OCCURRENCE, REMOVAL, AND TOXICITY OF PHARMACEUTICAL COMPOUNDS IN THE SELECTED DRINKING WATER SOURCES OF ISTANBUL

by

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ABSTRACT

OCCURRENCE, REMOVAL AND TOXICITY OF PHARMACEUTICAL COMPOUNDS IN THE SELECTED DRINKING WATER SOURCES OF ISTANBUL

Pharmaceutical compounds (PCs) after being ingested reach water bodies through the excreta of humans and animals, and depending on the effectiveness of treatment systems, they can end up in drinking water sources, posing a significant threat to public health. In this dissertation; occurrence, removal, fate and cytototoxicity of some PCs were investigated in the raw and treated drinking water samples collected from different water sources of Istanbul.

In the first part of the study, 55 PCs were analyzed in all water samples collected from 13 drinking water treatment plants (DWTP) in Istanbul, and 17 PCs were quantified. At least one or more PCs were determined in 77% of all samples. On average 70% of PCs found in raw water was eliminated in DWTPs. In 80% of the samples where PCs were detected, the parent pharmaceutical molecules have been degraded with a removal efficiency exceeding 99%.

In the second part, the extent of degradation of PCs were investigated on three molecules; sulfamethoxazole, carbamazepine, and clarithromycin. The main result is that all three tested pharmaceuticals were completely degraded into other organic transformation products after treatment.

In the third part, cytotoxicity of degradation products were investigated on human liver (HepG2) and human kidney (HK-2) cells. Significant cytotoxicity was observed in HK-2 and HepG2 cells after treatment in distilled water, whereas in raw water the observed cytotoxic activity disappeared.

All these suggest toxicity of degradation products of PCs generated in DWTPs need to be considered comprehensively in the risk assessment, process design and optimization.

ÖZET

ISTANBUL'UN SEÇİLMİŞ İÇME SUYU KAYNAKLARINDA FARMASÖTİK BİLEŞİKLERİN VARLIĞI, UZAKLAŞTIRILMASI VE TOKSİSİTESİ

Farmasötik bileşikler (FB'ler), tüketildikten sonra insanların ve hayvanların dışkıları aracılığıyla sucul ortamlara ulaşır ve arıtma sistemlerinin etkinliğine bağlı olarak içme suyu kaynaklarına kadar ulaşabilir, bu da halk sağlığı için önemli bir tehdit oluşturmaktadır.

Bu çalışma; İstanbul'un farklı içme suyu kaynaklarından alınan ham ve arıtılmış içme suyu örneklerinde bazı FB'lerin varlığı, uzaklaştırılması, akıbeti ve sitotoksisitesi incelenmiştir.

Çalışmanın ilk bölümünde, İstanbul'daki 13 içme suyu arıtma tesisinden (İSAT) toplanan tüm su örneklerinde 55 hedef FB analiz edilmiş ve 17 FB tespit edilmiştir. Tüm örneklerin %77'sinde en az bir veya daha fazla FB belirlenmiştir. Ham suda bulunan ana FB moleküllerinin ortalama %70'i İSAT'lerinde yok edilmiştir. FB'lerin tespit edildiği örneklerin %80'inde ana ilaç molekülleri, uzaklaştırma verimliliği %99'u aşan bir şekilde tamamen bozunmuştur.

Çalışmanın ikinci bölümünde, FB'lerin bozunma derecesi sulfamethoxazole, carbamazepine, ve clarithromycin molekülleri üzerinden incelenmiştir. Ana sonuç, üç örnek FB'nin tümünün arıtma sonrasında diğer organik dönüşüm ürünlerine tamamen bozunduğudur.

Çalışmanın üçüncü bölümünde, bozunma ürünlerinin insan karaciğeri (HepG2) ve böbrek (HK-2) hücreleri üzerindeki sitotoksisitesi incelenmiştir. Distile suda arıtma sonrasında HK-2 ve HepG2 hücrelerinde önemli sitotoksisite gözlenirken, ham suda yapılan deneylerde gözlenen bu sitotoksik etki ortadan kalkmıştır.

Tüm bunlar, İSAT'lerinde oluşan FB'lerin bozunma ürünlerinin toksisitesinin risk değerlendirme sürecinde ve proses tasarımı ve iyileştirmesinde kapsamlı bir şekilde dikkate alınması gerektiğini göstermektedir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Unit
°C	Degree Celcius	
Amu	Atomic Mass Unit	g/mol
C	Concentration	ng/L; mg/L, etc
Da	Atomic Mass Unit	g/mol
eV	Electron Volt	
$g.mol^{-1}$	gram per mol	
GHz	Giga hertz	
h	Hour	
K_H	Henry's coefficient	$atm.m^3$ / mol
L	Liter	
Log K_{ow}	Log Octanol Water Partition Coefficient	
Log p	Log Partition Coefficient	
m/z	Mass to Charge Ratio	
mg/L	Miligram Per Liter	
mg/mL	Miligram Per Mililiter	
min	Minute	
mm	Milimeter	
ng/L	Nanogram Per Liter	
ng/mL	Nanogram Per Mililiter	
nm	Nanometer	
p	Probability Value	
pKa	Acid Dissociation Constant	mol/L
ppb	Parts Per Billion	µg/L
ppt	Parts Per Trillion	ng/L
R^2	Coefficient of Determination	
rpm	Revolution Per Minute	
sd	Standard Deviation	
µL	Micro Liter	

Abbreviation	Explanation
ACC	Average Cell Count
ACN	Acetonitrile
AEC	Average Environmental Concentration
AGES	Austrian Agency For Health and Food Safety
AMR	Antimicrobial Resistance
AOP	Advanced Oxidation Processes
AT	After Treatment
BT	Before Treatment
CBZ	Carbamazepin
CHO-K1	Chinese Hamster Ovary Cells
CLA	Clarithromycin
COVID-19	Corona Virus Disease 2019
DDD	Defined Daily Doses
DEET	N,N-Diethyl-3-methylbenzamide
DMSO	Dimethyl sulfoxide
DW	Distilled water
EDTA	Ethylene di amin tetra acetic acid
EIC	Extracted Ion Chromatogram
EPA	Environmental Protection agency
ESI	Electron Spray Ionisation
EU	European Union
FAO	Food and Agricultural Organization
GAC	Granular Activated Carbon
HepG2	Hepatoblastoma Cell Line
HK2	Human Kidney (renal) Proximal Tubule Cell
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
ISKI	Istanbul Su ve Kanalizayon İdaresi
LC-MS/MS	HPLC with tandem Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MEC	Minimum Effect Concentration
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
OTC	Over The Counter

PC	Pharmaceutical Compound
PBS	Phosphate Buffered Saline Solution
PCsage	Passage
PR	Products (degradation)
R	Raw
RW	Raw Water
SMX	Sulfamethoxazole
SPE	Solid Phase Extraction
SRM	Selected Reaction Monitoring
T	Treated
TIC	Total Ion Chromatogram
ToF	Time of Flight
TW	Tap Water
UN	United Nations
UV	Ultraviolet
WHO	World Health Organization

1. INTRODUCTION

Unfortunately, water, which is the most crucial resource for the sustainability of ecosystems, is insufficient in both quantity and quality worldwide, particularly in certain regions of the world. Human activities have caused significant harm to the aquatic environment by releasing effluents into water bodies. As a result, emerging organic pollutants have been introduced into these water bodies. These pollutants are organic compounds that have been newly detected or identified in the environment due to advances in analytical techniques. They encompass a wide range of chemicals including pharmaceutical substances, personal care products, endocrine-disrupting chemicals, their metabolites, and transformation products, which enter, disperse, and persist in the environment (Lapworth et al., 2012a). These organic contaminants tend to accumulate in the aquatic environment and can have toxic effects on aquatic life. As a result, occurrence of organic micropollutants in surface waters and drinking waters is steadily increasing, posing a challenge for conventional water treatment plants that are not specifically designed to remove these substances (Heberer, 2002a). Moreover, they can be transferred to humans through the food chain or drinking water.

In 2009, a report was generated by a working group comprising experts in toxicology, analytical chemistry, water chemistry, health, water treatment engineering pharmacology, risk assessment bodies, regulators and policy makers, under the guidance of the World Health Organization (WHO). The report highlighted the necessity for more comprehensive research to improve our understanding of the transportation, occurrence, and fate of pharmaceuticals in the environment, with a specific focus on drinking (WHO, 2009) water sources

In relation to this thesis topic, the majority of scientific studies conducted so far have focused on the removal of pharmaceutical compounds (PCs) in drinking water treatment plants. However, limited research has been conducted on the degradation products formed during this removal process, as well as on whether these molecules are more or less toxic than the parent pharmaceutical molecules. Another significant aspect in terms of toxicology is that scientific studies have predominantly focused on the ecotoxicity of pharmaceutical compounds in water, while studies of PC in water on human toxicity have been scarce. It is important to investigate the formation of oxidation degradation products as they can differ from the parent compounds in terms of their chemical and physical properties, as well as their toxicity. Therefore, this study was designed to

integrate chemical and toxicological analyses of raw, treated, and pure water samples to gain insights into the presence and potential effects of these degradation products.

The main objective of this dissertation is to contribute increasing knowledge and a better understanding of the toxicity of pharmaceutical compounds (PCs) in drinking water via:

1. Screening the concentration of selected pharmaceutical compounds in both raw, treated and tap drinking water samples from Istanbul
2. Evaluate the removal capacity of the drinking water treatment plants for the elimination of the tested PCs
3. Evaluate the level of degradation of the PCs in the drinking water treatment process
4. Evaluate the cytotoxicity of the degradation products of the PCs in the drinking water treatment process.

