. Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim.

Means, J.L., Kucak, T., Crerar, D.A., 1980. Relative degradation rates of NTA, EDTA and DTPA and environmental implications. Environmental Pollution Series B, Chemical and Physical, 1(1), 45-60. Elsevier.

Metsärinne, S., Rantanen, P., Aksela, R., Tuhkanen, T., 2004. Biological and photochemical degradation rates of diethylenetriaminepentaacetic acid (DTPA) in the presence and absence of Fe(III). Chemosphere 55(3), 379–388. Elsevier.

Montagne A., Toga A.W., Zlokovic B.V.,2016. Blood-brain barrier permeability and gadolinium: benefits and potential pitfalls in research. JAMA Neurol. 73(1), 13–14. American Medical Association.

Nispel, F., Baumann, W., Hardes, G., 1990. Abbauversuche an DTPA in Modellkläranlagen. Abwasserreinigung 37,707–709.

Nörtemann, B., 2005. Biodegradation of chelating agents: EDTA, DTPA, PDTA, NTA and EDDS, in Nowack B., VanBriesen J.M (Eds), Biogeochemistry of Chelating Agents, ACS Symposium Series, Vol. 910, Chapter 8, pp 150–170. American Chemical Society

Pinnekamp, J., Merkel, W., 2008. Senkung des Anteils organischer Spurenstoffe in der Ruhr durch zusätzliche Behandlungsstufen auf kommunalen Kläranlagen – Gütebetrachtungen. Vergabe-Nr. 07/111.1 (IV-7-042 1 D 7); Senkung des Anteils organischer Spurenstoffe in der Ruhr durch zusätzliche Behandlungsstufen auf kommunalen Kläranlagen – Kostenbetrachtungen. Vergabe-Nr. 07/111.2 (IV-7-042 1 D 6). MUNLV (last accessed 27 April 2020).

Prasse, C., Wagner, M., Schulz, R., Ternes, T.A.,2012. Oxidation of the antiviral drug acyclovir and its biodegradation product carboxy-acyclovir with ozone: kinetics and identification of oxidation products. Environ. Sci. Technol. 46, 2169−2178. ACS Publications.

Quitana, J.B., Reemtsma, T., 2007. Rapid and sensitive determination of ethylenediamine-tetraacetic acid and diethylenetriaminepentaacetic acid in water samples by ion-pair reversed-phase liquid chromatography--electrospray tandem mass spectrometry. Journal of Chromatography A, 1145(1-2), 110-117. Elsevier.

Rämö, J., Sillanpää, M., 2001. Degradation of EDTA by hydrogen peroxide in alkaline conditions. Journal of cleaner production, 9(3), 191-195. Elsevier.

Randt, C., Wittlinger, R., Merz, W., 1993. Analysis of nitrilotriacetic acid (NTA), ethylenediamine-tetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) in water, particularly waste water. Fresenius' journal of analytical chemistry, 346(6), 728-731. Springer.

Sillanpää, M., Kokkonen, R., Sihvonen, M-L., 1995. Determination of EDTA and DTPA as their Fe (III) complexes in pulp and paper mill process and waste waters by liquid chromatography. Analytica chimica acta, 303(2-3), 187-192. Elsevier.

Sillanpää, M., Kurniawan, T.A., Lo, W.H., 2011. Degradation of chelating agents in aqueous solution using advanced oxidation process (AOP). Chemosphere 83(11), 1443–1460. Elsevier.

Sillanpää, M., Sihvonen, M.L., 1997. Analysis of EDTA and DTPA. Talanta 44(8), 1487–1497. Elsevier.

Staehlin, J., Hoigné, J., 1982. Decomposition of ozone in water: rate of initiation by hydroxide ions and hydroxide peroxide. Environmental Science and Technology 16, 676-682. ACS Publications.

Stemmler, K., Glod, G., von Gunten, U., 2001. Oxidation of metal-diethylenetriaminepentaacetate (DTPA)--complexes during drinking water ozonation. Water research, 35(8), 1877-1886. Elsevier.

Ternes, T.A., Stumpf, M., Steinbrecher, T., Brenner-Weiss, G., Haberer, K., 1996. Identifizierung und Nachweis neuer Metabolite des DTPA in Fliessgewässern und Trinkwasser. Vom Wasser 87, 1319–1322. VCH

Vogl, T.J., Balzer, J., Dresel, S., Grevers, G., Peer, F., Steger W., Wilimzig C., 2013. Kernspintomographie der Kopf-Hals-Region: Funktionelle Topographie – klinische Befunde – Bildgebung – Spektroskopie. Springer-Verlag Berlin Heidelberg GmbH.

Xian, J., Liu, M., Chen, W., Zhang, C., Fu, D., 2018. Chemometric study on the electrochemical incineration of diethylenetriaminepentaacetic acid using boron-doped diamond anode. Chemosphere 198, 257–265. Elsevier.

Chapter 6

Al-Ahmad, A., Daschner, F. D., & Kümmerer, K. (1999). Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria. Archives of environmental contamination and toxicology, 37(2), 158-163. Springer.

Dorival-Garcia, N., Zafra-Gomez, A., Navalón, A., González, J., & Vílchez, J. L. (2013). Removal of quinolone antibiotics from wastewaters by sorption and biological degradation in laboratory-scale membrane bioreactors. Science of the Total Environment, 442, 317-328. Elsevier.

Golet, E. M., Xifra, I., Siegrist, H., Alder, A. C., & Giger, W. (2003). Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. Environmental science and technology, 37(15), 3243-3249. ACS Publications.

Metsärinne, S., Rantanen, P., Aksela, R., Tuhkanen, T., 2004. Biological and photochemical degradation rates of diethylenetriaminepentaacetic acid (DTPA) in the presence and absence of Fe(III). Chemosphere 55(3), 379–388. Elsevier.

Pinnekamp, J., Merkel, W., Tacke, D., Herbst, H., Köster, S., Beier, S., Bergmann, A. & Mälzer, H. J. (2008). Senkung des Anteils organischer Spurenstoffe in der Ruhr durch zusätzliche Behandlungsstufen auf kommunalen Kläranlagen-Güte-und Kostenbetrachtungen. Abschlussbericht zum Forschungsvorhaben des Ministeriums für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz NRW. MUNLV.

Püttmann, W., Keil, F., Oehlmann, J., & Schulte-Oehlmann, U. (2008). Wassertechnische Strategien zur Reduzierung der Trinkwasserbelastung durch Arzneimittelwirkstoffe. Environmental Sciences Europe, 20(3), 209-226. Springer.

Tell, J., Caldwell, D. J., Häner, A., Hellstern, J., Hoeger, B., Journel, R., Mastrocco, F., Ryan, J.J., Snape, J., Straub, J.O. & Vestel, J. (2019). Science-based targets for antibiotics in receiving waters from pharmaceutical manufacturing operations. Integrated environmental assessment and management, 15(3), 312-319. Wiley Online Library.

Xifra, I. (2000). Behaviour of ciprofloxacin and norfloxacin during wastewater treatment. ETH, Zurich, Switzerland.

Appendix

I. List of abbreviations

AbwVO	Abwasserverordnung (English: Waste Water Ordinance)
AOP	Advanced oxidation process
AOX	Adsorbable Organic Halides
DIN	Deutsches Institut für Normung (English: German Institute for Standardization)
DNA	Deoxyribonucleic acid
DTPA	diethylenetriamine pentaacetic acid
CID	Collision induced dissociation
COD	Chemical Oxgen Demand
EC_{50}	Half maximal effective concentration
EMA	European medicinal agency
EN	European Norms
EU	European Union
eV	Electron volt
FDA	Food and Drug Administration
GOW	Gesundheitlicher Orientierungswert (English: health orientation value)
GWP	Global Warming Potential
HESI	Heated electrospray ionization
HRMS	High-resolution mass spectrometry
HPLC	High Performance Liquid Chromatography
Hz	Hertz
INFU	Institut für Umweltforschung
LC	Liquid Chromatography
LC_{50}	median lethal dose
LoD	Limit of detection
LTQ	Linear Trap Quadropole
MHz	Mega hertz
MSn	Multi Stage Mass Spectrometry
MW	Molecular Weight

NOEC	No observed effect level
NRW	North Rhine Westfalia
OECD	Organisation for Economic Co-operation and Development
PEC	predicted environmental concentration
pH	pondus Hydrogenii
PNEC	Predicted No Effect Concentration
TOC	Totol organic carbon
UBA	Umweltbundesamt (English: federal environmental agency)
UV	Ultraviolet
WHO	World Health Organization
WWTP	Waste water treatment plant

II. List of symbols

Latin symbols

a	specific area	[m²]
$A(O_3)$	absorbed ozone dose	[mg L^{-1}]
c(M)	concentration of the compound M	[g L^{-1}]
c_G	concentration in the gas phase	[g L^{-1} or mol L^{-1}]
c_L	concentration in the liquid phase	[g L^{-1} or mol L^{-1}]
c_L^*	saturation concentration	[g L^{-1} or mol L^{-1}]
d	diameter	[m]
$D(O_3)$	specific ozone consumption	[mg L^{-1}]
E	mass transfer enhancement-factor	[-]
G_A	Dilution Factor for the toxicity to algae	[-]
G_D	Dilution Factor for the toxicity to daphnia	[-]
G_L	Dilution Factor for the toxicity to luminous bacteria	[-]
G_M	Dilution Factor for the mutagenic potential	[-]
G_{Ei}	Dilution Factor for the toxicity to fish eggs	[-]
h	height	[m]
k	film mass transfer coefficient	[m s^{-1}]
k_{app}	apparent rate constant	[1 s^{-1}]
k_D	rate constant for direct ozone reaction	[1mol^{-1} s^{-1}]
k_L	liquid film mass transfer coefficient	[m s^{-1}]
k_La	liquid phase volumetric mass transfer coefficient	[m s^{-1}]
k_R	rate constant for indirect ozone reaction	[1mol^{-1} s^{-1}]
m_G	mass of gas phase	[kg]
m_L	mass of liquid phase	[kg]
m/z	mass to charge ratio	[kg/C]
Q_G	gas flow rate	[L s^{-1}]
Q_L	liquid flow rate	[L s^{-1}]
r	reaction rate	[g $L^{-1}s^{-1}$]
r_G	ozone consumption rate in gas phase	[g $L^{-1}s^{-1}$]
r_L	ozone consumption rate in liquid phase	[g $L^{-1}s^{-1}$]
R	linearity factor	[-]

t	time	[s]
T	temperature	[°C or K]
$\mathbf{V_L}$	liquid volume, reaction volumen	[L or m³]
$\mathbf{V_G}$	gas volumen	[L or m³]

Greek symbols

$\boldsymbol{\rho_G}$	Density gas phase	[kg m^{-3}]
$\boldsymbol{\rho_L}$	Density gas phase	[kg m^{-3}]

III. Supplementary Material

Chapter 3

Experimental setup

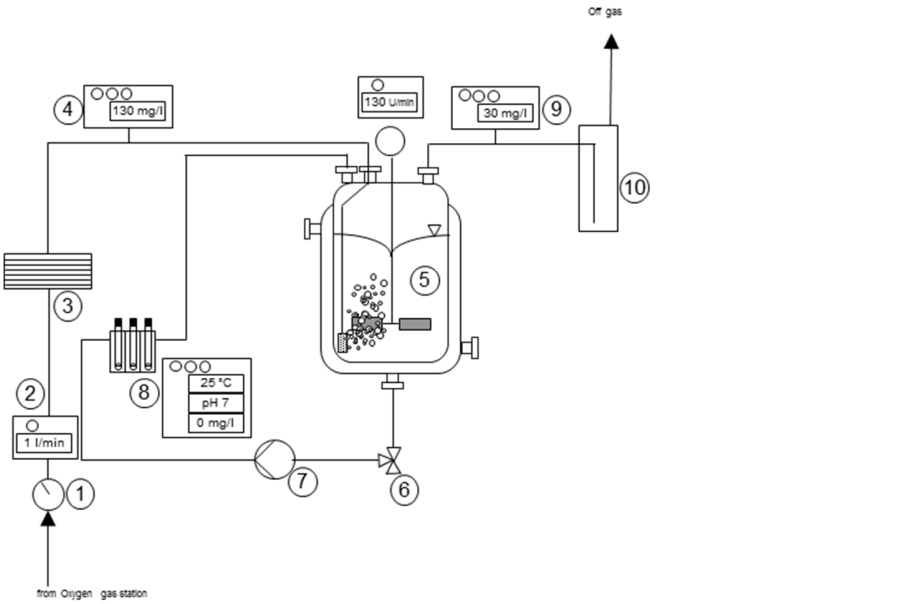


Fig. II-3-1: Experimental setup

Ozone consumption

$$A(O_3) = \sum_{i=1}^{n} \left[\frac{Q_G}{V_L(t_i)} (c_{Ozon,0}(t_i) - \overline{c_{Ozon,e}(t_i + \Delta t_i)}) \cdot \Delta t_i \right]$$

where:

$A(O_3)$, absorbed ozone dose (mg/L)
Q_G, gaseous mass flow (L/s)
V_L, reaction volume (L)
t, time (s)
$c_{Ozon,0}$, inlet ozone concentration in the gaseous phase (mg/L)
$c_{Ozon,e}$, outlet ozone concentration in the gaseous phase (mg/L)

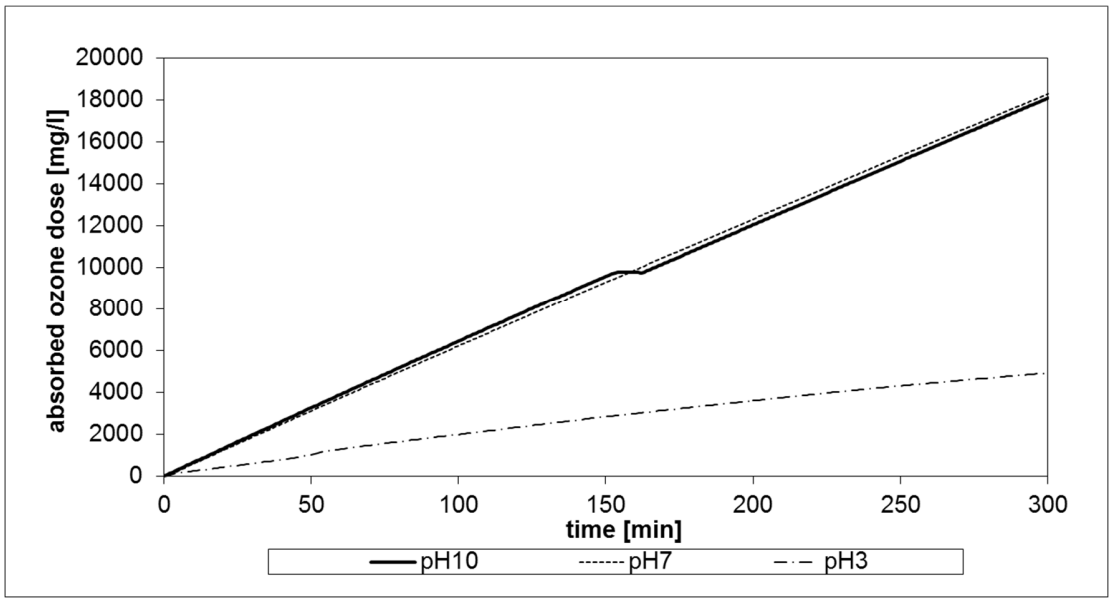


Fig. II-3-2: Absorbed ozone dose in course of ozonation of ciprofloxacin-containing waste water at pH 10, pH 7 and pH 3

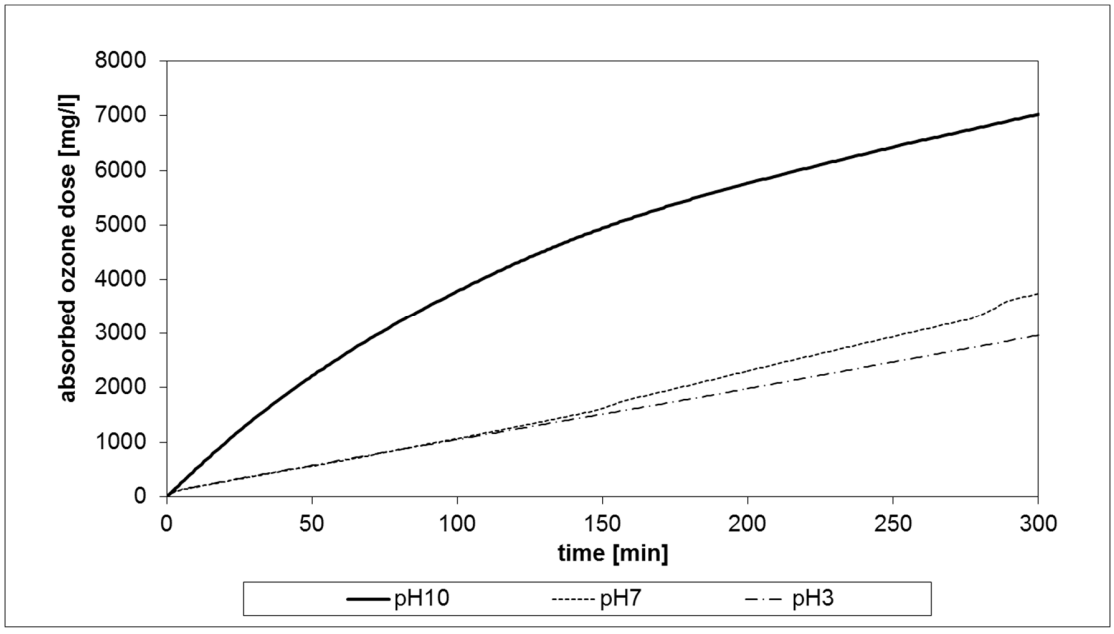


Fig. II-3-3: Absorbed ozone dose in course of ozonation of moxifloxacin-containing waste water at pH 10, pH 7 and pH 3