Istanbul, being a city that has served as the capital of some of the largest empires in the world with a history dating back 8,500 years, is distinct from other ancient cities in that it was not founded near a freshwater source such as a lake or river. Istanbul has always faced a significant challenge in terms of water supply throughout history. Thus due to the lack of a nearby water sources, water had to be transported to the city from distant locations. Furthermore, Istanbul has had to develop unique solutions such as aqueducts and cisterns to address this issue (ISKI Annual Report, 2022). However, in recent years, the emergence of climate change and drought, coupled with increased consumption, poses a higher risk of water scarcity for Istanbul, as it does for the rest of the world. Furthermore, quality point of view, increased frequency of extreme rainfalls due to climate change can lead to overflow of treatment plants and contamination of water sources consequently. Considering Istanbul's growing population and urbanization trend, it is crucial for a megacity like Istanbul to effectively manage the quantity, quality, and safety of the water cycle from its source to the end-users. This requires the exploration of new underground and surface water sources to enhance the existing water reserves, as well as proper utilization of water reuse and reclamation opportunities. While undertaking these efforts, it is essential to ensure the protection of public health by periodically monitoring the drinking water quality and safety in a risk-based approach, particularly addressing emerging hazards associated with pharmaceutical compounds, personal care products and endocrine disruptors. Implementing advanced technological developments may be necessary to mitigate these risks effectively.

2. BACKGROUND

2.1. Occurrence of Pharmaceutical Compounds in Drinking Waters

2.1.1. General

In most European countries, surface and groundwater sources are the primary sources of drinking water. However, ensuring the distribution of safe drinking water has become one of the crucial and complex challenges of our time, as these sources are susceptible to contamination. The quality of surface and groundwater can be influenced by various factors, both natural and human-induced. Pharmaceuticals are one of the significant anthropogenic contaminants found in the aquatic environment, leading to the degradation of water quality (Richardson & Kimura, 2020).

Pharmaceuticals play a crucial role in diagnosing and treating various illnesses, leading to their widespread usage. Globally, it is estimated that approximately 15 grams of medicinal drugs are used per person per year. However, in industrialized countries, this rate is believed to be higher, ranging from about 50 to 150 grams per person annually in line with the increasing production of drugs worldwide (Lenzen et al., 2020). According to a survey conducted by WHO in 2017, Turkiye is the biggest consumer of antibiotics in Europe with 38.18 DDD/ 1000 inhabitants per day versus the Netherlands that is the smallest consumer with 9.78 DDD/ 1000 inhabitants per day. DDD stands for “Defined Daily Doses” which means the assumed average maintenance dose per day. (WHO Report, 2018)

Socio-economic factors play an important role in the consumption of pharmaceuticals as well. For example, the economic crisis experienced in Greece between 2010 and 2014 resulted in remarkable shifts in pharmaceutical consumption patterns, particularly concerning the usage of psychoactive drugs. Significant increases were observed in wastewater samples, with antipsychotics, benzodiazepines, antihypertensives, and antidepressants showing surges of 35, 19, 13, and 11-fold, respectively. The COVID-19 pandemic and the following global economic crisis have also resulted in a significant rise in the consumption of psychotropic drugs (Benistand et al., 2022). Additionally, the consumption of illicit drugs such as methadone, ecstasy, and methamphetamine exhibited striking increases of 7, 5, and 2-fold, respectively (Thomaidis et al.,

2016). It is noteworthy that pharmaceutical consumption patterns also display variations corresponding to seasonal changes (Castiglioni et al., 2006).

More than 200 different pharmaceutical compounds (PCs) have been detected in surface water, groundwater, and sewage systems (Taheran et al., 2016). The concentration levels, effects, and fate of these compounds on human health and wildlife are still not fully understood. However, it has been determined that these compounds have the potential to induce aquatic toxicity, promote the development of antibiotic resistance in pathogenic microorganisms, exhibit genotoxic and endocrine activity effects (Martín et al., 2012). Furthermore, the release of PCs into the environment is not currently regulated or addressed adequately within existing water quality standards (Ma et al., 2017).

Despite numerous scientific studies on the presence of PCs in wastewater and the environment, research specifically focused on the occurrence of pharmaceuticals in drinking water is limited both globally and in Turkey. The fact that PCs can directly affect human health and the extensive use of pharmaceuticals worldwide make this issue a matter that needs to be addressed with great care. In a very comprehensive review, the global distribution of the pharmaceuticals can be clearly seen (Figure 2.1.) both in terms of drug classes and regions (aus der Beek et al., 2016). Generally, there is a scarcity of comprehensive data regarding the presence of pharmaceuticals in tap or drinking water. This data gap is particularly pronounced in developing and emerging countries. The majority of detections have been reported from Western European countries, and the United States where over 30 distinct pharmaceutical compounds have been identified.

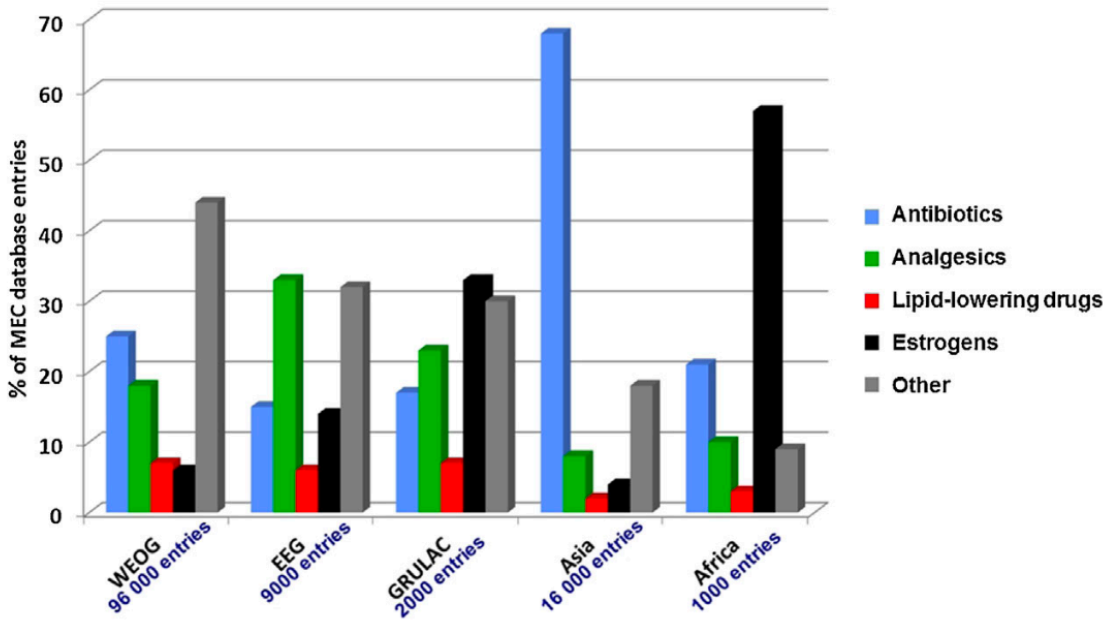


Figure 2. 1. Distribution of various drug classes in different regions of the world.

MEC= Measured Env.Concentration, EEG= Eastern Eur. Group, GRULAC=Latin America Group, WEOG=Western Eur. Group, (adapted from aus der Beek et al., 2016).

In order to control the pharmaceuticals risk effectively, first of all it is essential to determine which PCs are present in drinking water sources. Subsequently, the identification of the sources of contamination becomes crucial. By accurately identifying the specific PCs and their origins, appropriate measures can be taken to address and mitigate the contamination.

PCs are biologically active compounds produced for the diagnosis, treatment, alleviation of certain diseases, disorders, abnormal physical conditions or their symptoms that can be seen in humans and animals. The term pharmaceutical encompasses a wide range of classes of compounds of variable nature, function, behavior and activity (Bila & Dezotti, 2003). Pharmaceuticals are classified based on their respective application fields. For instance, common classifications include antiepileptics, antihistamines, antibiotics, antibacterials, painkillers and antipyretics, beta-blockers, cholesterol drugs, chemotherapy drugs, and others. These drugs have been detected in the ecosystem through various research studies (Derksen et al., 2004). The primary concern with pharmaceuticals lies not in their acute toxicity but in their chronic toxicity (Richardson & Bowron, 2011). The molecular weights of the generally researched PCs are typically between 200-100 Dalton which are called small molecules. Due to their high water solubility and biological activity, these compounds have the potential to be present in wastewater and readily mix with natural waters. This characteristic enables their easy dispersion in the aquatic environment (Kümmerer, 2004).

Once PCs are used for therapeutic purposes, they enter the wastewater treatment plant through excretion in feces and urine, ultimately mixing with the aquatic environment. These compounds can be present in their original form or as metabolites without undergoing significant changes. According to reports, over half of the 2056 drinking water treatment plants in the USA may potentially receive effluents from wastewater treatment plants as a source of drinking water (Rice & Westerhoff, 2015). Studies have indicated that OTC (over-the-counter) drugs such as aspirin, ibuprofen, paracetamol, and naproxen are frequently detected in the aquatic environment. Among anti-inflammatory drugs, diclofenac and some antibiotics like clarithromycine, sulfamethoxazole, trimethoprim, and ciprofloxacin have been identified as having the highest acute toxicity. As a result, they are listed in different versions of the EU Watch Lists. These antibiotics are recognized as contaminants of emerging concern under the Water Framework Directive in the European Union and it has been the subject of extensive research (Buser et al., 1998; EC Decision 2018/840, 2018; EC Decision 2015/495, 2015; EC Decision 2022/1307, 2022).

In a study, estimated concentrations of β-lactam antibiotics in hospital wastewater range from 20 to 80 µg/L, while ciprofloxacin concentrations range from 2 to 83 µg/L. In municipal wastewater, the concentration of antibiotics approaches approximately 50 µg/L (Y. Wang et al., 2014). Carbamazepine, an antiepileptic drug, has been identified as the most commonly detected PC in surface water. Germany has reported the highest concentrations of carbamazepine in its surface waters, followed by France and Finland. The presence of a significantly elevated concentration of carbamazepine in surface water may indicate the occurrence of open defecation in close proximity to the river area, as carbamazepine is primarily utilized in human pharmaceuticals (Jurado et al., 2014; Zhou et al., 2011).

2.1.2. Sources of Pharmaceutical Compounds in Drinking Water

The major sources of pharmaceuticals in drinking water are wastes from human use (domestic), hospitals, pharma industry, and veterinary use, including landfill sites. The cycle starts with the consumption and excretion, followed by the waste water treatment plant, surface water, ground water and back again to the human body via drinking water. Pharmacokinetic studies exhibit that an appreciable proportion of the administered pharmaceuticals are excreted via feces and urine and thus are present in domestic wastewater (Forth et al., 1996). Other important contributors to the cycle are the rivers transferring the waste from pharmaceutical industries and the landfills leaking pharmaceuticals to the underground water (Zühlke et al., 2004).

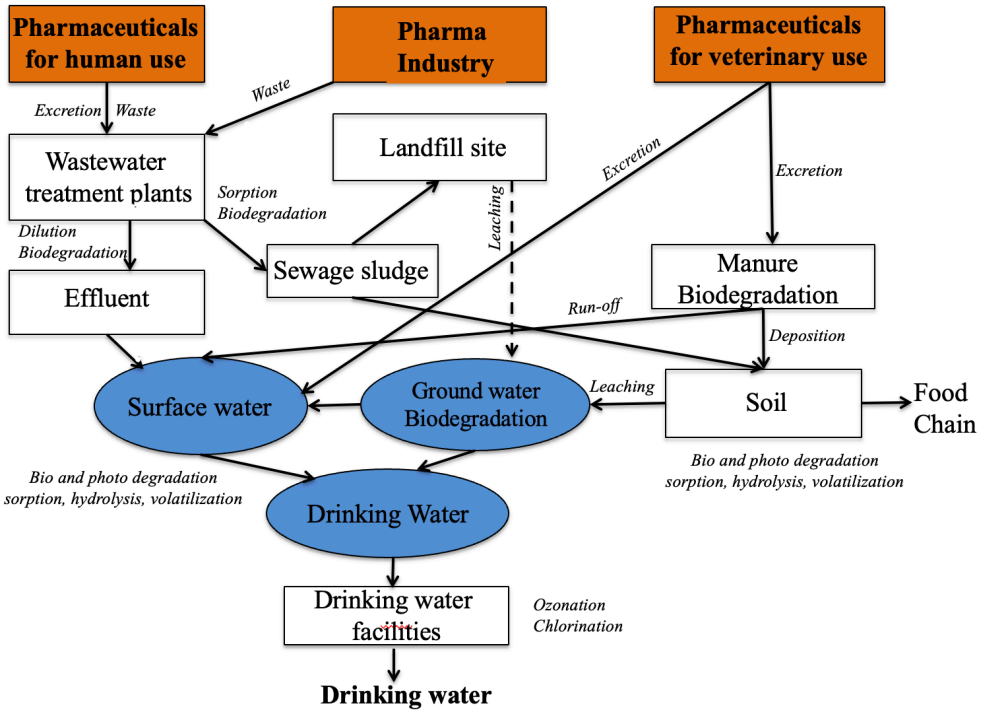


Figure 2. 2. An overview of sources and transport of pharmaceuticals and main trasnformations in freshwater environment (adapted from Farré et al., 2008)

Figure 2.2. shows how freshwater environments can be contaminated with PCs through various pathways schematically. One significant pathway involves the absorption of pharmaceuticals by the human body during medical use, followed by distribution, metabolism, and excretion. These pharmaceuticals are then released into the sewage system or septic tank. After sewage treatment, the wastewater may be used for irrigation, with the bio-solids (treated sludge) potentially being applied as fertilizer to agricultural land (Yang et al., 2015). This introduces pharmaceuticals into the soil. Another source of pharmaceuticals in the environment is through the manufacturing processes, where wastewater from production facilities directly enters waste water treatment plants (Fick et al., 2009).

After treatment, the sludge is deposited on the soil as fertilizer, while the liquid effluent is discharged into freshwater environments. Additionally, pharmaceuticals can enter groundwater through leaching from the soil, posing a potential threat to drinking water. Moreover, runoff from land treated with digested sludge for agricultural purposes can also introduce pharmaceuticals into freshwater environments (Nikolaou et al., 2007). The release of veterinary pharmaceuticals into the environment occurs when animal wastes, whether in solid or liquid form, are used as fertilizers and sprayed onto agricultural fields. These veterinary pharmaceuticals, along with their metabolites, can contaminate the soil and potentially enter the food chain. As a result, agricultural runoff can transport these compounds into freshwater systems, which can further leach into groundwater (Farré et al., 2008). In addition, personal care products, which are applied externally, are primarily discharged through activities such as showering, bathing, swimming, and washing sinks. These personal care products can pass through wastewater treatment plants and ultimately reach the environment.

In addition to human exposure to pharmaceutical compounds from the environment as drinking water, an important source has been added in recent years. Due to severe drought and water scarcity occurring in certain regions of the world as a result of climate change, the use of reclaimed wastewater in agriculture has been increasing. As a consequence, there is a significant entry of pharmaceutical compounds into agroecosystems, and it is expected that the use of reclaimed water will further increase in the coming years. In a study conducted in Israel, a quantifiable level of pharmaceutical compounds was found in 99.6% of agricultural food products irrigated with treated wastewater (secondary, tertiary, soil-aquifer treatments). Among the tested food products, leafy green plants showed the largest number and highest concentration of pharmaceuticals. On the other

hand, some plants have the potential for increased concentrations of pharmaceutical compounds in their bodies through bioconcentration (Ben Mordechay et al., 2021). This further reinforces the importance of the relationship between environmental pollution and food safety, highlighting the need for more research in this field.

In surface waters, the concentrations of pharmaceuticals in the aqueous phase can be reduced through various processes such as dilution, sorption, biodegradation, and phototransformation. The degree of dilution depends on the proportion of sewage effluent present in the receiving water, and it can vary significantly among different receiving waters and seasons. For instance, in the research conducted by Kolpin and colleagues (2004), higher concentrations of pharmaceuticals were observed in a river during low-flow seasons compared to high-flow seasons, attributed to a reduced level of dilution during low-flow conditions (Kolpin et al., 2004). Numerous studies have indicated that a significant number of pharmaceuticals are resistant to biodegradation during conventional biological treatment processes. Additionally, these pharmaceutical compounds are not effectively adsorbed by wastewater sludge (Ingerslev & Halling-Sørensen, 2000; Kümmerer et al., 1997). In line with these findings according to a study conducted in Berlin, it has been found that the main reason for the presence of pharmaceuticals in surface and ground water is the waste water treatment plants (Heberer, 2002b).

Extensive investigations have been conducted in various locations worldwide, focusing on pharmaceutical residues in drinking water, particularly in groundwater resources. These studies have involved the analysis of pharmaceuticals alongside other micropollutants, primarily endocrine disruptors. The following list outlines some of the most comprehensive surveys conducted in this field:

- Denmark, 1993-2001, 7671 drinking water resources (Juhler & Felding, 2003)
- USA, 2008, 74 drinking water resources (Focazio et al., 2008)
- European research, 2010, 164 drinking water resources (Loos et al., 2010)
- USA, 2011, 1230 drinking water resources (Fram & Belitz, 2011)
- UK, 2011, 2644 drinking water resources (Stuart et al., 2011)
- Turkey, Büyükçekmece Istanbul (Aydin & Talinli, 2013)
- Turkey, Samsun (Üstün-Odabaşı et al., 2020)

The effluents are discharged into surface waters following waste water treatment. In cases where surface water is not available, the effluents may also be infiltrated into the ground. Due to

dilution, the concentrations of pharmaceuticals in surface waters are generally lower compared to waste water treatment effluents.

Previous studies have shown that the concentrations of the pharmaceuticals analyzed in surface waters were typically below 200 ng/L (Bendz et al., 2005; Glassmeyer et al., 2005; Kolpin et al., 2004; Wiegel et al., 2004). However, there are certain locations where concentrations of specific compounds have reached several µg/L (Bound & Voulvoulis, 2006; Metcalfe et al., 2003; Stackelberg et al., 2004).

After these results, societies and the media took action and studies were started to determine the amount of pharmaceuticals in which natural waters and drinking water sources in developed countries. After these chemicals are taken into the organism, the investigation of their chronic and acute effects on the endocrine system is carried out by toxicologists, ecologists, biochemists and medical professionals (Kleywegt et al., 2019).