Calculation of rate constants

Rate constants were calculated according to the following equations:

$$c(M_t) = c(M_{t=0}) \exp^{-k_{ges} \cdot c(O_3) \cdot t} \quad \text{(C.1)}$$

Simplification:

$$c(M_t) = c(M_{t=0}) \exp^{-k_{app} \cdot t} \quad \text{(C.2)}$$

$$\ln \frac{c(M_t)}{c(M_{t=0})} = k_{app} \cdot t \quad \text{(C.3)}$$

time	0	15	30	60	120	180	300
concentration [mg/l]	3244.750759	3067.721544	2834.877474	2062.143734	1463.777705	560.0479033	61.38858075
c/c_0 [%]	100.0%	94.5%	87.4%	63.6%	45.1%	17.3%	1.9%
$-\ln(c/c_0)$	0.00	0.06	0.14	0.45	0.80	1.76	3.97

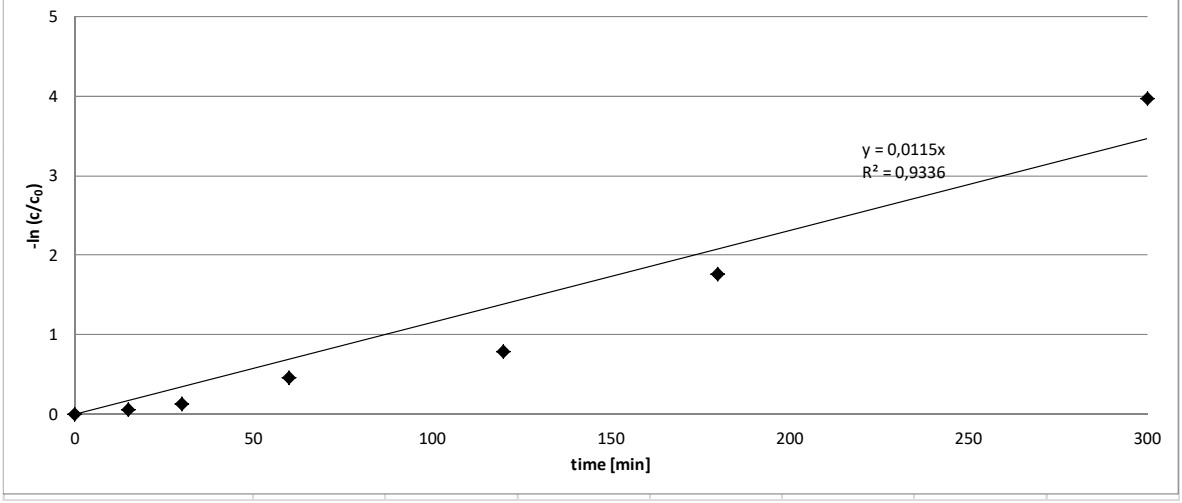


Fig. II-3-4: Determination of rate constant of ciprofloxacin degradation in ozonated process water

time	0	15	30	60	120	180	300
concentration [mg/l]	5637.522013	1955.879984	18.45324079	0.251962093	0	0	0
c/c_0 [%]	100.0%	34.7%	0.3%	0.0%	0.0%	0.0%	0.0%
$-\ln(c/c_0)$	0.00	1.06	5.72	10.02	-	-	-

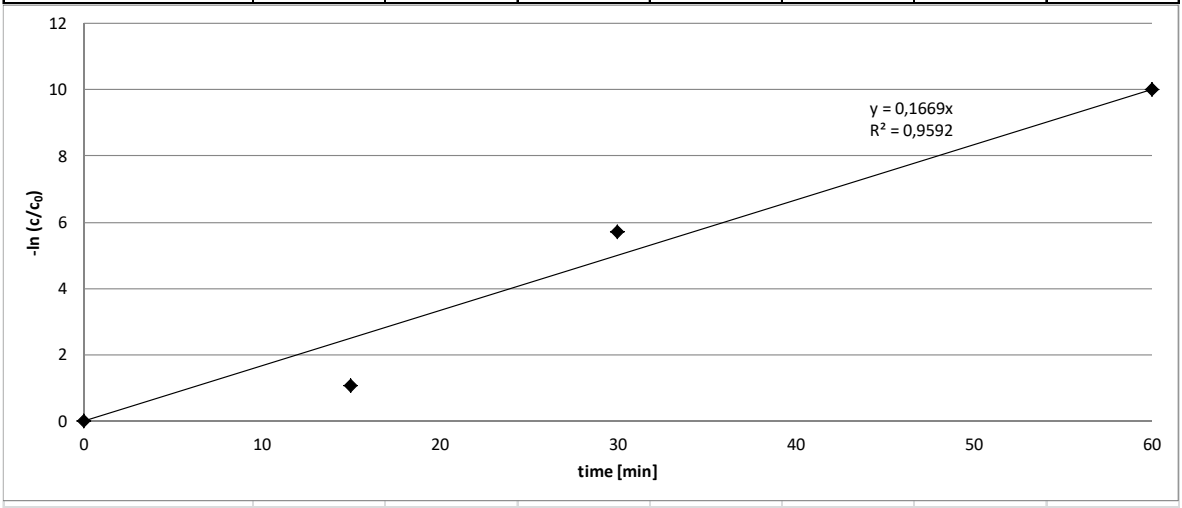
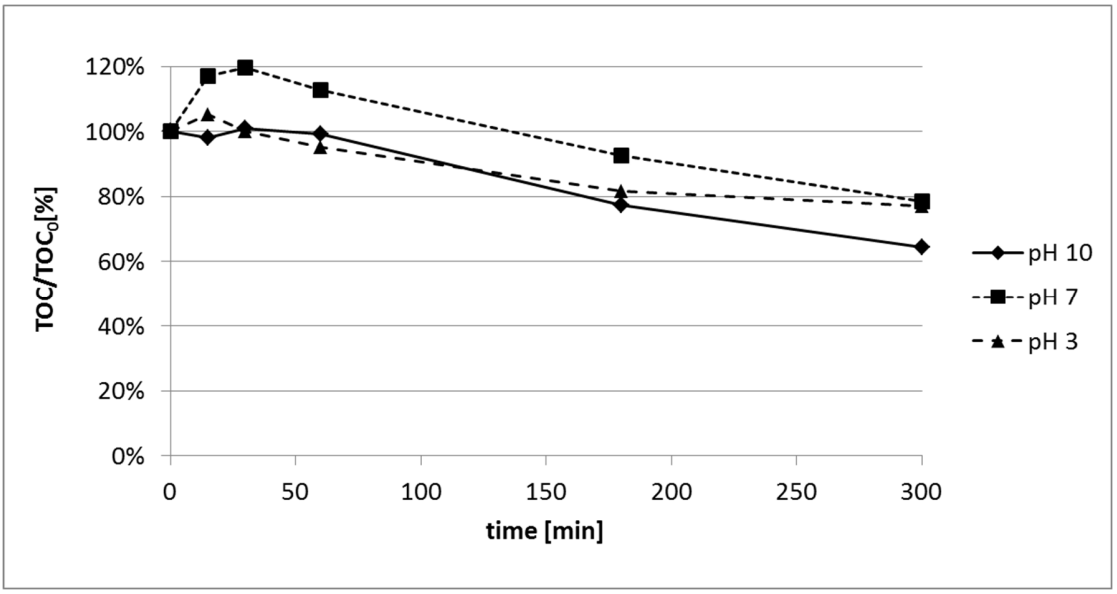


Fig. II-3-5: Determination of rate constant of moxifloxacin degradation in ozonated process water (only three measurement points due to fast degradation)

TOC



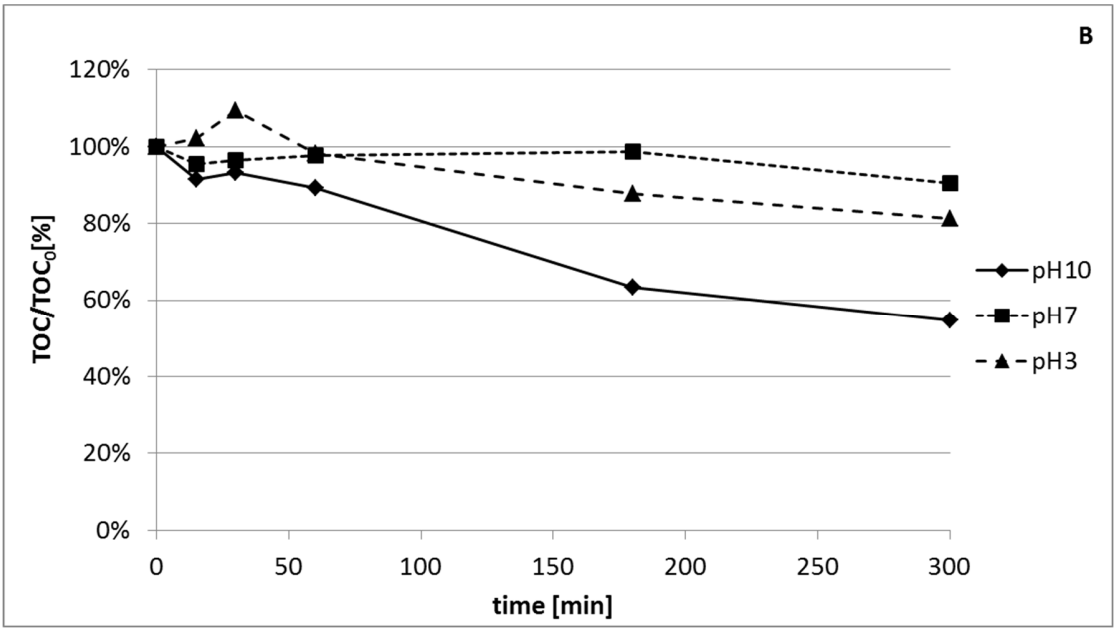


Fig. II-3-6: Degradation of TOC in ciprofloxacin (A) and moxifloxacin (B) containing waste water over time (relative to concentration at time 0 min)

TOC decreased to about 60% under basic conditions and to 80-90 % under neutral/ acidic conditions for both investigated process waste waters. The incipiently increase in TOC during the first 60 min is founded in the initial high concentration of the target compound and the associated by-products, resulting in partial precipitation. These precipitated compounds cannot be detected with the current TOC measurement.