In a comprehensive study of aus der Beek and colleagues (2016), they gathered data on Minimum Effect Concentrations (MECs) for human and veterinary pharmaceutical compounds in various environmental matrices worldwide by means of a systematic survey on 1016 original publications and 150 review articles (aus der Beek et al., 2016). These matrices included surface water, groundwater, tap/drinking water, manure, soil, and others, resulting in the creation of a comprehensive database. While acknowledging the heterogeneity of data sources, a simplified assessment of data quality was conducted. The findings from this database revealed that pharmaceuticals and their transformation products have been detected in the environment of 71 countries spanning all continents (Figure 2.3., Figure 2.4.). To facilitate analysis, these countries were categorized into the five regions recognized by the United Nations (UN). The compiled database consists of 631 distinct pharmaceutical compounds, each identified at MECs above the detection limit of the employed analytical methods, thereby uncovering distinct regional trends. Notably, sixteen compounds were detected across all five UN regions. For example, the anti-inflammatory drug diclofenac was found in environmental matrices in 50 countries, with concentrations exceeding predicted no-effect concentrations in several locations. Urban wastewater emerged as the predominant emission pathway for pharmaceuticals on a global scale, although local sources such as industrial production, hospitals, agriculture, and aquaculture also contributed significantly to their release.

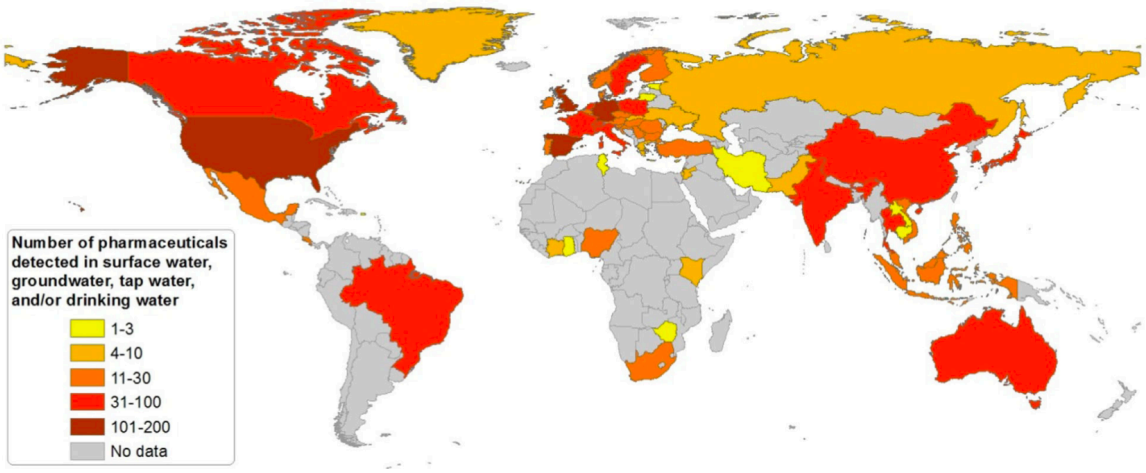


Figure 2. 3. Number of PCs detected in surface waters, ground water and drinking water

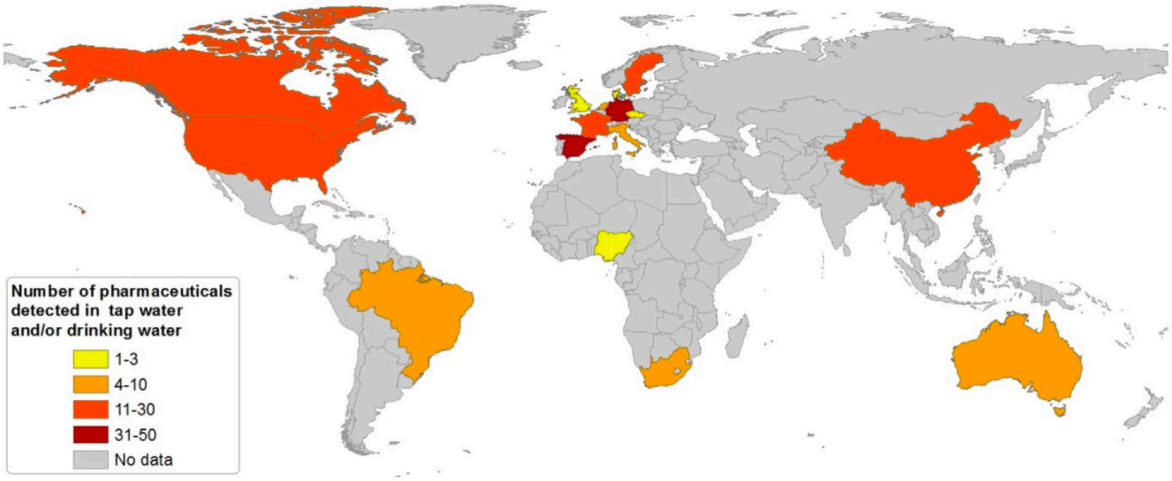


Figure 2. 4. Number of PCs detected in drinking water, (adapted from aus der Beek et al., 2016)

2.2. Removal of Pharmaceutical Compounds in Drinking Water Treatment Processes

The most rational, economical, and obvious way to combat PCs in drinking water is, of course, to prevent the treatment of these pollutants in wastewater facilities and avoid their discharge into the receiving environment. This includes preventing illegal discharges and ensuring that waste water from animal farms do not mix with water sources. In other words, it means solving the problem at its source. However, achieving this goal in real life is not always feasible everywhere. Therefore, in order to keep the public health risks associated with pharmacological compounds in drinking water under control, there is a need to understand the fate of pharmaceuticals molecules in drinking water treatment plants, increase our knowledge in this area, and design, establish, and operate efficient drinking water treatment facilities accordingly.

PCs due the demands of their molecular design are relatively stable molecules. Therefore, the conventional waste water treatment plants of active sludge are insufficient for the removal of these type of pollutants (Joss et al., 2005, 2006). As a result, PCs present in the effluent of waste water treatment plants have the potential to be transported to drinking water treatment plants and eventually reach tap water. Despite the low levels of these compounds in the range of nanograms per liter (ng/L) in tap water, serious concerns among scientists persist. The long-term impact of these concentrations especially as the combined effect with other molecules, metabolites, and transformation products on human health is not yet fully understood. The most effective approach to safeguarding individuals from these potential effects is firstly to reduce the contamination via improving the removal capabilities of the waste water treatment plants and then followed by further removal of these compounds in drinking water treatment plants efficiently (Charuaud et al., 2019). Advanced Processes have to be considered as efficient approach for the elimination of PCs.

2.2.1. Conventional Drinking Water Treatment

The traditional treatment process for obtaining potable water from groundwater often includes basic steps such as aeration, coagulation, flocculation, sand filtering and disinfection steps (Figure 2.5.). If drinking water is to be obtained from surface water, since it contains relatively more organic and inorganic pollutants, more effective steps are needed (Chang et al., 2017). In processes involving advanced treatment, it may include one or more of the color and odor and micro pollutant removal, oxidation, adsorption and membrane systems. Generally speaking conventional drinking water treatment methods fall short of effectively removing PCs from water (Zwiener, 2007).

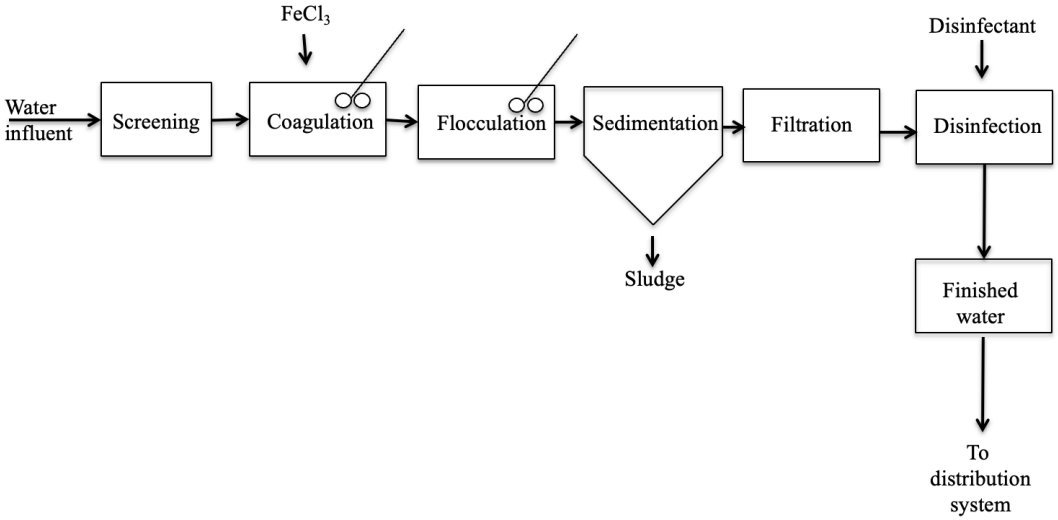


Figure 2. 5. Schematic diagram of primary-treatment processes

2.2.2. Steps of Conventional Drinking Water Treatment Process

Coagulation is a widely used process because in general it is an effective method for removing natural organic substances and turbidity in drinking water (Matilainen et al., 2002). According to the results of some studies in the literature, the removal efficiency of pharmaceuticals by coagulation in drinking water has generally remained low (Westerhoff et al., 2005). The low removal efficiency of PCs by coagulation can be attributed to the generally low hydrophobicity, different molecular sizes, low concentration and charge of pharmaceuticals, and the lack of mechanism to adsorb into the flock.

Chlorination is the most common and cost-effective method used all over the world for disinfection and oxidation of drinking water in conventional treatment plants. Although chlorine dioxide and chlorine are not as effective as ozone, they can provide oxidation of pharmaceuticals (Huber et al., 2005). Compounds containing electron-rich functional groups such as amine and phenol react with chlorine dioxide and chlorine. Diclofenac and sulfamethoxazole react rapidly especially with free chlorine at neutral pH (Westerhoff et al., 2005). The primary active form of chlorine in treated water is hypochlorous acid (HOCl), which is generated through the reaction between chlorine gas and water (2.1).

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^- \quad (2.1)$$

As a weak acid hypochlorous acid ionizes further as shown below (2.2)

$$HOCl \rightleftharpoons H^+ + OCl^- \quad (2.2)$$

These equilibrium reactions are of course pH and temperature dependant. Hypochlorous acid, in addition to its disinfection properties, can react with naturally occurring organic matter in water, leading to the formation of a significant number of disinfection and oxidation byproducts, but it does not lead to significant mineralization of compounds. One group of the byproducts is the commonly referred to as trihalomethanes. Several studies have demonstrated the effectiveness of chlorination in removing various pharmaceuticals, including antibiotics. Specifically, chlorination has been shown to effectively degrade sulfonamides, trimethoprim, fluoroquinolones, macrolides, and β-lactams. Chlorine dioxide exhibits superior oxidative properties compared to chlorine, thereby facili tating more effective oxidation of pharmaceutical compounds. Studies have demonstrated that chlorine dioxide can achieve comprehensive eradication of phenazone, diclofenac, dimethylaminophenazone, and propiphenazone. The removal efficacy of gemfibrozil,

cyclophosphamide, and glibenclamide was found to be negligible, indicating minimal elimination of these pharmaceutical compounds. Furthermore, no removal was observed for ibuprofen, bizafibrate, diazepam, and carbamazepine, suggesting their persistence in the system. Nevertheless, carbamazepine exhibited a noteworthy reduction when subjected to ozonation treatment.

Toxic degradation products point of view, when certain pharmaceuticals such as sulfamethoxazole, metoprolol, and acetaminophen are subjected to chlorination, they can generate chloramines, which are considered toxic. On the other hand, diclofenac, when chlorinated, forms several non-chloramine byproducts. Additionally, chlorination of acetaminophen can give rise to the formation of highly toxic compounds such as N-acetyl-p-benzoquinone imine and 1,4-benzoquinone (Bedner & MacCrehan, 2006). These findings highlight the importance of considering the potential formation of toxic byproducts during chlorination treatment of pharmaceutical compounds. This is an important concern for the experimental part of the thesis related to the toxicity of the degradation products generated during the drinking water treatment processes.

2.2.3. Advanced Oxidation Processes

Advanced oxidation processes (AOPs) are defined as oxidative water treatment processes that take place under pressure and temperature conditions similar to the environment. These processes involve the generation of a significant amount of hydroxyl radicals (·OH), which are then able to react with the organic compounds present in the medium (Glaze & Kang, 1989).

The application potential of advanced oxidation processes (AOPs) is significant, primarily due to the high reactivity with 2.80 V reduction potential and low selectivity of hydroxyl (·OH) radicals. However, it is important to consider the presence of natural organic matter and the low concentrations of pharmaceutical compounds when utilizing these treatments, as ·OH radicals have the ability to oxidize both PCs and the natural organic matter. AOPs encompass a range of catalytic and non-catalytic processes that utilize the strong oxidizing capacity of hydroxyl radicals (·OH). These processes differ in how the hydroxyl radical is generated. They rely on the in situ production of hydroxyl radicals, which exhibit rapid reactivity with most organic compounds (Haag & Yao, 1992). This radical is generated in sufficient quantities to effectively interact with organic compounds (Brillas et al., 2009).

AOPs can be classified based on the source of hydroxyl radical as shown below:

- Photolysis: UV light
- Ozone based processes: O_3, O_3/UV, O_3/ H_2O_2, O_3/ H_2O_2 /UV
- H_2O_2-based processes: H_2O_2/UV, H_2O_2/ Fe^{2+}
- Heterogeneous photocatalytical processes :TiO_2/UV, TiO_2/UV/ H_2O_2Sonochemical oxidation,
- Electrochemical oxidation

2.2.2.1. Ozonation. The ozonation process is the most effective method used in the oxidation of organic substances in drinking water. The oxidation mechanism of ozone is a complex process that occurs through two pathways: direct reaction with dissolved ozone (O_3) and indirect oxidation through the formation of radicals (·OH). The prevalence of these mechanisms during the degradation of a compound depends on various factors, including the nature of the contaminant, ozone dosage, and pH of the medium. The rate of ozone decomposition depends also on the amount of natural organic matter and alkalinity in the water. Carbonate and bicarbonate ions inhibit the decomposition of ozone. While natural organic matter increases the decomposition of ozone, it also causes the depletion of hydroxyl radicals (von Gunten, 2003). Typically, under acidic conditions (pH < 4), direct ozonation is the dominant process, as described by the below reaction (2.3):

$$3O_3 + OH^- + H^+ \rightarrow 2OH + 4O_2 \quad (2.3)$$

In contrast, at pH values greater than 9, the indirect pathway becomes the predominant mechanism. Generally, in ozonation processes, degradation rates increase with higher pH levels, as elevated pH promotes the decomposition of ozone into free radicals, as shown below (2.4):

$$O_3 + OH^- \rightarrow O_2 + HO_2^- \quad (2.4)$$

Additionally, other chemical reactions involved in the indirect oxidation with ozone include (2.5-2.9):

$$O_3 + HO_2^- \rightarrow HO_3 + O_2^- \quad (2.5)$$

$$HO_2 \rightarrow H^+ + O_2^- \quad (2.6)$$

$$O_2^- + O_3 \rightarrow O_2 + O_3^- \quad (2.7)$$

$$O_3^- + H^+ \rightarrow HO_3 \quad (2.8)$$

$$HO_3 \rightarrow \cdot OH + O_2 \quad (2.9)$$

Under alkaline conditions, an additional fast side-reaction needs to be considered (2.10):

$$HO + O_3 \rightarrow HO_2\cdot + O_2 \quad (2.10)$$

This side-reaction leads to the rapid formation of hydroperoxyl radicals (E◦ = 1.65 V) at the expense of hydroxyl radicals (E◦ = 2.80 V), resulting in a reduction in the overall oxidation capacity (Ternes et al., 2003). The oxidation potential of molecular ozone is 2.07 V. Molecular ozone reactions are selective between ozone (O_3) and organic molecules containing nucleophilic moieties like carbon-carbon double bonds, aromatic rings, and functional groups with sulfur, phosphorus, nitrogen, and oxygen atoms. In general, electron-donating groups enhance the oxidation by ozone whereas electron-withdrawing groups reduce the reaction rates.

Therefore, the primary targets of ozone attack are compounds containing double bonds, such as -C = C- or -N = N- bonds, and aromatic rings substituted with electron-donating groups such as OH and NH_2. Conversely, aromatic molecules substituted with electron-withdrawing groups such as COOH, NO_2 and Cl show lower reactivity towards ozone (Langlais et al., 1991; von Gunten, 2003). On the other hand, hydroxyl radicals exhibit non-selective reactivity towards various organic and inorganic compounds, involving hydrogen abstraction, electrophilic addition, and electron transfer reactions. Ultimately, these reactions lead to the complete mineralization of organic compounds depending on the process conditions (Oppenlaender, 2003). Various operational parameters significantly affect the formation of ozone and its subsequent transformation into hydroxyl radicals (·OH). These parameters include the chemical structure and concentration of the pollutant, the quality of the effluent, pH (as mentioned earlier), and temperature. It is crucial to consider these factors when optimizing ozone-based oxidation processes.

Typically, when the ozonation process utilizes low ozone doses, it does not result in complete elimination or mineralization of organic pharmaceutical compuınds. Instead, partially oxidized molecules known as degradation or transformation products such as organic acids, aldehydes, and ketones and more complex derivatives are formed during this process. These degradations products may possess higher toxicity than their parent compounds. In a specific study, researchers investigated 39 emerging organic contaminants during ozonation and identified 227 degradation products. Surprisingly, 87% of these degradation products were even more resistant to biodegradation compared to the original compounds (Gulde et al., 2021). However, these partially oxidized molecules can be completely eliminated through mineralization when higher excess of ozone is applied (Chu, 2000).

Another important aspect to consider is the need for proper mass transfer during ozone-based oxidation. It is crucial to ensure the transfer of ozone molecules from the gas phase to the liquid

phase to enable the attack on the chemical bonds of organic molecules to occur. This highlights the importance of efficient gas-liquid mass transfer in ozone-based oxidation processes.

Many scientific studies have demonstrated that a variety of pharmaceutical compounds, including antibiotics such as Amoxycillin, Clarithromycin, Flumequin can be eliminated at rates ranging from 90% to 100% through the ozone-based ozonation method (Huber et al., 2005; Lange et al., 2006; H. Wang et al., 2019). Beta-blockers such as bisoprolol, salbutamol and betaxalol can also be completely removed by ozonation in drinking water. These compounds contain secondary amino groups and weak aromatic rings that are likely reactive sites from ozonation. In addition, propranolol, metoprolol, sotalol, atenolol and acebutolol beta blokers are more resistant to ozonation due to their specific functional groups (Huerta-Fontela et al., 2011). In a study conducted by (Abellán et al., 2008), pathways for the degradation of sulfamethoxazole were proposed based on the molecular identification of degradation products of sulfamethoxazole by LC/MS and MS^n in Figure 2.6. In the present study, a thorough degradation of sulfamethoxazole was accomplished within a 10-minute reaction time. Ozone primarily targets sulfamethoxazole through the amine group of the aniline ring, resulting in the formation of nitro-aromatic compounds in certain instances.