MS^n spectra of new identified ciprofloxacin degradation products

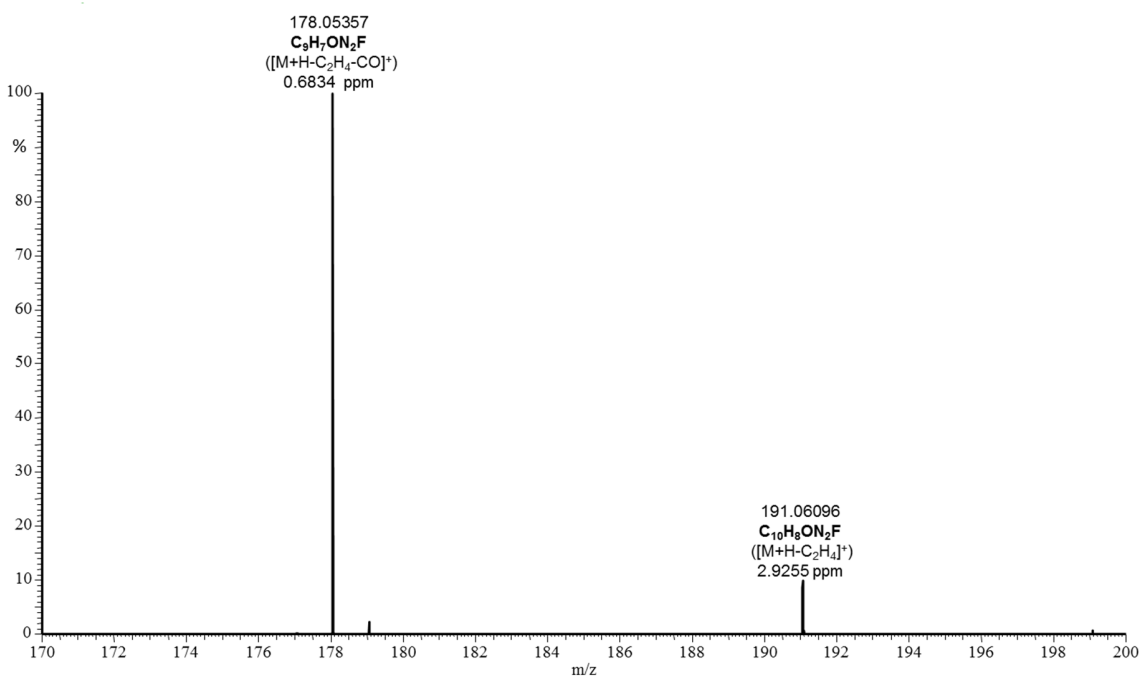


Fig. II-3-7: MS² spectrum of MW218

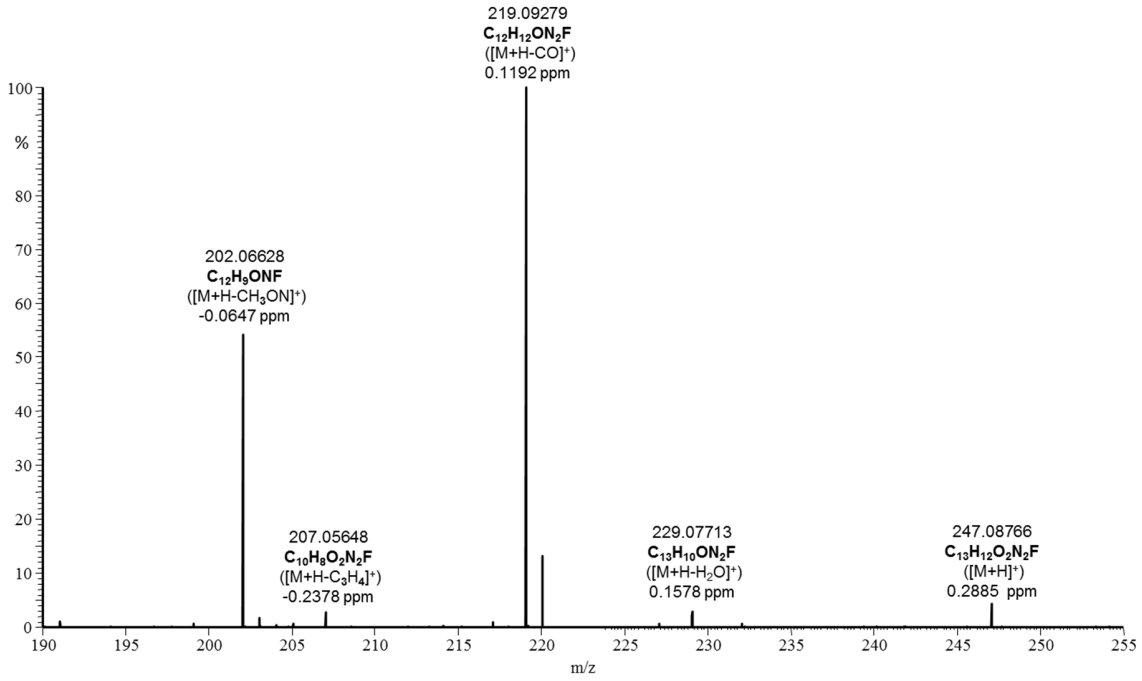


Fig. II-3-8: MS² spectrum of MW246

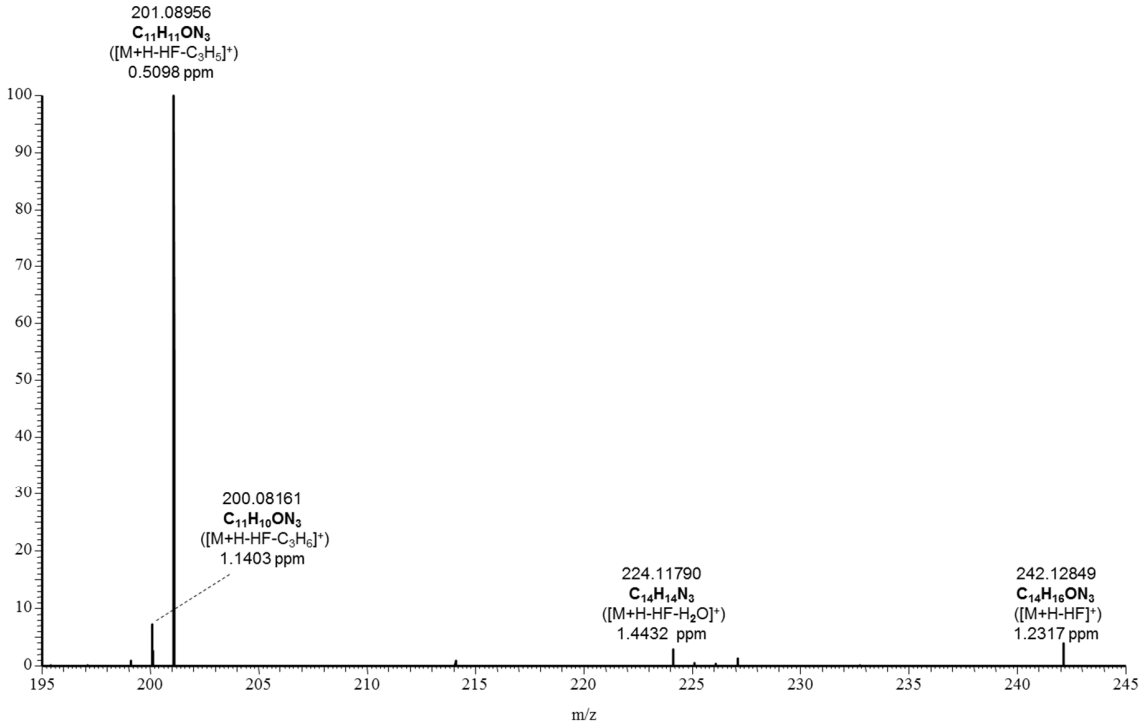


Fig. II-3-9: MS^3 spectrum of MW261

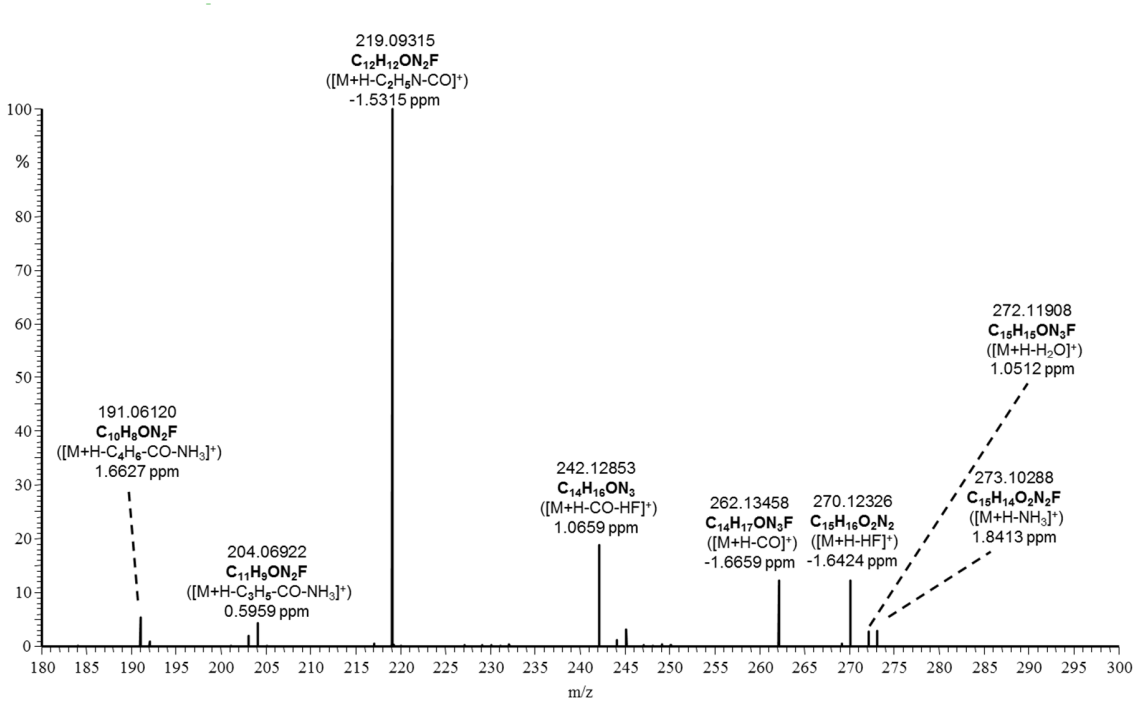


Fig. II-3-10: MS^2 spectrum of MW289

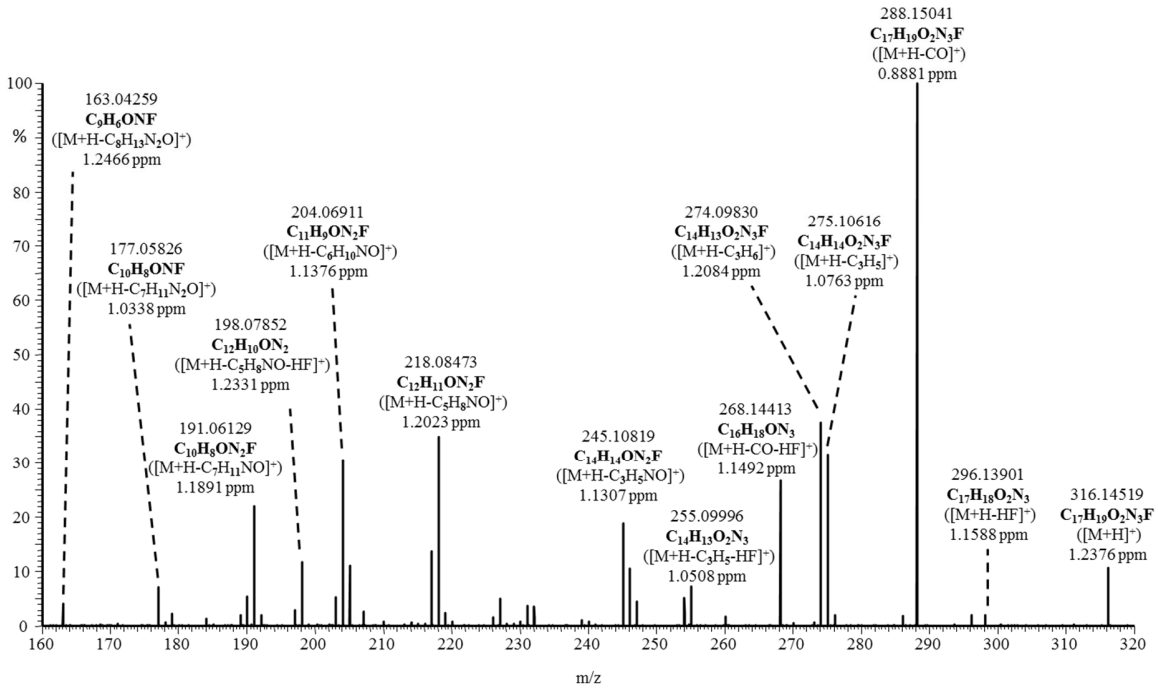


Fig. II-3-11: MS^2 spectrum of MW315

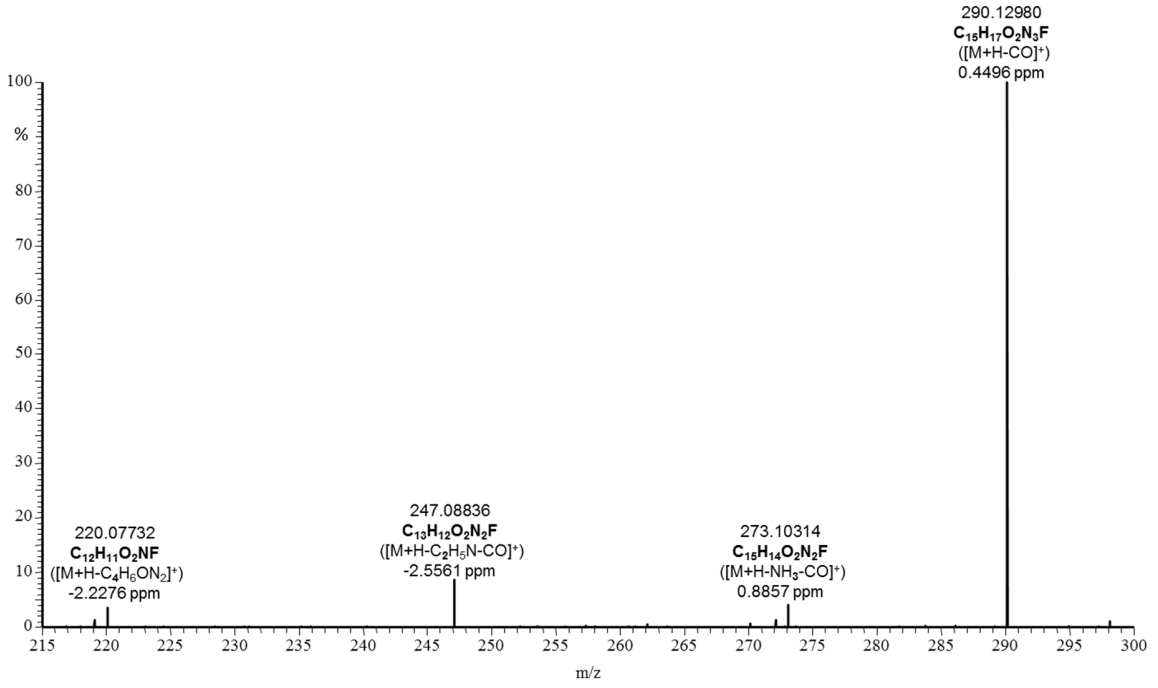


Fig. II-3-12: MS^2 spectrum of MW317

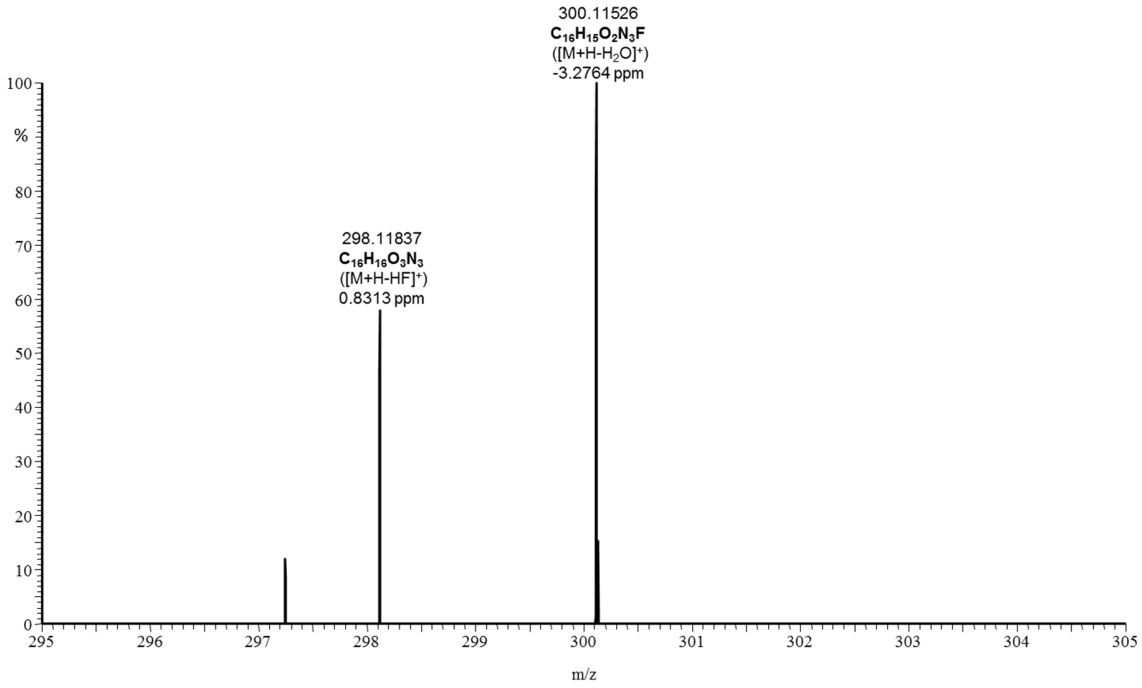


Fig. II-3-13: MS^2 spectrum of MW317

MS^n spectra of new identified moxifloxacin degradation products

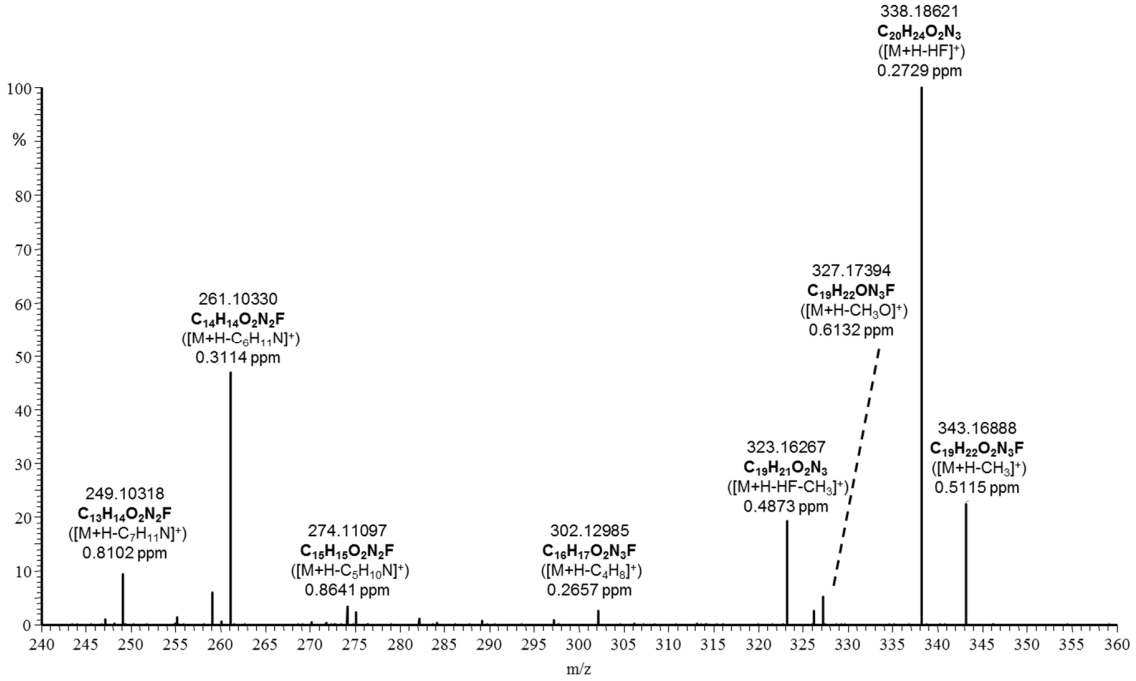


Fig. II-3-14: MS^2 spectrum of MW357

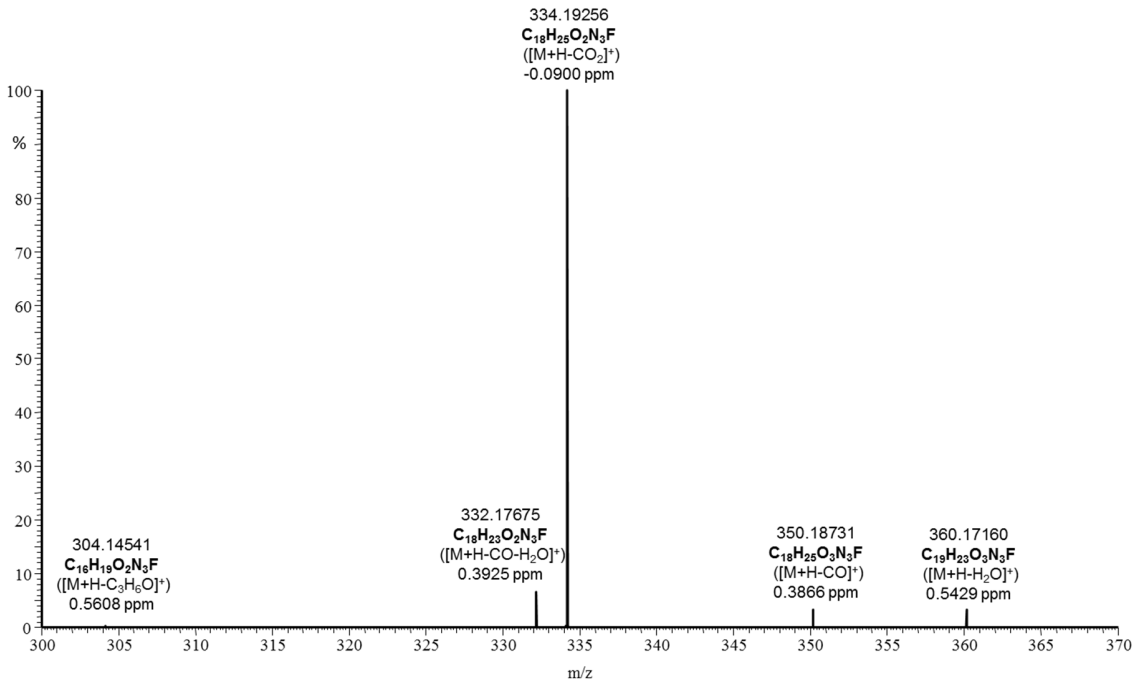


Fig. II-3-15: MS^2 spectrum of MW377

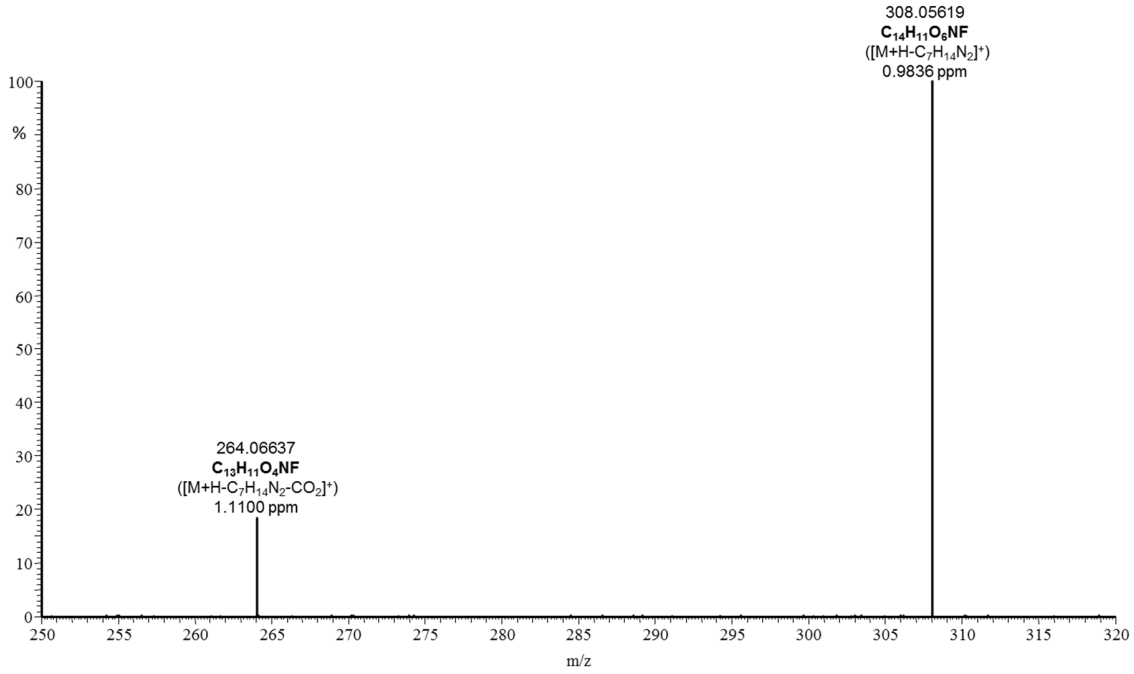


Fig. II-3-16: MS^2 spectrum of MW433

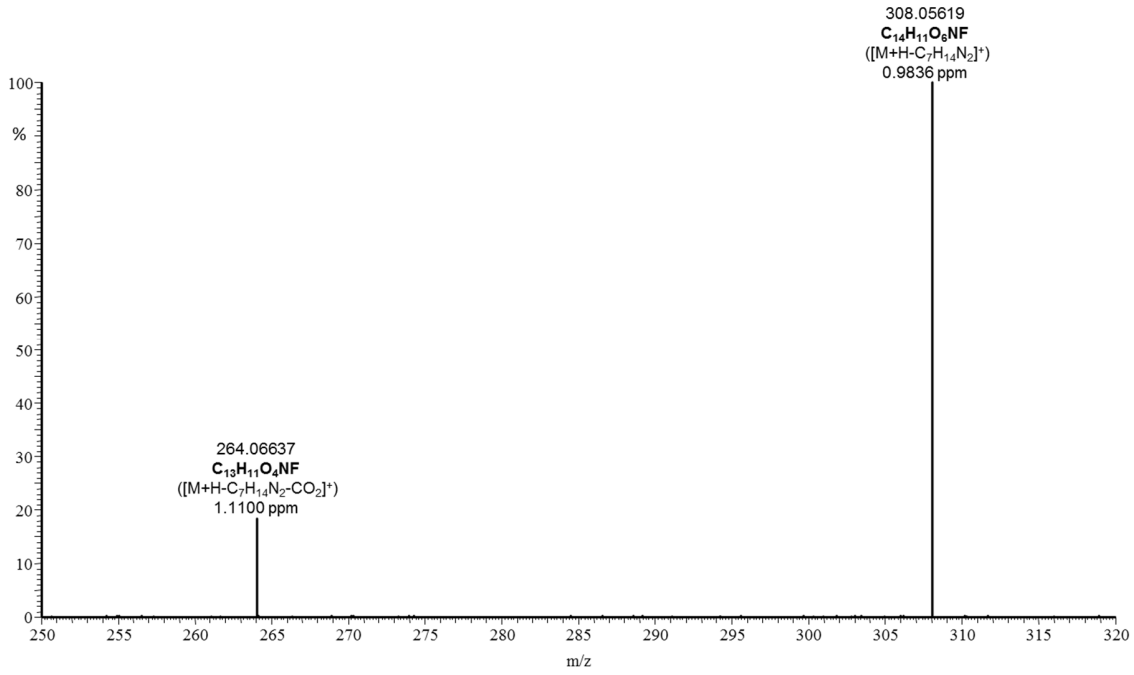


Fig. II-3-17: MS^2 spectrum of MW477

Transformation products

Transformation products of ciprofloxacin

Molecular formular	Molecular weight	t_R[a]	$[M+H]^+$	Error[b]	$[M+Na]^+$	Error[c]	Highest content A/A_{0Cipro}	Remarks
	[M]	[min]		[ppm]		[ppm]	[%]	
$C_{17}H_{18}O_3N_3F$	**331**	**5.05**	**332.14051**	**-0.0453**	**354.122500**	**-0.1812**	**100% at 0 min**	**reference standard**
$C_{12}H_{11}O\ N_2F$	*218*	*4.20-4.50*	*219.0927*	*0.5319*	*241.074500*	*1.1968*	*9.7% at 180 min*	*New*
$C_{13}H_{11}O_2\ N_2F$	*246*	*7.5-8.1*	*247.087750*	*-0.0772*	*269.069650*	*0.1057*	*3.0% at 180 min*	*New*
$C_{14}H_{16}O\ N_3F$	*261*	*0.66-1.3*	*262.13495*	*0.2489*	*284.116950*	*0.0383*	*3.3% at 60 min*	*New*
$C_{13}H_{11}O_3\ N_2F$	262	10.1-10.8	263.082650	-0.0153	285.064550	0.1564	10.8% at 300 min	previously published (An et al., 2010; Burhenne et al., 1997; Calza et al., 2008; Cardoza et al., 2005; de Witte et al., 2008; de Witte et al., 2009; Paul et al., 2010; Tuerk et al., 2012; Wang et al., 2010)
$C_{16}H_{18}O\ N_3F$	**287**	**1.0-1.9**	**288.15064**	**0.0871**	**310.132350**	**0.9055**	**48.3% at 0 min**	**reference standard**
$C_{15}H_{16}O_2\ N_3F$	289	5.5-6.9	290.12988	0.1729	312.111600	0.9512	6.8% at 180 min	prev. publ. with different structure (de Witte et al., 2008)
$C_{14}H_{11}O_4\ N_2F$	290	10.4-10.8	291.077550	0.0379	313.059450	0.1931	2.0% at 180 min	previously published (Paul et al., 2010)
$C_{14}H_{14}O_3\ N_3F$	291	2.8-3.3	292.10913	0.2233	--	--	0.1% at 30 min	previously published (Tuerk et al., 2012)
$C_{15}H_{10}O_3\ N_3F$	*299*	*12.5-12.9*	*300.078*	*-0.3511*	*322.059650*	*0.6353*	*0.5 % at 300 min*	*No structure identified*
$C_{16}H_{18}O_2\ N_3F$	*303*	*4.2-4.9*	*304.145550*	*0.0990*	*326.127450*	*0.2474*	*2.7% at 120 min*	*No structure identified*
$C_{15}H_{16}O_3N_3F$	**305**	**2.6-3.9**	**306.12479**	**0.1803**	**328.106650**	**0.4588**	**12.9% at 120 min**	**reference standard**
$C_{17}H_{19}O_3\ N_3$	**313**	**3.1-3.9**	**314.14987**	**0.1501**	**336.131650**	**0.6770**	**3.3% at 0 min**	**reference standard**
$C_{17}H_{18}O_2\ N_3F$	315	9.5-10.2	316.14553	0.1587	338.127150	1.1900	17.8% at 180 min	prev. publ. with different structure (de Witte et al., 2009)
$C_{16}H_{16}O_3\ N_3F$	*317*	*4.0-5.1*	*318.124850*	*-0.0158*	*340.106750*	*0.1261*	*1.9% at 180 min*	*New*
$C_{15}H_{14}O_4\ N_3F$	*319*	*11.0-12.1*	*320.104150*	*-0.1254*	*342.086050*	*0.0157*	*1.6% at 180 min*	*No structure identified*
$C_{16}H_{16}O_4\ N_3F$	333	10.7-10.8	334.11969	0.2101	356.101550	0.4653	11.1% at 30 min	previously published (Calza et al., 2008; de Witte et al., 2008; Paul et al., 2010; Wang et al., 2010)
$C_{17}H_{16}O_4\ N_3F$	345	9.7-10.2	346.119750	0.0290	368.101750	-0.1304	1.9% at 120 min	previously published (Burhenne et al., 1997)
$C_{18}H_{21}O_3\ N_3F$	345	6.7-7.6	346.156150	-0.0145	368.138150	-0.1738	0.6% at 0 min	No structure identified
$C_{17}H_{18}O_4\ N_3F$	347	9.3-9.6	348.135450	-0.1152	370.117350	0.0144	3.4% at 120 min	previously published (Calza et al., 2008; de Witte et al., 2008; Tuerk et al., 2012)