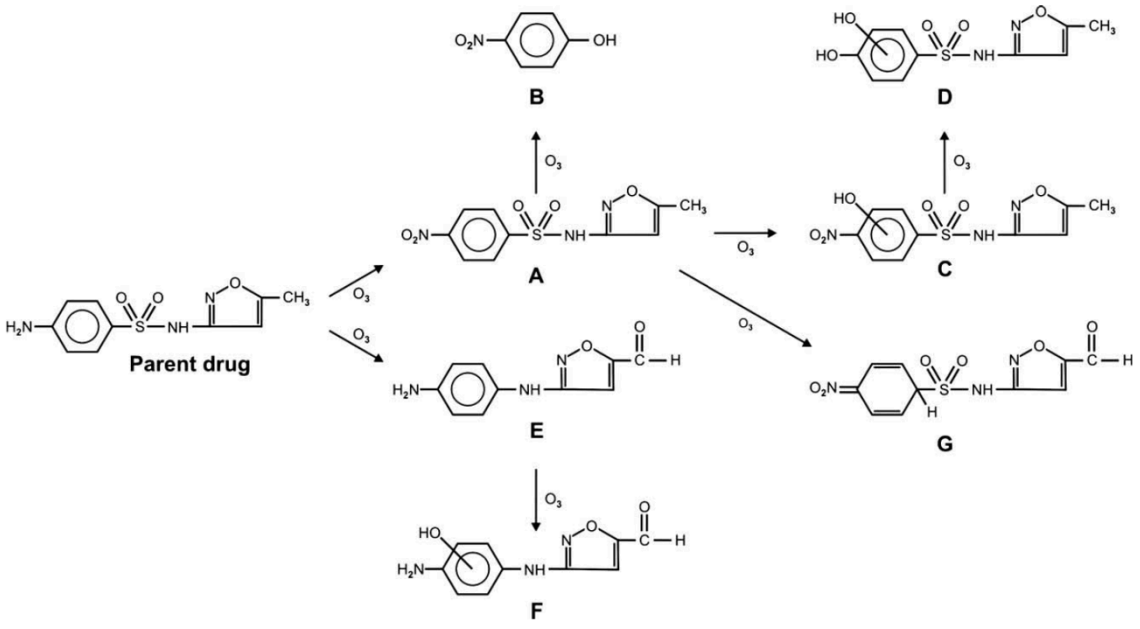


Figure 2. 6. Proposed degradation pathways for sulfamethoxazole during ozonation

In another study performed by Dwivedi and colleagues in 2018, three transformation products of carbamazepine (CBZ) after ozonation were identified and a pathway was proposed in Figure 2.7. (Dwivedi et al., 2018). Ozone exhibits two distinct mechanisms for reacting with CBZ (Fig. 2; I): direct attack by ozone (O_3) or indirect attack via the formation of hydroxyl radicals (·OH).

However, when ozone is present in excess, it primarily acts through direct attack on the C4 = C5 position of the CBZ ring. This results in the formation of firstly highly unstable intermediates and then followed by rearrangement and transformation into stable products at the end which were identified as BQM, BQD, and BaQD corresponding to the products VI, VII, and VIII, respectively.

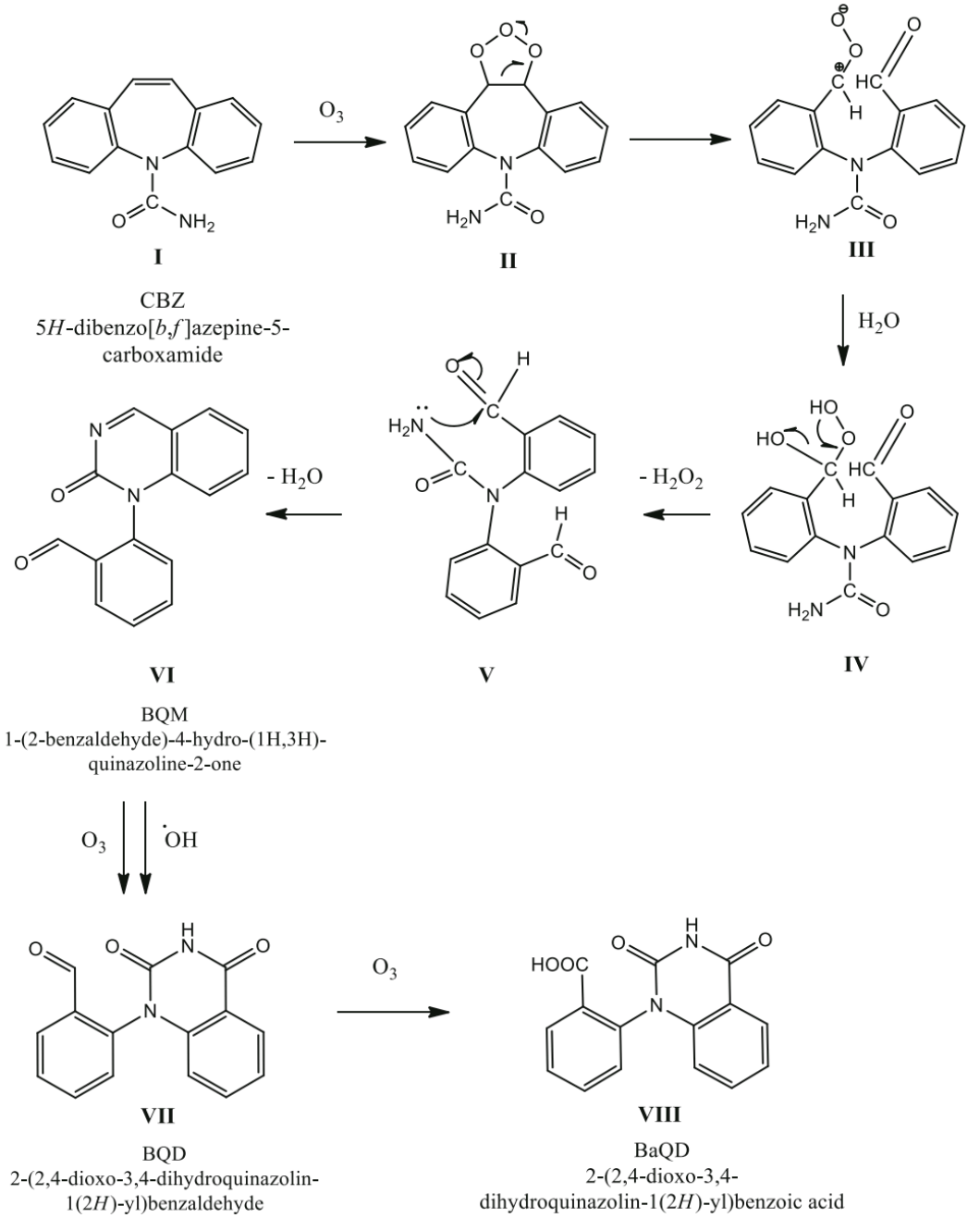


Figure 2. 7. Proposed degradation pathway of CBZ after ozone treatment

Figure 2.8. shows the degradation product of Clarithromycin-N-oxide where clarithromycin reacts at its dimethylamino group and yields the corresponding N-oxide that does not possess the essential basic properties of the tertiary -NH_2 group necessary for binding. As a result clarithromycin-N-oxide is no longer biologically active (Sein et al., 2009).

Due to the highly fast reaction between the CBZ molecule and ozone, the complete elimination of CBZ can be achieved even at very low ozone doses.

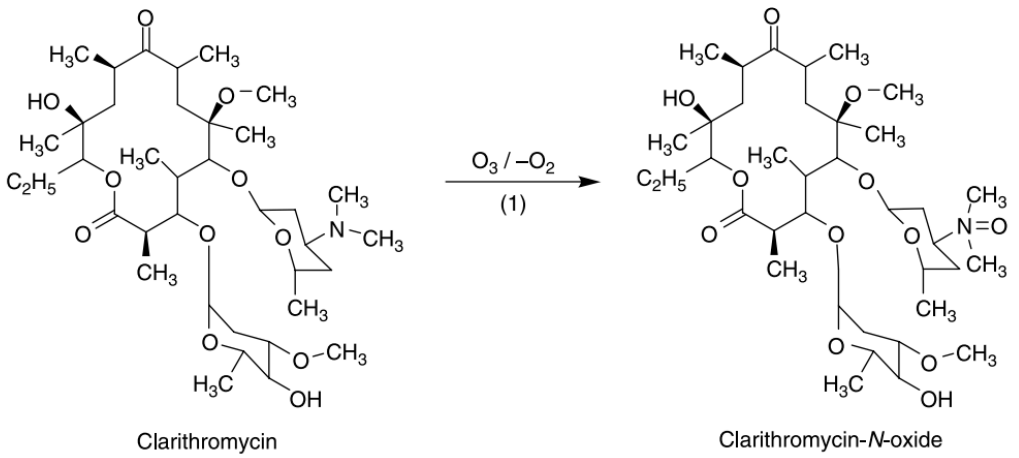


Figure 2. 8. Degradation product of Clarithromycin

In general the ozonation process can be enhanced by combining with H_2O_2 and/or UV (O_3/UV, O_3/H_2O_2 or O_3/H_2O_2 /UV processes).