$C_{16}H_{16}O_5\ N_3F$	349	10.4-10.8	350.11454	0.3867	372.096450	0.4870	2.4% at 120 min	previously published (de Witte et al., 2008)
$C_{18}H_{18}O_4\ N_3F$	359	12.6-13.1	360.135550	-0.3898	382.117350	0.0139	11.7% at 120 min	previously published (Cardoza et al., 2005; Tuerk et al., 2012)
$C_{17}H_{16}O_5\ N_3F$	361	8.0-8.7	362.11475	-0.2077	384.096450	0.4708	2.9% at 180 min	previously published (Paul et al., 2010; Wang et al., 2010)

Tab. II-3-1: Transformation products of ciprofloxacin identified by LC/HRMS. retention time based on MS detection. Difference between measured and theoretical mass for the ionization with $(H^+)^b$ and $(Na^+)^c$. Highest content of transformation product based on the initial ciprofloxacin concentration and dedicated sampling time. Transformation products for which reference substances were available are presented in bold. new identified substances in italic. previously identified substances in normal font

Transformation products of moxifloxacin

Molecular formular	Molecular weight	t_R[a]	$[M+H]^+$	Error[b]	$[M+Na]^+$	Error[c]	Highest content A/A_{0Cipro}	Remarks
	[M]	[min]		[ppm]		[ppm]	[%]	
$C_{21}H_{24}O_4N_3F$	**401**	**10.9-11.8**	**402.18225**	**0.2742**	**424.163950**	**0.8849**	**100% at 0 min**	**reference standard**
$C_{14}H_{13}O_4N_2F$	*292*	*12.6-13.1*	*293.09325*	*-0.1335*	*315.075150*	*0.0205*	*3.2% at 15 min*	*No structure identified*
$C_{15}H_{13}O_4N_2F$	*304*	*10.9-11.5*	*305.093250*	*-0.1283*	*--*	*--*	*<0.1% at 15 min*	*No structure identified*
$C_{14}H_{10}O_6NF$	*307*	*11.2-12.9*	*308.056450*	*0.1368*	*--*	*--*	*5.2% at 30 min*	*No structure identified*
$C_{14}H_{12}O_7NF$	*325*	*11.8-12.3*	*326.067*	*0.1754*	*348.049350*	*-1.0706*	*0.2% at 30 min*	*new*
$C_{18}H_{18}O_3N_3F$	*343*	*11.0-12.0*	*344.140500*	*-0.0146*	*--*	*--*	*0.4% at 0 min*	*No structure identified*
$C_{20}H_{24}O_2N_3F$	*357*	*11.1-11.2*	*358.192150*	*1.0639*	*--*	*--*	*<0.1% at 0 min*	*new*
$C_{18}H_{20}O_4N_3F$	*361*	*11.0-12.0*	*362.151050*	*0.0277*	*--*	*--*	*<0.1% at 0 min*	*No structure identified*
$C_{19}H_{24}O_4N_3F$	*377*	*10.7-11.1*	*378.182350*	*0.0265*	*--*	*--*	*0.5% at 15 min*	*new*
$C_{20}H_{18}O_6N_3F$	*415*	*11.4-13.0*	*416.125250*	*-0.0241*	*--*	*--*	*0.6% at 15 min*	*No structure identified*
$C_{21}H_{24}O_5N_3F$	*417*	*10.4-11.8*	*418.177250*	*0.0599*	*--*	*--*	*0.6% at 15 min*	*No structure identified*
$C_{20}H_{24}O_7N_2F$	*423*	*11.0-15.0*	*424.164050*	*-0.0449*	*--*	*--*	*<0.1% at 0 min*	*No structure identified*
$C_{21}H_{24}O_6N_3F$	*433*	*10.7-11.2*	*434.172250*	*-0.1385*	*--*	*--*	*13.4% at 15 min*	*new*
$C_{21}H_{22}O_7N_3F$	*447*	*11.5-12.0*	*448.151450*	*0.0112*	*--*	*--*	*0.7% at 15 min*	*new*

Tab. II-3-2: Transformation products of moxifloxacin identified by LC/HRMS. retention time based on MS detection. Difference between measured and theoretical mass for the ionization with (H^+)[b] and (Na^+)[c]. Highest content of transformation product based on the initial moxifloxacin concentration and dedicated sampling time. Transformation products for which reference substances were available are presented in bold, new identified substances in italic, previously identified substances in normal font

Chapter 4

Calculation of ozone consumption

Ozone consumption values were calculated according to the following equation:

$$A(O_3) = \sum_{i=1}^{n} \left[\frac{Q_G}{V_L(t_i)} (c_{Ozon,0}(t_i) - \overline{c_{Ozon,e}(t_i + \Delta t_i)}) \cdot \Delta t_i \right]$$

where:

$A(O_3)$, absorbed ozone dose (mg/L)
Q_G, gaseous mass flow (L/s)
V_L, reaction volume (L)
t, time (s)
$c_{Ozon,0}$, inlet ozone concentration in the gaseous phase (mg/L)
$c_{Ozon,e}$, outlet ozone concentration in the gaseous phase (mg/L)

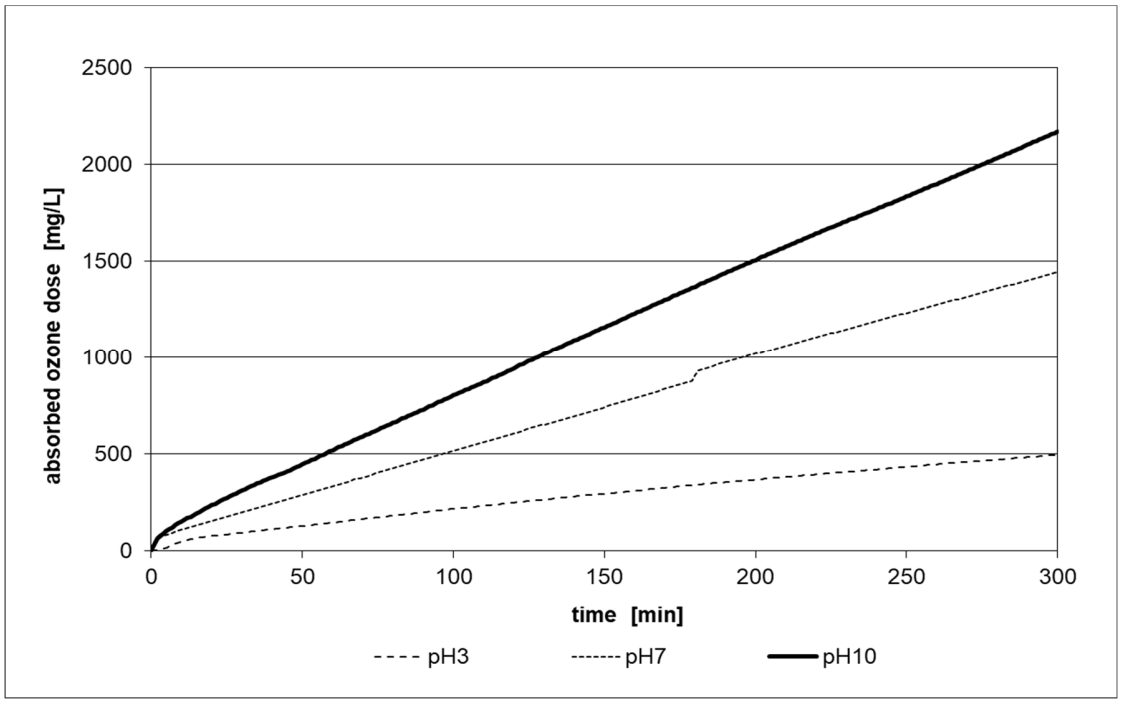


Fig. II-4-1: Absorbed ozone dose in course of ozonation of rivaroxaban-containing waste water at pH 3, pH 7 and pH 10 (measured every minute)

Calibration curves of rivaroxaban and acetoxamide

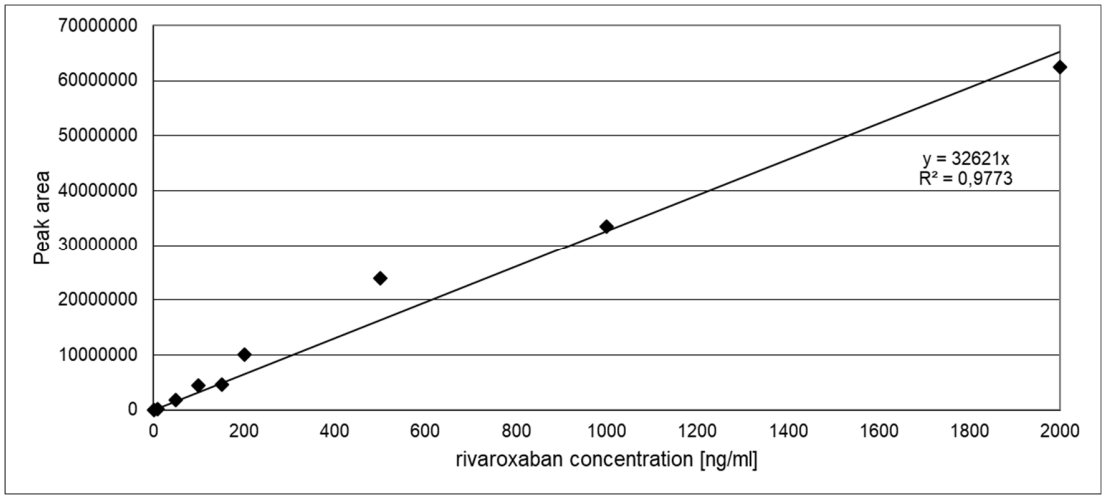


Fig. II-4-2: Calibration curve of rivaroxaban

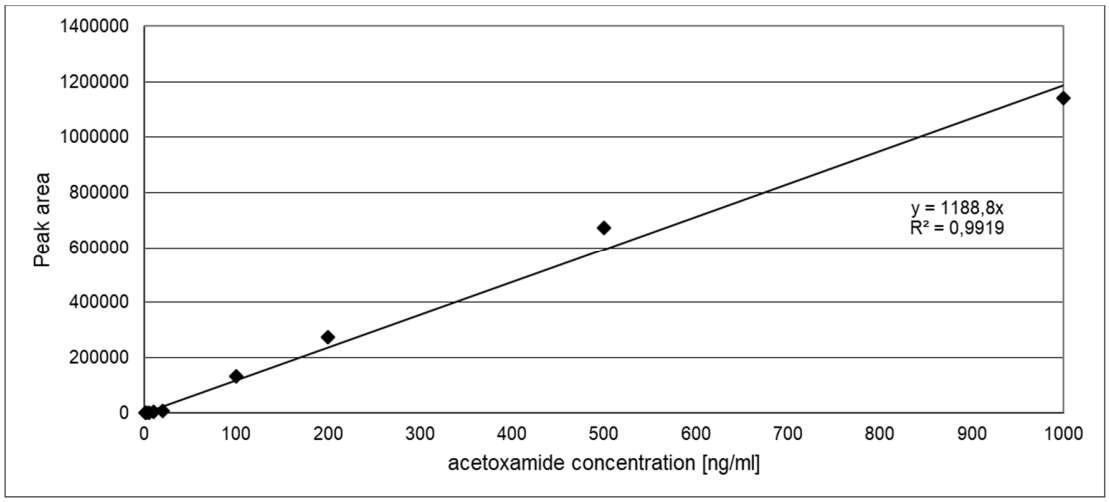


Fig. II-4-3: Calibration curve of acetoxamide

Calculation of ozonation rate constants

The reaction rate constants were calculated according to the following equations:

$$c(M_t) = c(M_{t=0}) \exp^{-k_{ges} \cdot c(O_3) \cdot t}$$

Simplification:

$$c(M_t) = c(M_{t=0}) \exp^{-k_{app} \cdot t}$$

$$\ln \frac{c(M_t)}{c(M_{t=0})} = k_{app} \cdot t$$

where:

c(M), concentration of Product M

$c(O_3)$, concentration of ozone

k, reaction rate constant

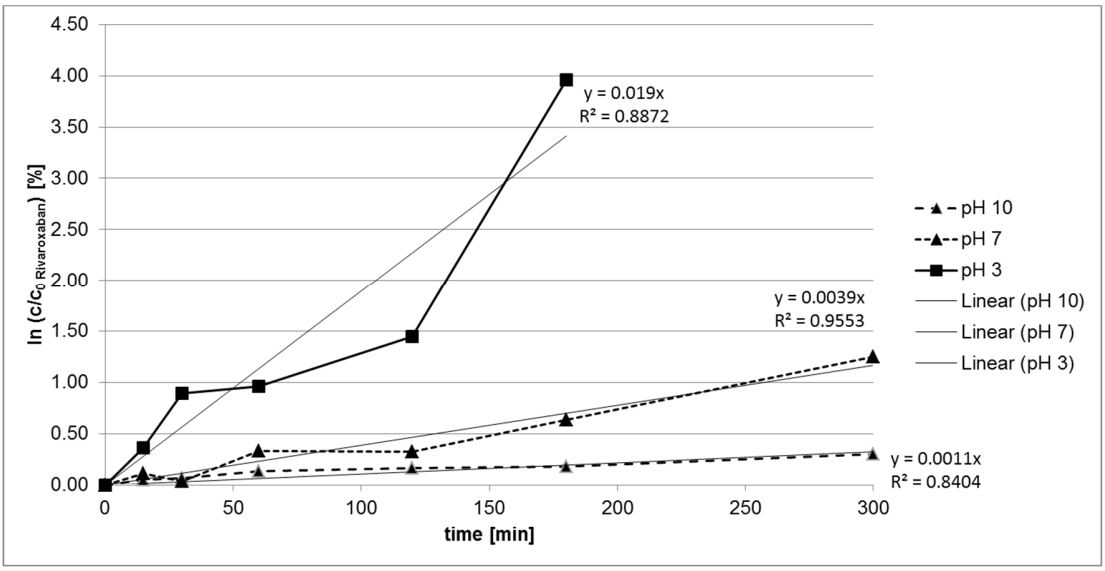


Fig. II-4-4: Determination of rate constants of rivaroxaban degradation in ozonated process water under various reaction conditions

TOC content profiles

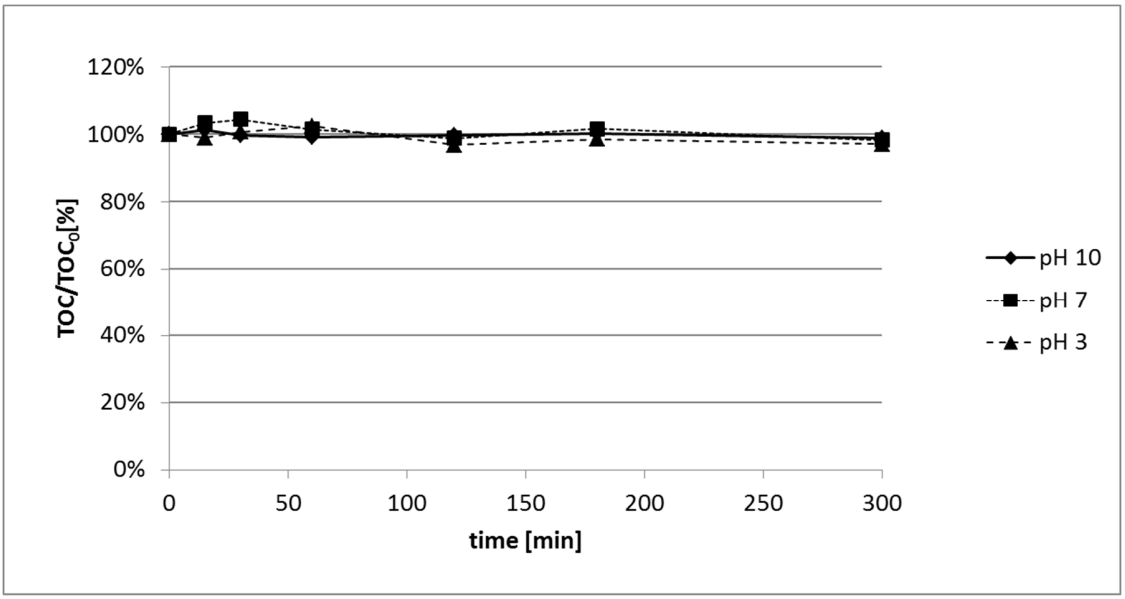


Fig. II-4-5: Relative TOC content in rivaroxaban production process waste water over time (relative to rivaroxaban concentration at time 0 min, 3000-9000 mg/L)

MS spectra of rivaroxaban and its most prominent transformation products (at their highest concentration)

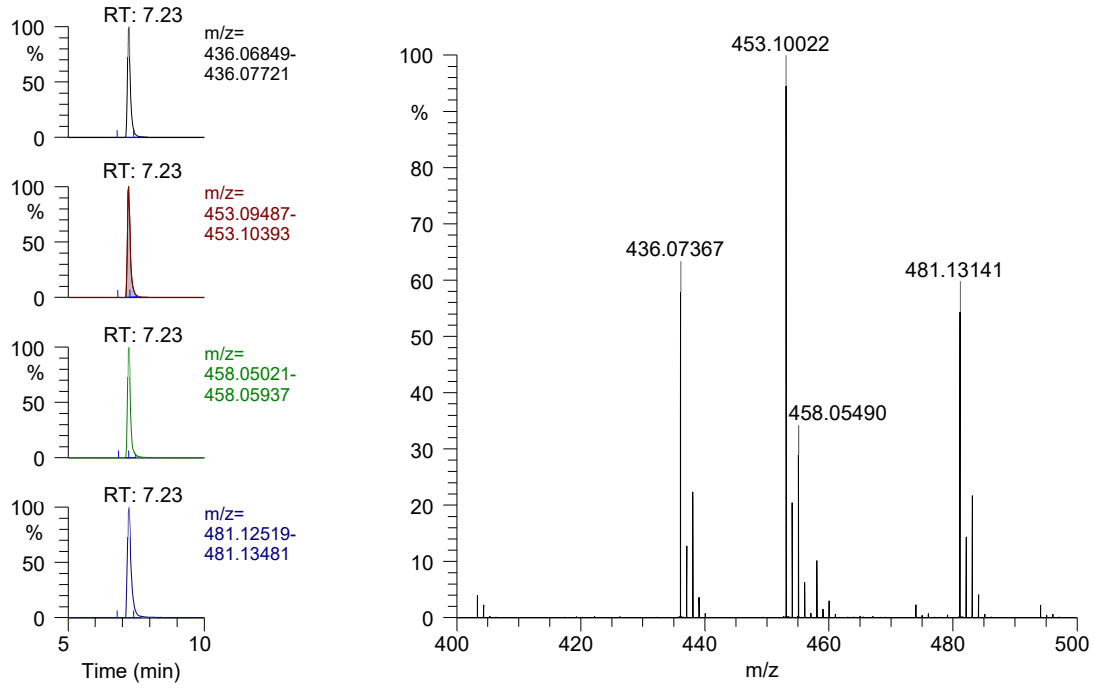


Fig. II-4-6: MS spectrum of rivaroxaban (MW435, pH 3, 0 min)

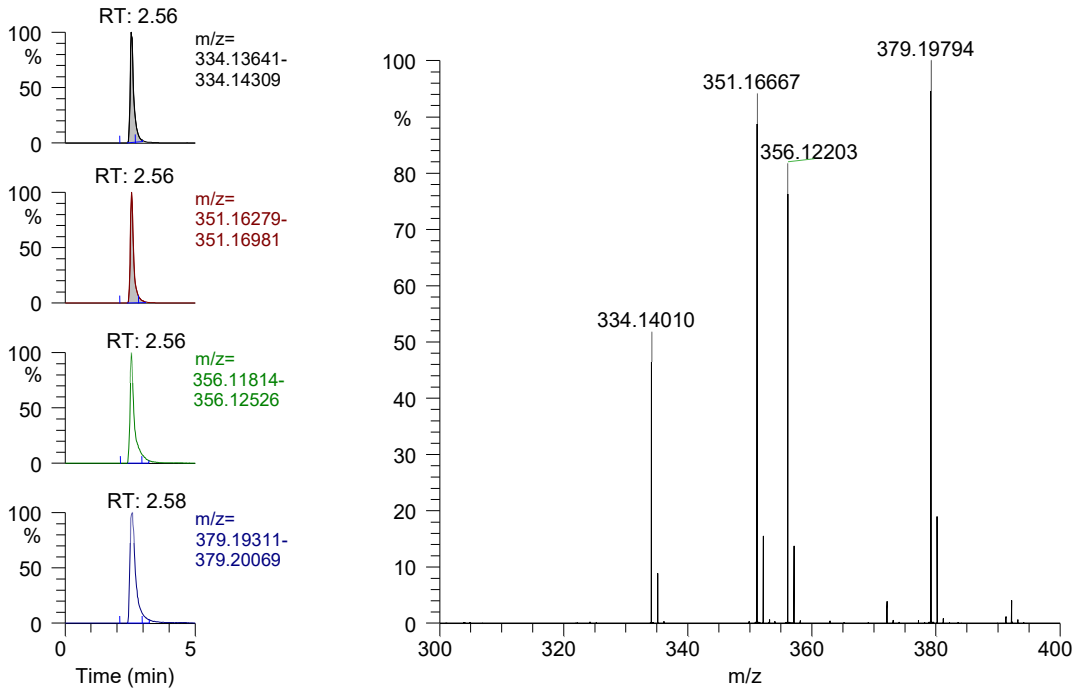


Fig. II-4-7: MS spectrum of acetoxamide (MW333, pH 7, 180 min)

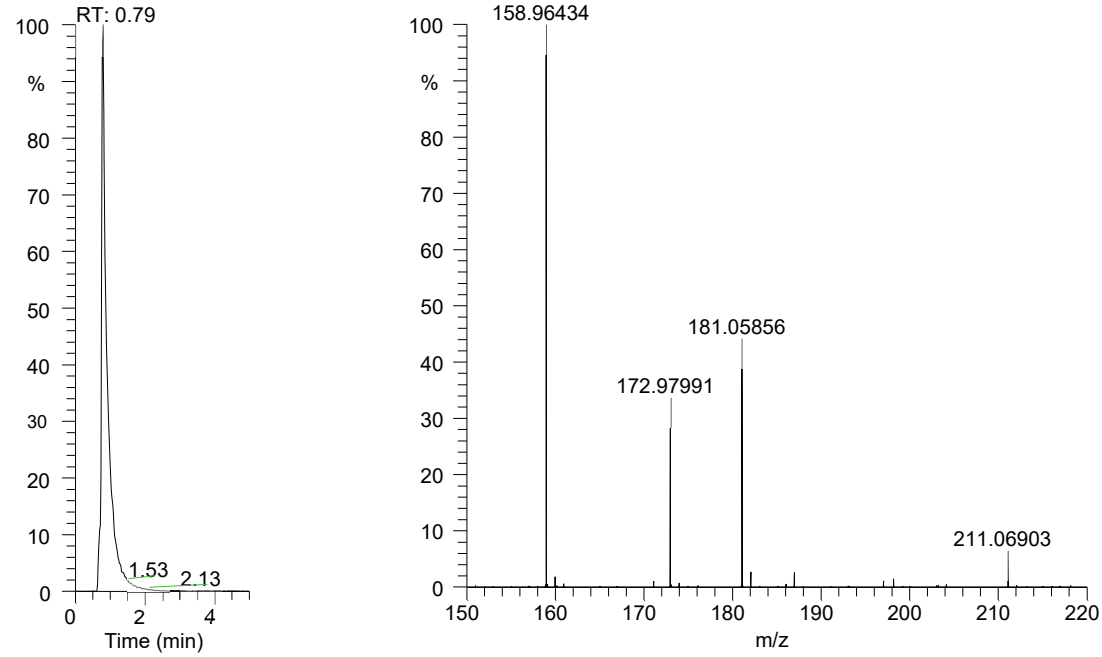


Fig. II-4-8: MS spectrum of oxazolinone acetamide (MW158, pH 7, 300 min)

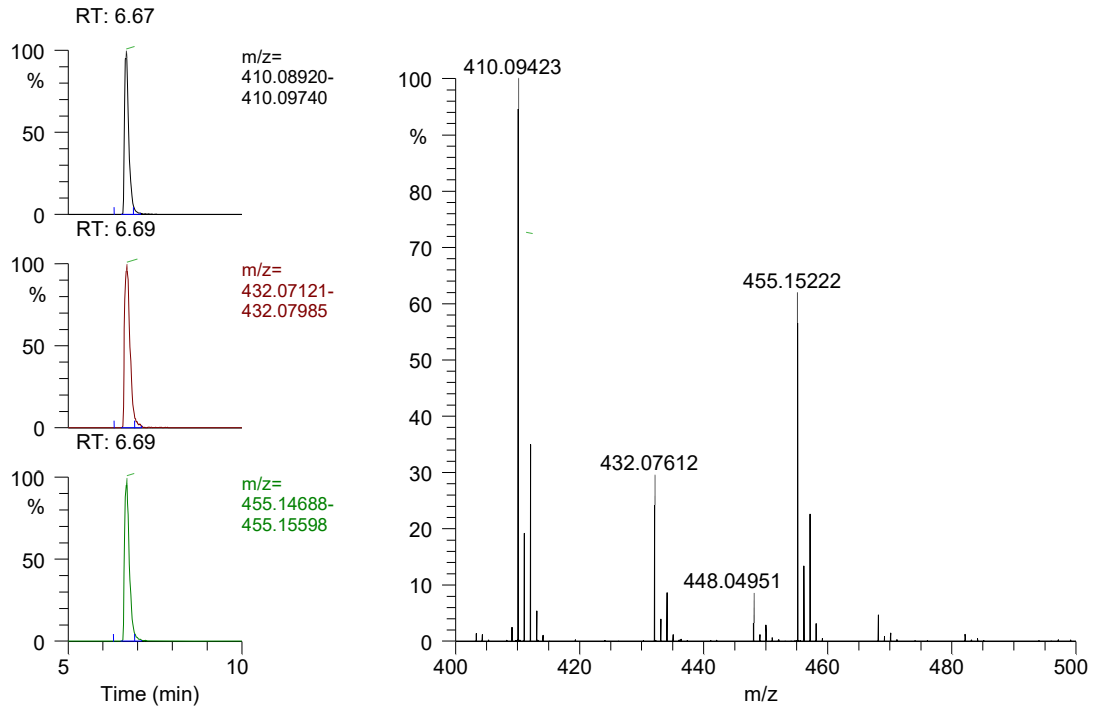


Fig. II-4-9: MS spectrum of MW409 (pH 10, 0 min)