2.2.2.2. Other Advanced Processes.

Activated Carbon. Activated carbon adsorption is a widely used drinking water treatment process for treating drinking water especially in Western Europe. It effectively eliminates many PCs, but some, particularly polar compounds which have low adsorption affinity are not easily removed by activated carbon (Lipp et al., 2010). In drinking water treatment plants, this process is typically implemented through granular activated carbon (GAC) filter beds. Natural water sources often contain a variety of organic substances and different compounds, leading to a competitive adsorption scenario on the surface of GAC (Newcombe & Drikas, 1997). Therefore, it is important to note that studies demonstrating pharmaceutical's adsorption on activated carbon surfaces in distilled water under laboratory conditions may yield different results compared to those observed in actual drinking water treatment plants. A study conducted on a real-scale drinking water treatment plant, incorporating advanced treatment processes, investigated the removal of pharmaceutical and personal care products (PCs). The findings revealed that acebutolol, diazepam, and diltiazem, despite their hydrophilic nature, were completely removed in the GAC filters situated after the ozonation unit. Furthermore, hydrochlorothiazide, carbamazepinepoxide, irbesartan, valsartan, phenytoin, and venlafaxin compounds exhibited a removal efficiency exceeding 75% (Perrich, 2018).

Membrane filtration. This technology has emerged as a promising water treatment method in recent years. In membrane filtration, pollutants are separated from water based on their molecular size and interactions with the membrane surface. In certain cases, organic pollutants adhere to the membrane surface or other particles in the water, leading to their retention by the membrane filter. The effective removal of pharmaceuticals by membrane filters relies on factors such as molecular size, charge, and hydrophobicity. Microfiltration and ultrafiltration, which operate at low pressure, generally remove pharmaceuticals by adsorption onto colloids or particles present in the water. However, the removal of steroid hormones, acidic drugs, and beta-blockers is typically ineffective using these membrane filters. On the other hand, membranes such as reverse osmosis and nanofiltration exhibit high efficiency in removing pharmaceuticals due to their small pore diameters. Nanofiltration is a membrane separation process that utilizes a pressure gradient as the driving force with a pore size on the scale of 1 nm, which corresponds to a molecular weight cut-off typically ranging from 100 to 5000 Da (Licona et al., 2018). Whereas Reverse Osmosis membranes have much smaller pore sizes, typically around 0.1 nm, and the molecular weight cutoff of reverse osmosis membranes is usually below 100 Da, effectively blocking the passage of almost all ions, and larger organic molecules. These membranes are highly selective, producing high-quality, low total dissolved solid water by rejecting the majority of contaminants including larger drug molecules present in the feed water (Foureaux et al., 2019). In a study to investigate the removal of 20 pharmaceuticals from surface waters using the nanofiltration method results revealed that the removal efficiency of pharmaceuticals in nanofiltration is influenced by the charge of the compound (Verliefde et al., 2007). Positively charged compounds exhibited low rejection values, whereas negatively charged compounds showed high rejection values. Neutral compounds demonstrated moderate removal rates. Another study conducted by Yoon et al. in 2006 reported that the retention of pharmaceuticals in nanofiltration is influenced by both the hydrophobicity and particle size of the compound (Yoon et al., 2006). In contrast, in ultrafiltration, the retention of pharmaceuticals is primarily determined by their hydrophobicity.

While, these advanced processes offer only a marginal enhancement in removal efficiency when compared to ozone treatment (Patel et al., 2019), they do not generate any degradation or transformation products in the treated water.

2.2.4. Water Management in Istanbul

The water management in Istanbul aims to provide safe and sufficient amount of drinking and utility water, to treat wastewater to minimize environmental impact, and to protect water resources and drinking water basins. According to the 2022 Istanbul Water and Sewerage Administration (ISKI) annual activity report (ISKI Report, 2022), ISKI supplies an average of 3 million cubic meters of water per day, equivalent to 1.1 billion cubic meters of water per year, to 16 million Istanbul residents through 24 drinking water treatment plants. Total capacity of the drinking water treatment plants of Istanbul is 5 Mio m^3 /day.

57.5% of the produced water is obtained from reservoirs and catchment areas, while the remaining 42% is sourced from regulators and wells. All of the drinking water treatment plants withdraw their raw water from surface waters, except Danamandıra, İhsaniye, and Hallaçlı plants in Büyükçekmece basin where the raw water is withdrawn from groundwater wells' whose depth is between 80 to 100 meters below ground level. Water sources and transmission lines are located within the boundaries of six provinces (Istanbul - Kırklareli - Tekirdağ - Kocaeli - Sakarya - Düzce). 60% of water resources are on the Asian Side, while 40% are on the European Side. In contrast, 60% of the population resides on the European Side, and 40% on the Asian Side. Water reservoirs have been constructed in various regions of Istanbul to balance water consumption, save energy, and minimize water interruptions caused by failures and power outages.

Raw water obtained from water sources is treated in drinking water treatment plants and delivered to end users. Water transmission and distribution are structured in two parts. The first part involves the transmission of raw water and treated water through pipes with a diameter of Ø400 mm or larger, water reservoirs, and booster (pumping) stations. The second part involves the distribution of water to end users through network pipes with a diameter smaller than Ø400 mm.

Water is distributed to a large part of the European Side from Kâğıthane, İkitelli, and Büyükçekmece, while it is distributed to the Asian Side from Ömerli and Elmalı facilities. With the operation of the Cumhuriyet Water Treatment Plant located on the Asian Side, raw water from the Melen River is treated and transferred to the European side through the Boğaz Kuzey Tunnel. Some of the treated water produced at the Ömerli Drinking Water Treatment Plant is conveyed to Fatih, Zeytinburnu, Bakırköy, and Bahçelievler districts on the European side through the existing Salacak-Sarayburnu Boğaz Güney Conveyance Line. The accumulated waters in water sources are transported to drinking water treatment plants through transmission lines which consist of 2,825 km

of steel and ductile iron pipes and 227 km of other pipes, tunnels, channels, and galleries. The hilly geography of Istanbul and the long-distance transport of water to the city result in high energy demand. Booster systems are installed at every stage of the drinking water pipelines to ensure pressurized water from the source to the taps. Raw waters in drinking water sources reach the drinking water treatment plants through transmission lines for the necessary treatment processes. Except Büyükçekmeçe and Cumhuriyet plants, ozonation system is used in most of the large plants for drinking water treatment to meet drinkable standards. After the treatment process, water reaches Istanbul residents through transmission lines and the drinking water distribution system. Ductile iron pipes are used in the drinking water distribution network in Istanbul (ISKI, 2023)

The drinking water treatment plants of ISKI primarily utilize either the conventional drinking water treatment process or the ozonation-assisted conventional drinking water treatment process. The sample flow chart for the conventional drinking water treatment process is presented in Figure 2.9 while Figure 2.10 depicts the flow chart for the ozonation-assisted drinking water treatment process. In order to remove the off-taste and odor that occur in the tap water as a result of the serious decrease in water levels in the water sources supplying the drinking water treatment plants in Istanbul or particularly during the summer months due to algal blooms, powder activated carbon is used when necessary during the treatment process.

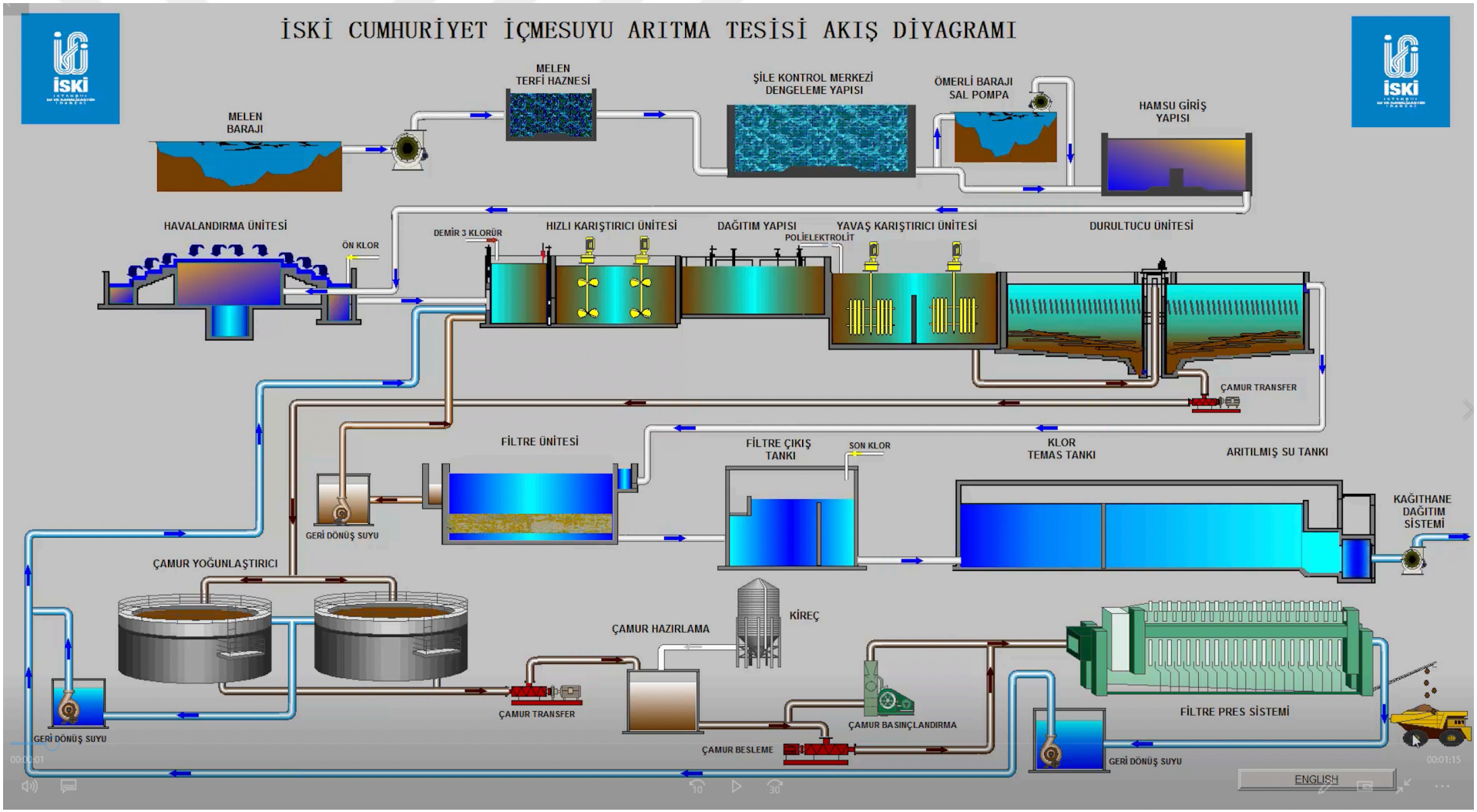


Figure 2. 9. Flow chart of ISKI's conventional drinking water treatment process (Cumhuriyet Plant) (Courtesy of ISKI)