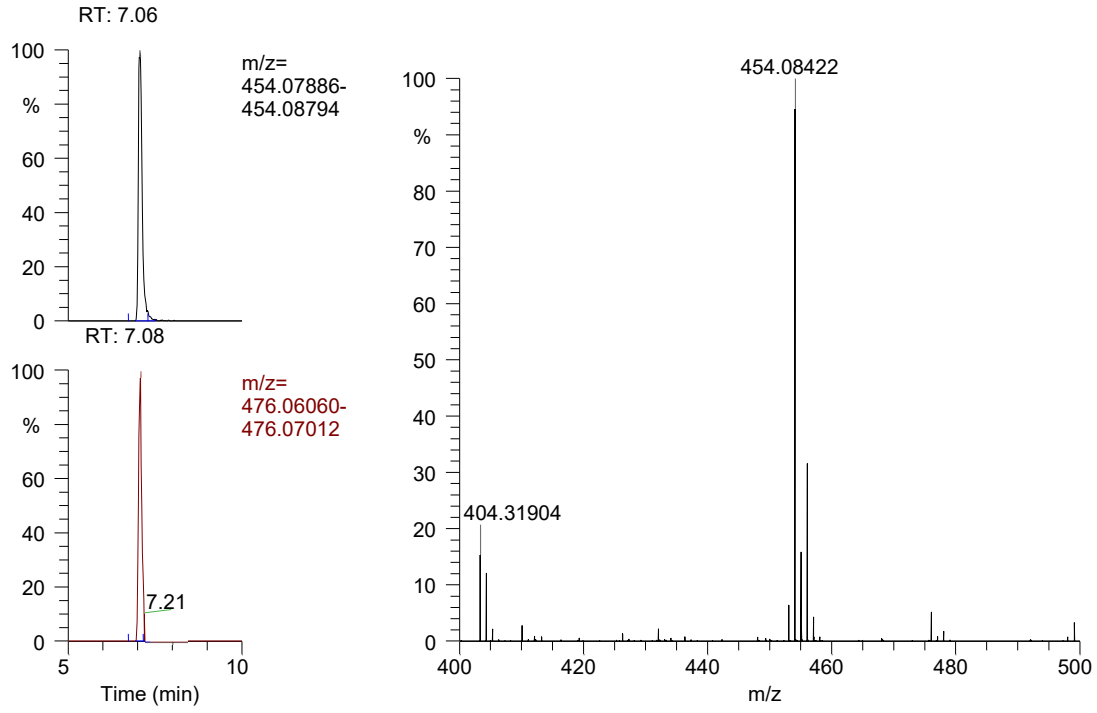


Fig. II-4-10: MS spectrum of MW453 (pH 10, 0 min)

MS^n spectra of rivaroxaban and acetoxamide

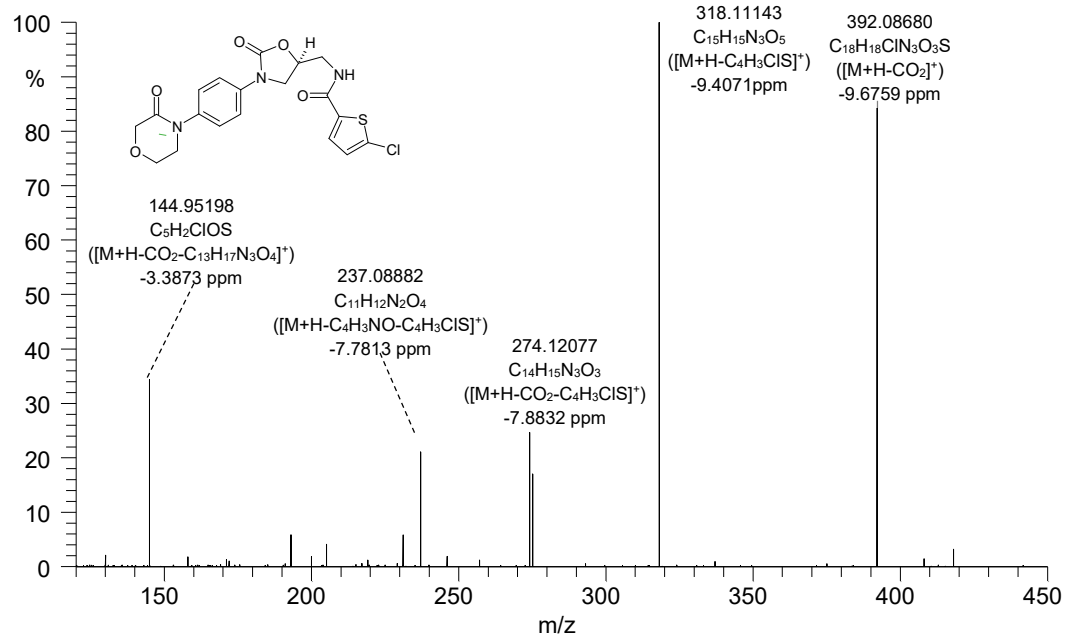


Fig. II-4-11: MS² spectrum of rivaroxaban (MW435)

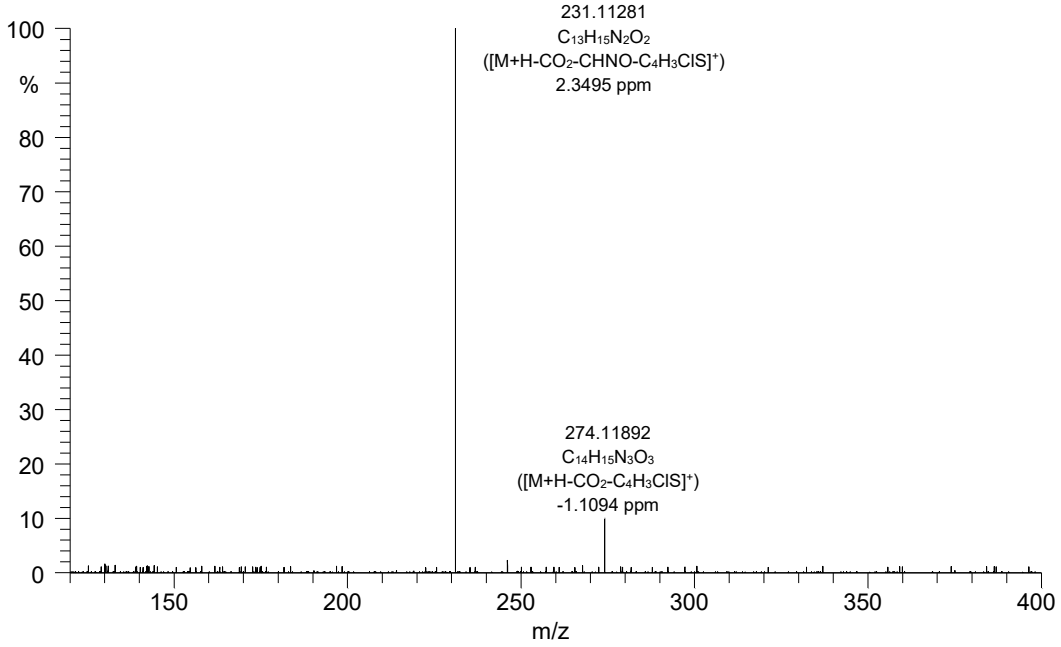


Fig. II-4-12: MS^3 spectrum of rivaroxaban (MW435, 436→392)

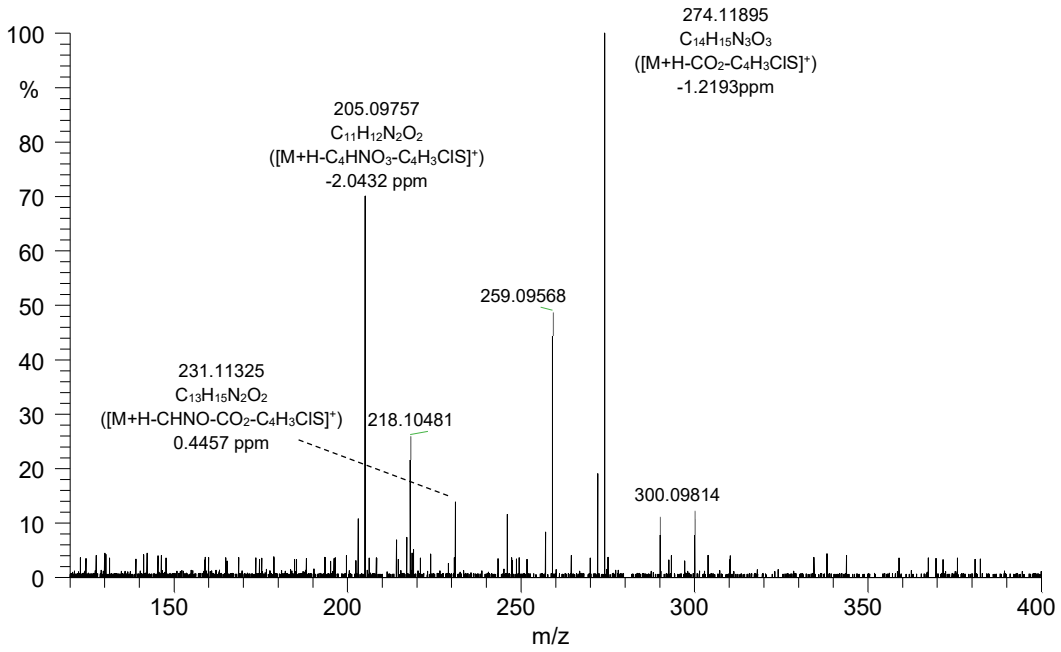


Fig. II-4-13: MS^3 spectrum of rivaroxaban (MW435, 436→318)

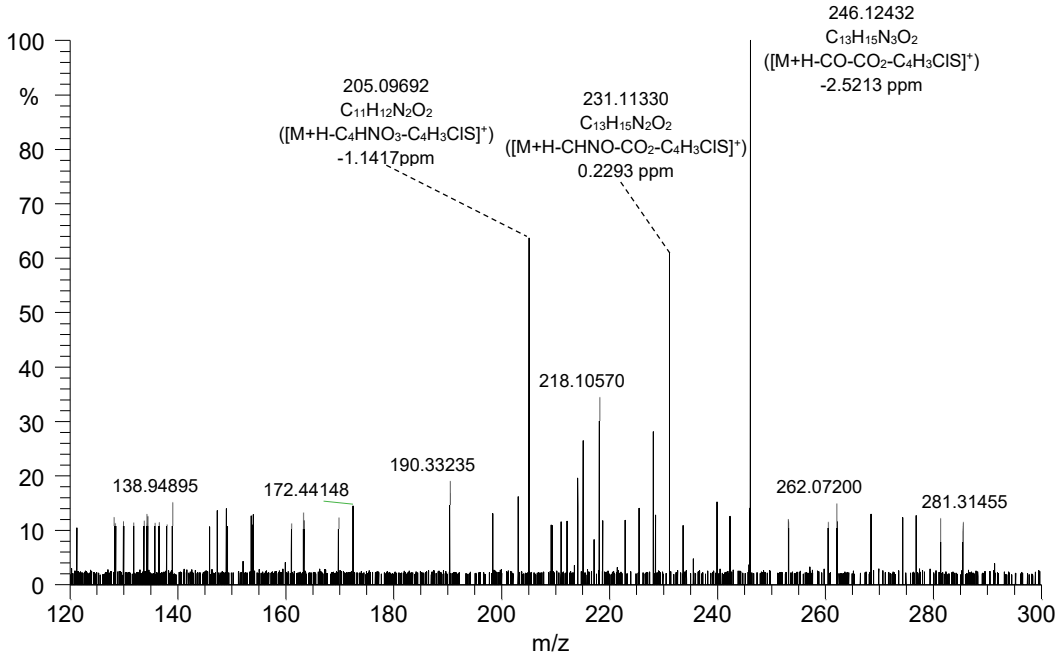


Fig. II-4-14: MS³ spectrum of rivaroxaban (MW435, 436→275)

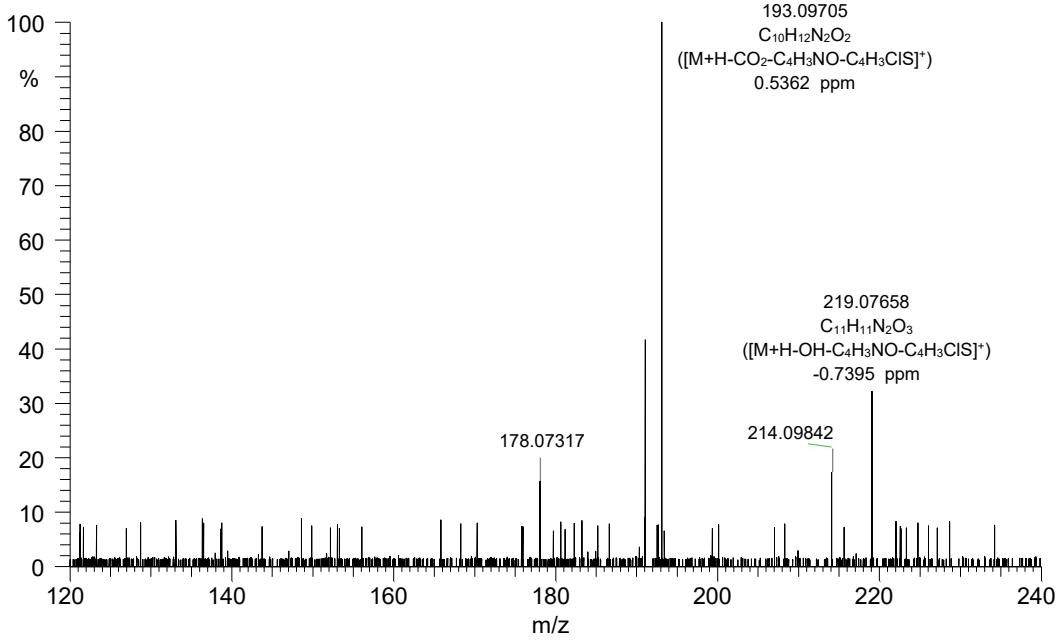


Fig. II-4-15: MS³ spectrum of rivaroxaban (MW435, 436→237)

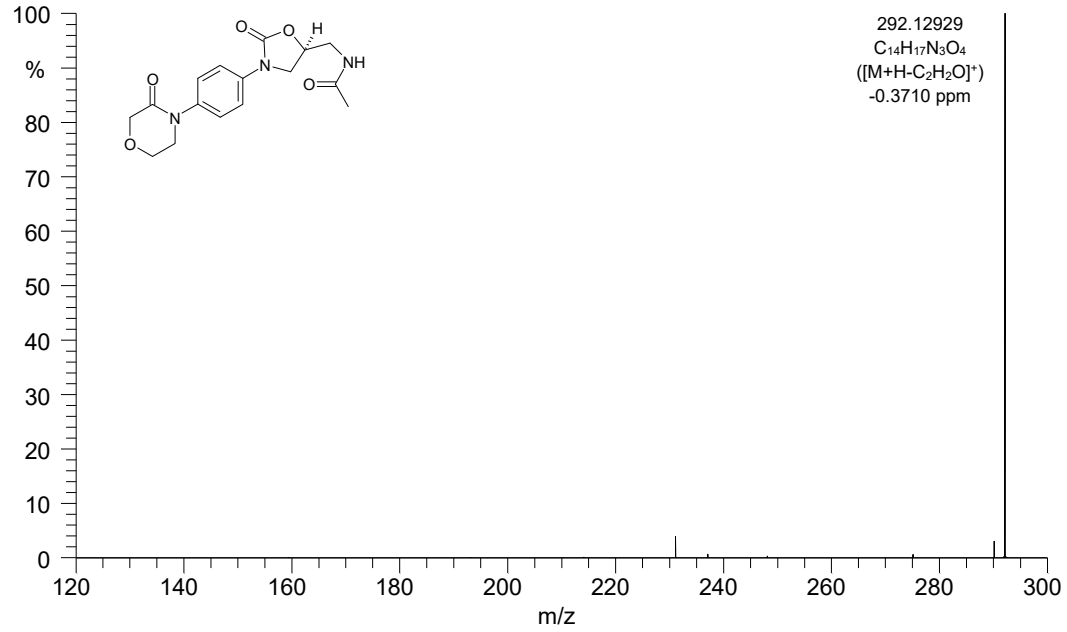


Fig. II-4-16: MS^2 spectrum of acetoxamide (MW333)

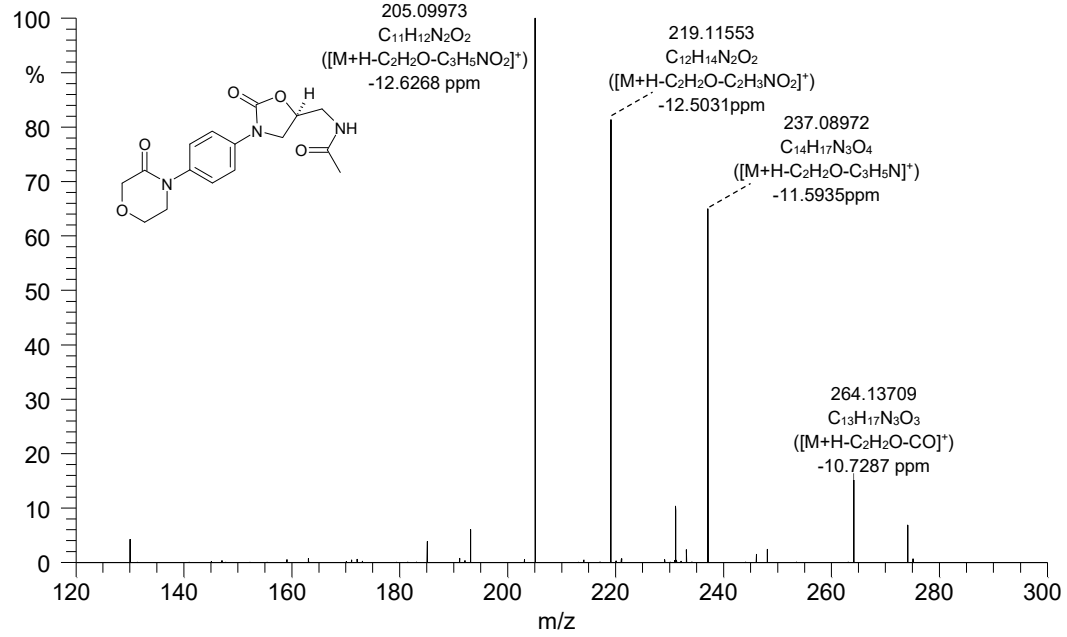


Fig. II-4-17: MS^3 spectrum of acetoxamide (MW333, 334→292)

List of all rivaroxaban transformation products

Molecular formula	MW	RT	Max concentration	$[M+H]^+$ Error [a]	$[M+NH4]^+$ Error [b]	$[M+Na]^+$ Error [c]	$[M+Me_2NH_2]^+$ Error [d]	Remarks
	[g/mol]	[min]	[%]					
$C_6H_{10}N_2O_3$	158	0.76 -0.81	12.1 (pH 7, 300 min)	159.076350 0.4302 ppm	176.10295 0.1075 ppm	181.058680 -2.0055 ppm	204.134250 0.1075 ppm	Oxazolinone acetamide
C_5H_4ClNOS	161	-	-	Not identified	Not identified	Not identified	Not identified	Previously published by Lang et al. (2009)
$C_5H_3ClNO_2S$	162	-	-	Not identified	Not identified	Not identified	Not identified	Previously published by Lang et al. (2009) and Weinz et al. (2009)
$C_7H_6ClNO_3S$	219	-	-	Not identified	Not identified	Not identified	Not identified	Previously published by Lang et al. (2009) and Weinz et al. (2009)
$C_{14}H_{14}N_2O_5$	290	-	-	Not identified	Not identified	Not identified	Not identified	Previously published by Lang et al. (2009) and Weinz et al. (2009)
$C_{14}H_{17}N_3O_4$	291	4.77 -4.79	-	292.129170; 0.0412 ppm	Not identified	Not identified	Not identified	Previously published by Lang et al. (2009) and Weinz et al. (2009)
$C_{14}H_{16}N_2O_5$	292	-	-	Not identified	Not identified	Not identified	Not identified	Previously published by Lang et al. (2009) and Weinz et al. (2009)
$C_{14}H_{14}N_2O_6$	306	7.42	-	307.092280 0.5979 ppm	324.11887 0.4639 ppm	329.074210 0.6469 ppm	352.150230 0.2679 ppm	Previously published by Lang et al. (2009) and Weinz et al. (2009)
$C_{16}H_{19}N_3O_5$	**333**	**2.55-2.56**	**33.4 (pH 7, 300 min)**	**334.139950 -0.6094 ppm**	**351.16656 -0.7925 ppm**	**356.121900 -0.6244 ppm**	**379.197850 -0.7625 ppm**	**Acetoxamide**
$C_{17}H_{18}ClN_3O_4S$	395	-	-	Not identified	Not identified	Not identified	Not identified	Previously published by Lang et al. (2009) and Weinz et al. (2009)
$C_{19}H_{19}N_3O_5S$	401	6.53	-	402.111450 0.9175 ppm	419.13807 0.7405 ppm	424.093580 0.4562 ppm	447.169580 0.2169 ppm	Previously published by Wingert et al. (2016)
$C_{18}H_{18}ClN_3O_4S$	407	-	-	Not identified	Not identified	Not identified	Not identified	Previously published by Lang et al. (2009)
$C_{18}H_{20}ClN_3O_4S$	409	6.67 -6.69	21.3 (pH 10, 0 min)	410.094140 -1.3665 ppm	-	432.075930 -0.9876 ppm	455.152110 -1.6622 ppm	Previously published by Ramisetti et al. (2014) and Wingert et al. (2016)
$C_{19}H_{18}ClN_3O_5S$	**435**	**7.20 -7.26**	100 (0 min)	**436.073570 -1.6641 ppm**	**453.10015 -1.7354 ppm**	**458.054860 -0.1586 ppm**	**481.131410 -1.6434 ppm**	**Rivaroxaban**
$C_{19}H_{18}ClN_3O_6S$	451	-	-	Not identified	Not identified	Not identified	Not identified	Previously published by Lang et al. (2009) and Weinz et al. (2009)
$C_{19}H_{20}ClN_3O_6S$	453	7.06 -7.08	2.9 (pH 10, 0 min)	454.084060 -1.4324 ppm	-	476.065270 0.1898 ppm	499.141430 -0.3752 ppm	Previously published by Lang et al. (2009); Ramisetti et al. (2014); Weinz et al. (2009); Wingert et al. (2016)
$C_{19}H_{18}ClN_3O_7S$	467	-	-	Not identified	Not identified	Not identified	Not identified	Previously published by Lang et al. (2009) and Weinz et al. (2009)

Tab. II-4-1: Transformation products of rivaroxaban identified by LC-HRMS. Differences between measured and theoretical mass for the ionization with $(H^+)^a$, $(NH_4^+)^b$, $(Na^+)^c$ and $(Me_2NH_2^+)^d$. Compounds for which reference standards were available are presented in bold.

Chapter 5

Calculation of ozone consumption

Ozone consumption values were calculated according to the following equation:

$$A(O_3) = \sum_{i=1}^{n} \left[\frac{Q_G}{V_L(t_i)} (c_{Ozon,0}(t_i) - \overline{c_{Ozon,e}(t_i + \Delta t_i)}) \cdot \Delta t_i \right] \qquad \text{(S2.1)},$$

where:

$A(O_3)$, absorbed ozone dose (mg/L)
Q_G, gaseous mass flow (L/s)
V_L, reaction volume (L)
t, time (s)
$c_{Ozon,0}$, inlet ozone concentration in the gaseous phase (mg/L)
$c_{Ozon,e}$, outlet ozone concentration in the gaseous phase (mg/L)

Calculation of ozonation rate constants

The reaction rate constants were calculated according to the following equations:

$$c(M_t) = c(M_{t=0}) \exp^{-k_{ges} \cdot c(O_3) \cdot t} \qquad \text{(S3.1)}$$

Simplification:

$$c(M_t) = c(M_{t=0}) \exp^{-k_{app} \cdot t} \qquad \text{(S3.2)}$$

$$\ln \frac{c(M_t)}{c(M_{t=0})} = k_{app} \cdot t \qquad \text{(S3.3)},$$

where:

c(M), concentration of Product M
$c(O_3)$, concentration of ozone
k, reaction rate constant

TOC content profiles

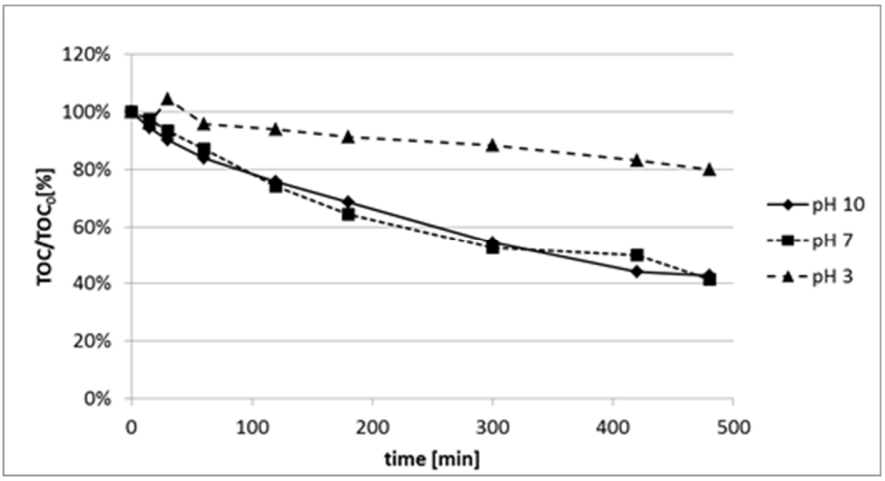


Fig. II-5-1: Relative TOC content in DTPA-containing waste water over time (relative to concentration at time 0 min)

MS^n spectra of DTPA and its transformation products

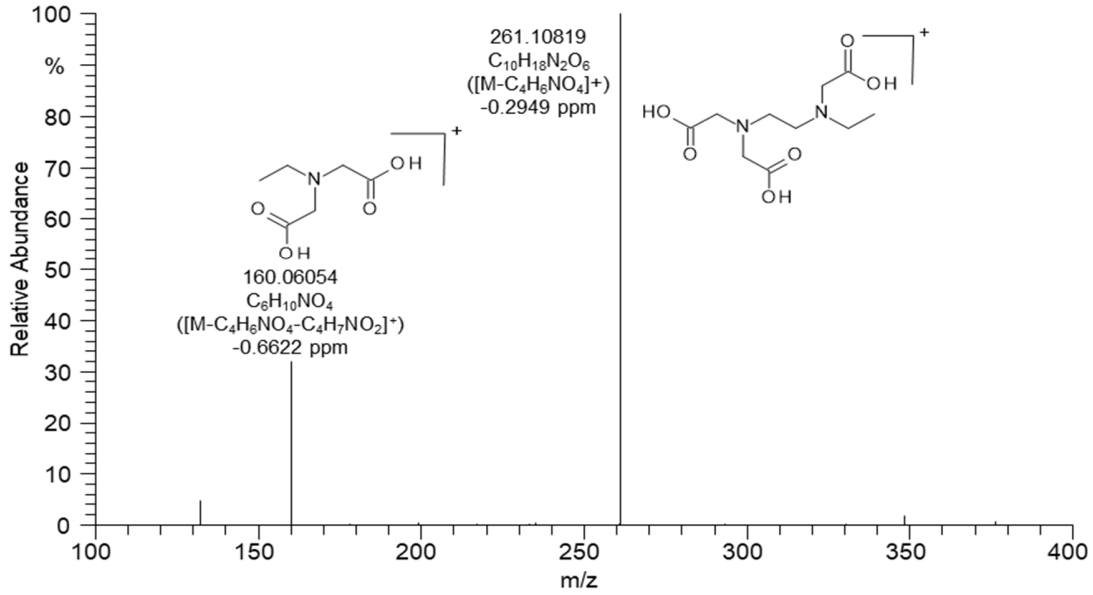


Fig. II-5-2: MS² spectrum of DTPA (MW393, $[C_{14}H_{23}N_3O_{10}]+H^+$)

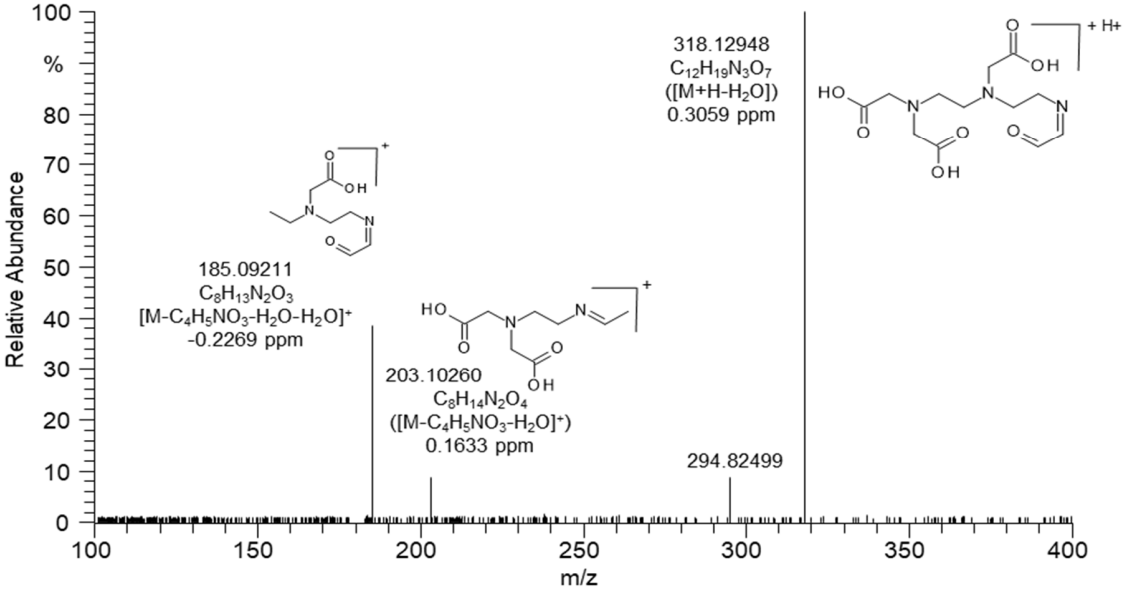


Fig. II-5-3: MS² spectrum of DT4A (MW335, $[C_{12}H_{21}N_3O_8]+H^+$)

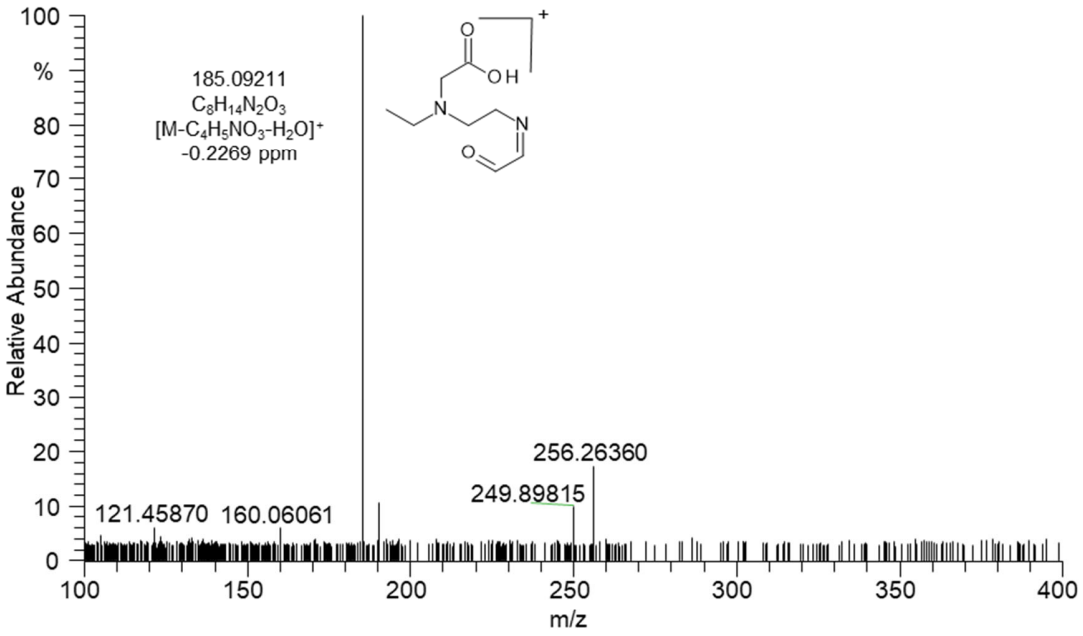


Fig. II-5-4: MS² spectrum of MW317, $[C_{12}H_{19}N_3O_7]+H^+$

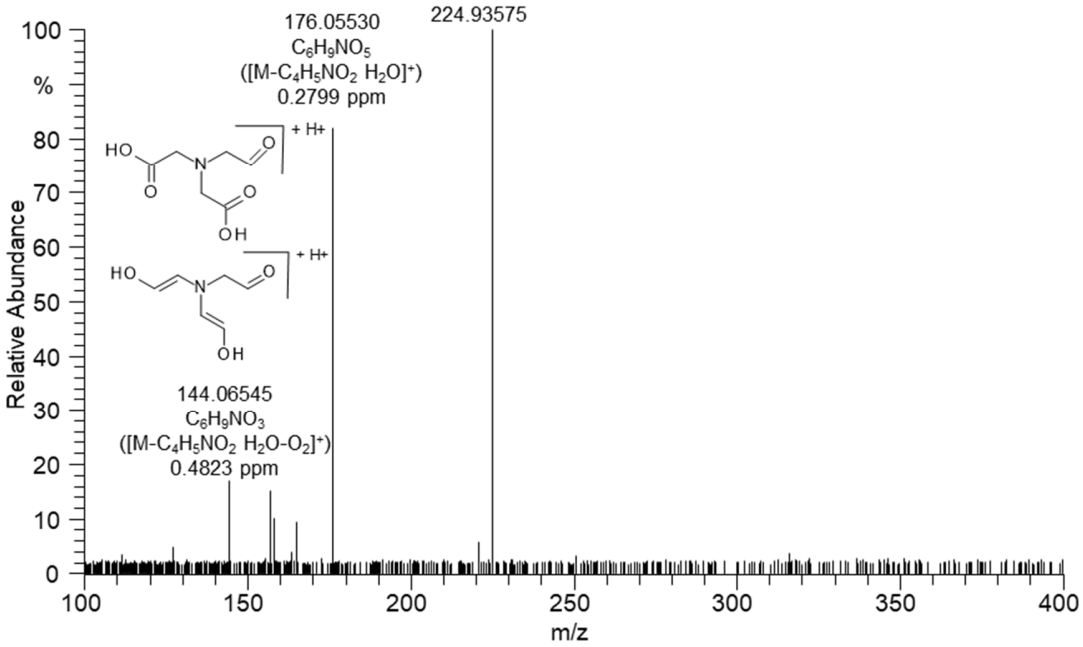


Fig. II-5-5: MS² spectrum of EDTA (MW292, $[C_{10}H_{16}N_2O_8]+H^+$)

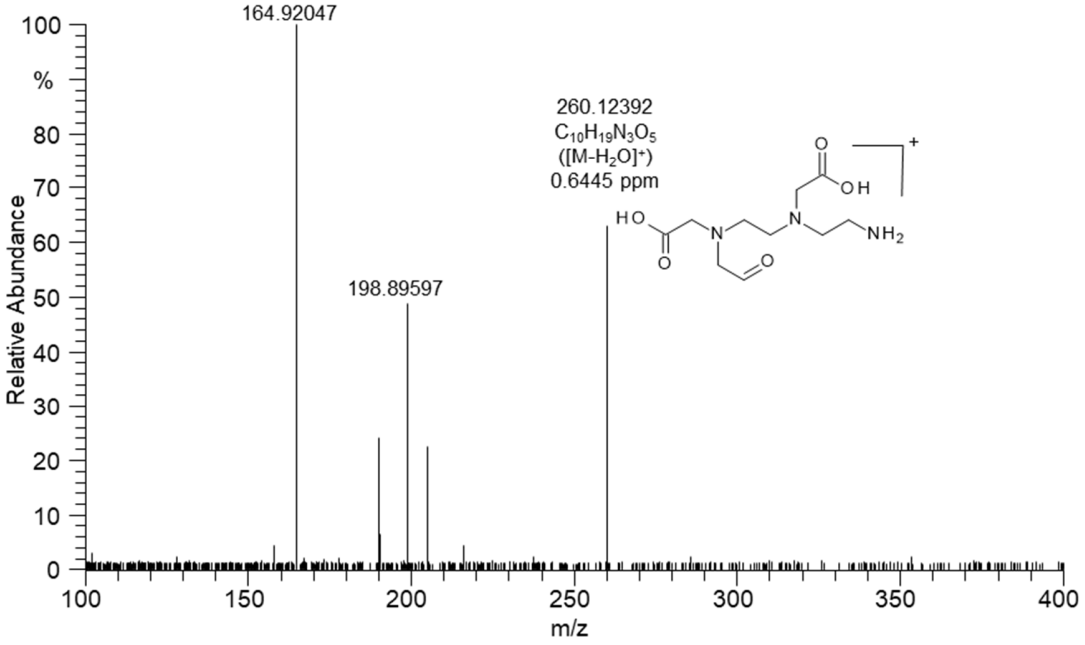


Fig. II-5-6: MS^2 spectrum of DT3A (MW277, $[C_{10}H_{19}N_3O_6]+H^+$)

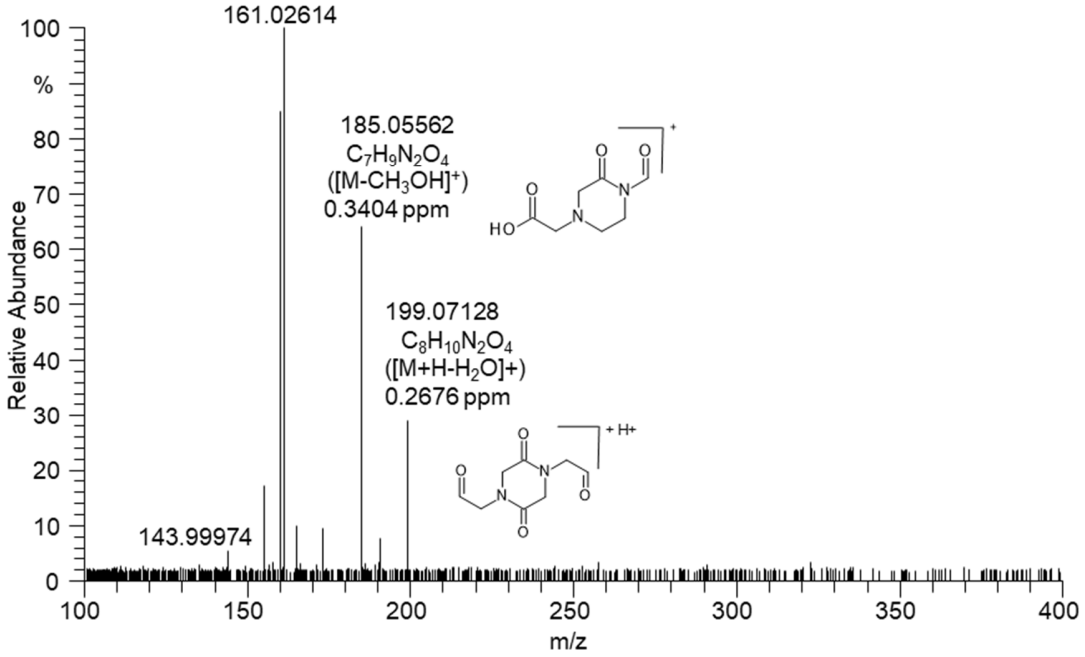


Fig. II-5-7: MS^2 spectrum of KPDA (MW216, $[C_8H_{12}N_2O_5]+H^+$)

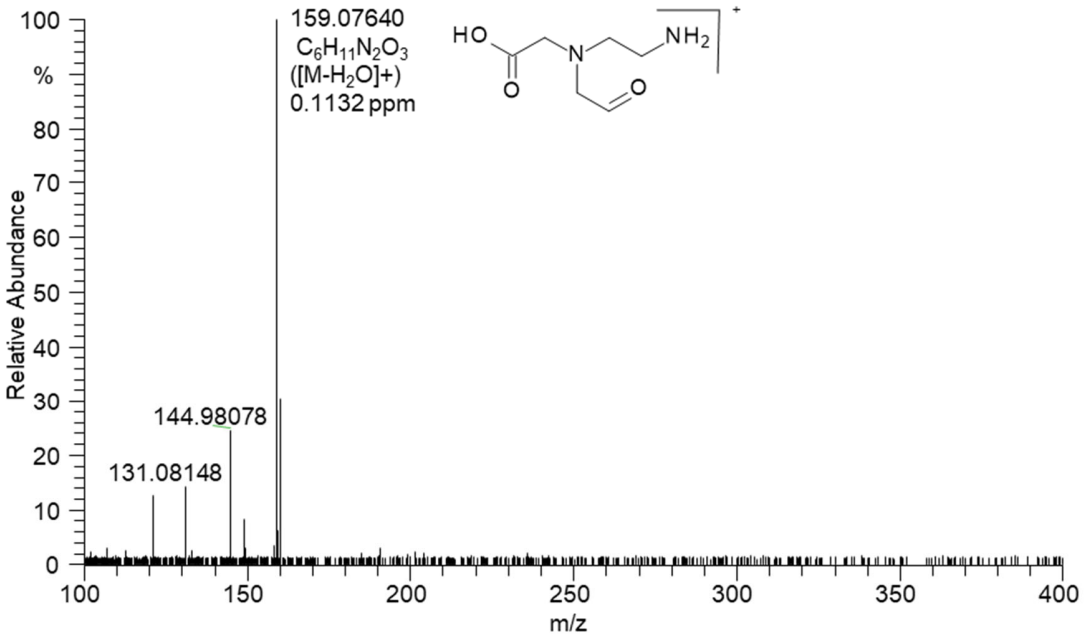


Fig. II-5-8: MS² spectrum of N,N-EDDA (MW176, $[C_6H_{12}N_2O_4]+H^+$)

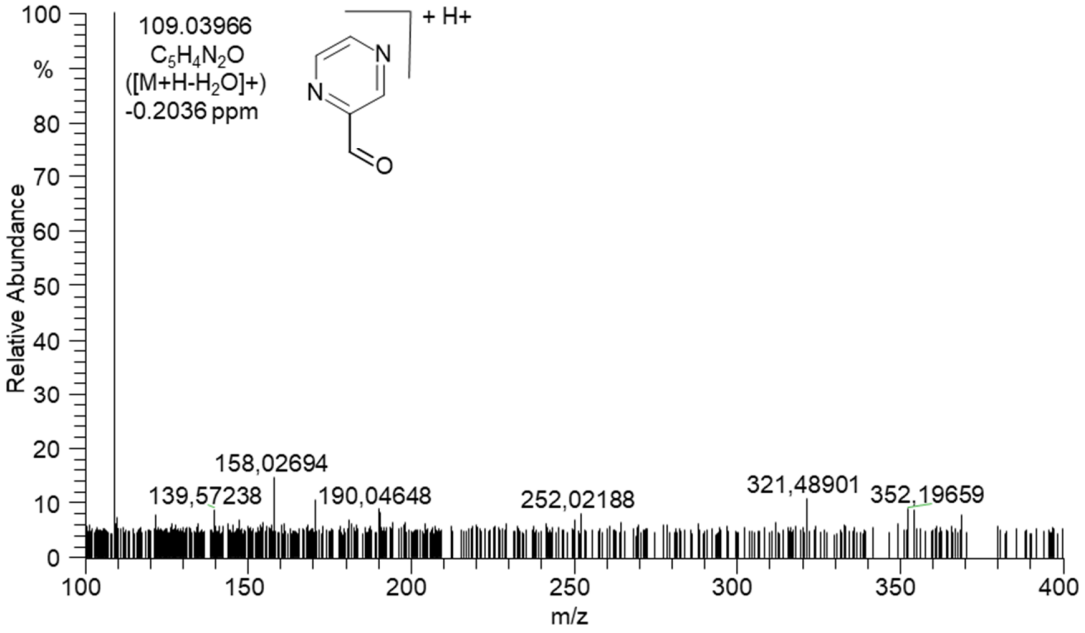


Fig. II-5-9: MS² spectrum of MW126, $[C_5H_6N_2O_2]+H^+$

DTPA and its transformation products, comprehensive list

Molecular formula	MW	$[M+H]^+$; Error[a]	$[M+NH4]^+$; Error[b]	$[M+Na]^+$; Error[c]	Remarks
	[g/mol]				
$C_5H_6N_2O_2$	126	127.05021; -0.0555 ppm	Not identified	149.03225; -0.8092 ppm	Newly identified transformation product
N,N-EDDA $C_6H_{12}N_2O_4$	176	177.08699; -0.0398 ppm	Not identified	199.069; -0.4089 ppm	Previously published by Noertemann (2005) and Metsaerinne et al. (2004)
KPDA $C_8H_{12}N_2O_5$	216	217.082; -0.4721 ppm	Not identified	239.06385; -0.0324 ppm	Previously published by Ternes et al. (1996)
DT3A $C_{10}H_{19}N_3O_6$	277	278.13465; -0.0433 ppm	295,161; 0.7614 ppm	Not identified	Previously published by Metsaerinne et al. (2004) and Xian et al. (2018)
EDTA $C_{10}H_{16}N_2O_8$	292	293.09794; 0.0103 ppm	Not identified	315.07991; -0.0753 ppm	Available as standard reference compound
$C_{12}H_{19}N_3O_7$	317	318.1295; 0.2428 ppm	Not identified	340.11155; -0,0883 ppm	Newly identified transformation product
DT4A $C_{12}H_{21}N_3O_8$	335	336.1401; 0,1253 ppm	353,167; -0.9220 ppm	358,1221; -0,0388 ppm	DT4A Previously published by Noertemann (2005), Metsaerinne et al. (2004) and Xian et al. (2018)
DTPA $C_{14}H_{23}N_3O_{10}$	393	394.14585; -0.5799 ppm	Not identified	416.12756; -0,0178 ppm	Available as standard reference compound

Tab. II-5-1: Transformation products of DTPA identified by LC-HRMS. Difference between measured and theoretical mass for the ionization with $(H^+)^a$, $(NH_4^+)^b$ and $(Na^+)^c$.

Ecological assessment

	Incineration			Ozonolysis + biological waste water treatment		
	Evaporation	Incineration		Ozonolysis	Biolog. Treatment	
Steam	1.2		$t/t_{Waste\ water}$			$t/t_{Waste\ water}$
Cooling water	86.4	19.2	$t/t_{Waste\ water}$			$t/t_{Waste\ water}$
Electrical power	10.0	-37.6	$kWh/t_{Waste\ water}$	201.2	9.8	$kWh/t_{Waste\ water}$
Natural gas		208.2	$kWh/t_{Waste\ water}$			$kWh/t_{Waste\ water}$

Tab. II-5-2: Calculated utility demands

	Specific CO_2 emissions	
Steam	200	kg CO_2 /t
Cooling water	0.15	kg CO_2 /t
Electrical power	0.5	kg CO_2 /kWh
Natural gas	0.183	kg CO_2 /kWh

Tab. II-5-3: Specific CO_2 emissions

	Incineration	Ozonolysis + biological waste water treatment	
Steam	245.5	0.0	kg $CO_2/t_{Waste\ water}$
Cooling water	15.8	0.0	kg $CO_2/t_{Waste\ water}$
Electrical power	-13.8	105.5	kg $CO_2/t_{Waste\ water}$
Natural gas	38.1	0.0	kg $CO_2/t_{Waste\ water}$

Tab. II-5-4: CO_2 emissions per metric ton of process waste water

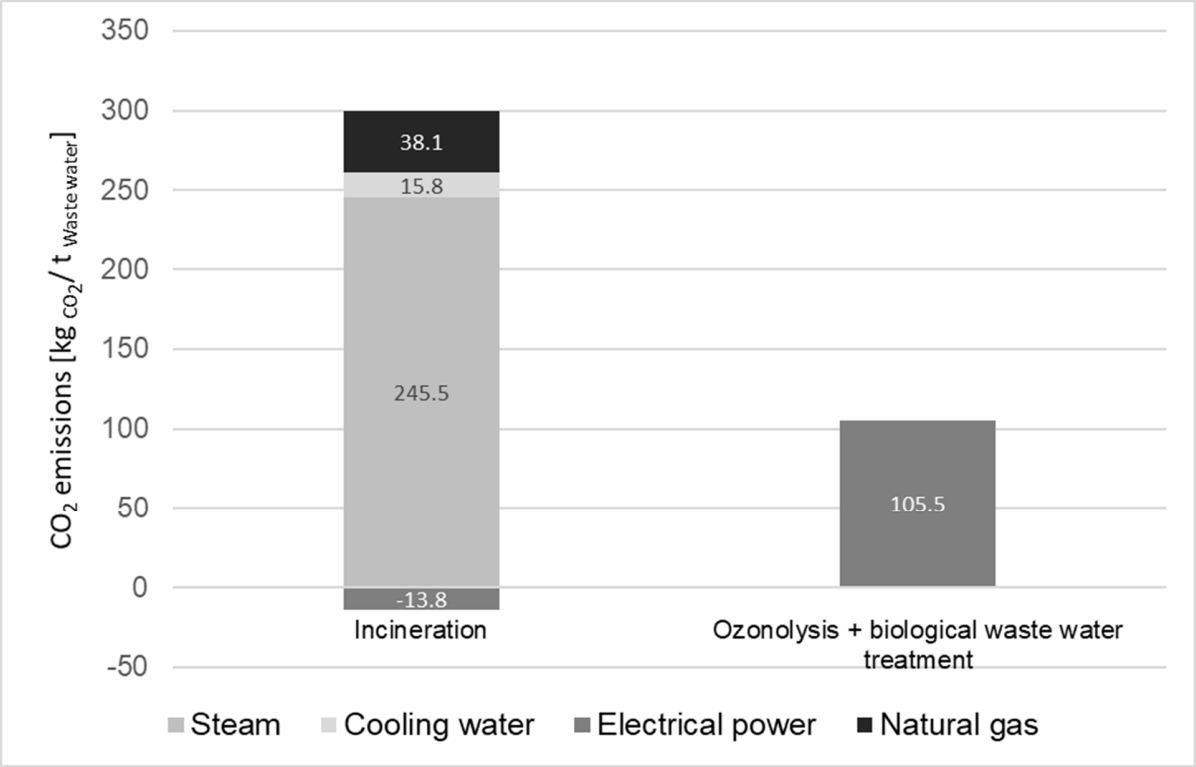


Fig. II-5-10: CO_2 emissions per metric ton of process waste water

IV. List of Original Contributions

Peer reviewed articles

Daoud, F., Pelzer, D., Zuehlke, S., Spiteller, M., Kayser, O, 2017. Ozone pretreatment of process waste water generated in course of fluoroquinolone production. Chemosphere 185, 953–963. Elsevier.

Daoud, F., Zuehlke, S., Spiteller, M., Kayser, O, 2020. Ozonation of rivaroxaban production waste water and comparison of generated transformation products with known in vivo and in vitro metabolites. Sci. Total Environ. 714, 136825. Elsevier.

Daoud, F., Zuehlke, S., Spiteller, M., Kayser, O, 2021. Elimination of diethylenetriamine-pentaacetic acid from effluents from pharmaceutical production by ozonation. Ozone: Science & Engineering, 1-13. Taylor & Francis.