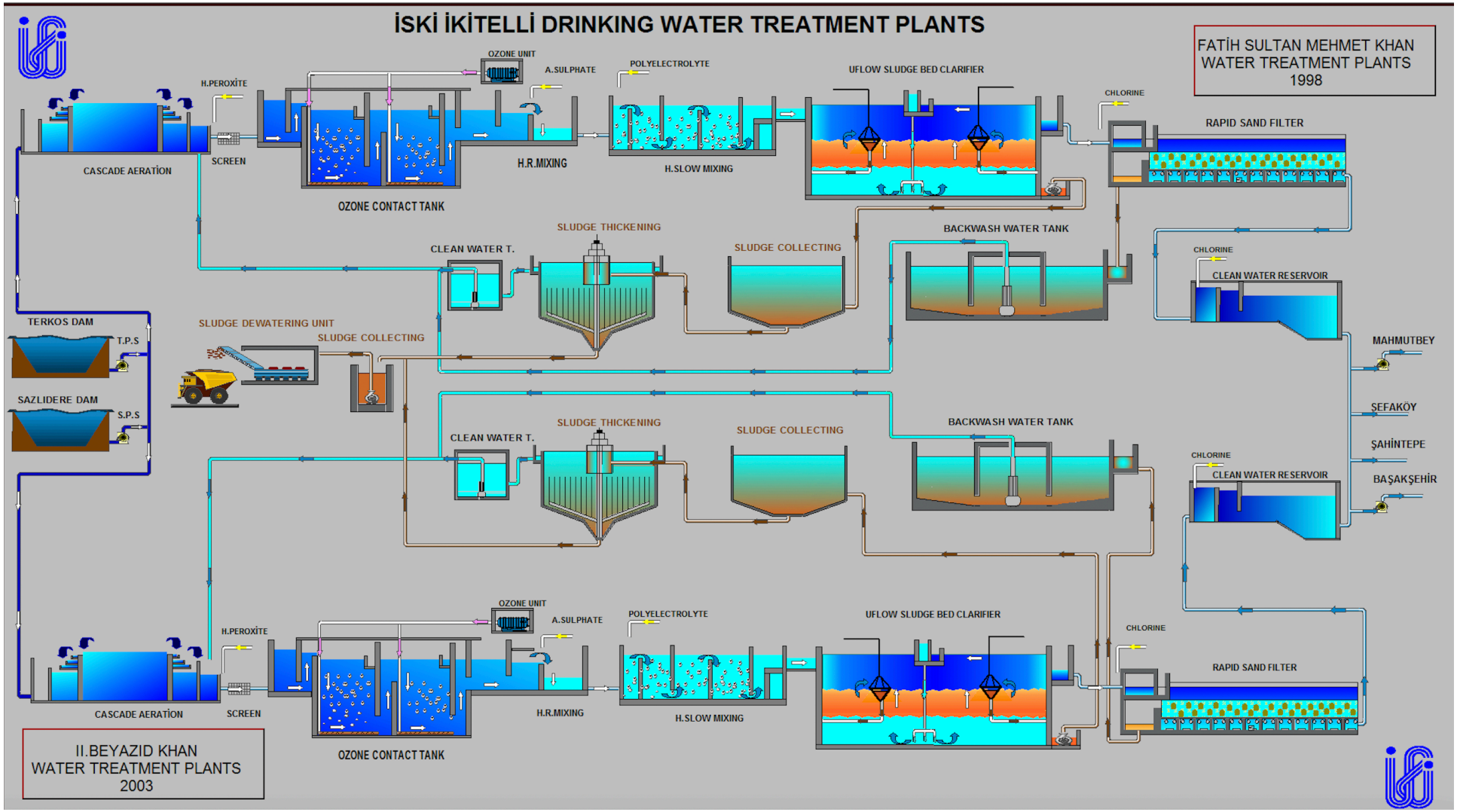


Figure 2. 10. Flow chart of ISKI's conventional drinking water treatment process with ozonation (İkitelli Plant) (Courtesy of ISKI)

Regarding waste water, in Istanbul's 90 wastewater treatment plants, an average of 4.1 million cubic meters of wastewater is treated daily, amounting to 1.48 billion cubic meters per year. Only 40% of the wastewater undergoes biological and advanced biological treatment, while 60% is subjected to physical treatment only. All treated wastewater is discharged into the sea through deep-sea outfalls. In advanced biological treatment plants, water that has been treated and reclaimed for nutrients such as carbon (C), phosphorus (P), and nitrogen (N) is utilized for irrigation and industrial purposes, accounting for 2% of the total treated water.

2.3. Analysis of PCs in Drinking Water

The quantification of micro pollutants, particularly pharmaceutical compounds in water samples, poses a major challenge in environmental analysis due to the need for high levels of sensitivity, selectivity, efficiency, and of course reliability. Due to its good selectivity and sensitivity, as well as its ability to detect a wide range of polar and nonpolar substances, liquid chromatography coupled with tandem mass spectrometry, triple-quadrupole and quadrupole-time of flight instruments are nowadays the method of choice for the ultra trace multi component screening and identification of organic microcontaminants including pharmaceutical compounds in water (Lambropoulou & Nollet L.M.L, 2014). In combination with analyte enrichment using solid-phase extraction (SPE) on polymer-functionalized adsorbent materials, organic compounds can generally be quantified down to the low ng/L range. Despite the advantages of LC-MS/MS, the method has its limitations. In practice, Triple-Quadrupole devices are predominantly used. This device technology can only capture one ion transition at a time in the selected reaction monitoring (SRM) mode. While this has the advantage of obtaining a signal with high intensity, it requires performing multiple experiments consecutively when measuring multiple compounds (Khan et al., 2012).

Electrospray ionization and atmospheric pressure chemical ionization are commonly used ionization methods in LC-MS analysis due to the high polarity of pharmaceutical molecules. These methods allow for the direct ionization of highly polar compounds in aqueous samples, making them suitable for the analysis of pharmaceuticals. In pharmaceutical analyses in water samples, the signal intensity of analytes can be significantly reduced due to matrix effects, leading to lower limits of detection. To address this, chelating agents can be utilized during sample preparation to further eliminate interfering matrix chemicals, resulting in improved analytical outcomes. LC analysis detectors frequently employed for pharmaceuticals include time-of-flight mass spectrometry, mass spectrometry, Mass Spec/Mass Spec (MS/MS), ultraviolet, and fluorescence techniques .

In a multicomponent method, the challenge lies in being able to co-concentrate neutral, cationic, and anionic molecules, which can differ greatly in terms of their substance properties such as molecular size and polarity, by a factor of 1000 in one enrichment step (Hernández et al., 2005).

Sample concentration and clean-up play crucial roles in the analysis of micropollutants in water. However, the enrichment steps involved are labor-intensive and time-consuming, especially when dealing with large volumes of samples. To tackle this issue, an automated on-line solid-phase extraction (SPE) system coupled to a liquid chromatography (LC) instrument through a column-switching system can be employed. This approach offers several advantages over conventional techniques, including faster analysis, enhanced efficiency and improvements in method selectivity, specificity, and reproducibility by eliminating time-consuming evaporation and reconstitution steps, as well as minimizing the need for manual sample handling. This shift can be attributed to the need for handling low analyte concentrations commonly encountered in environmental samples. The concentration factor, which typically ranges from 100 to 2000, is a critical parameter in these methods (Patel et al., 2019). In addition to LC separation, ESI ionization, and mass spectrometric detection, the enrichment of analytes also has a significant impact on the overall method's performance (Fontanals et al., 2007). On-line SPE brings about significant benefits such as high sample throughput, reduced solvent usage, shorter sample preparation times, and the ability to work with smaller sample volumes. Notably, in one instance, an on-line method was demonstrated to be 16 times more economical than an alternative off-line method (Trenholm et al., 2009). SPE allows for simultaneous extraction and clean-up. Different sorbents, including ion-exchange, C-18, non-polar, and polymeric phases, have been employed in SPE cartridges for the extraction and clean-up of target pharmacuetical compounds (Batt & Aga, 2005; Stolker et al., 2004). Waters Oasis HLB and Oasis MCX sorbents have also been used for preconcentration of both polar and non-polar compounds.

Another significant advancement in the field is the widespread use of high-resolution mass spectrometry (HRMS) systems, which have greatly facilitated the identification of transformation/degradation products of organic pollutants and enhanced the screening capabilities for unknown substances. This development represents a substantial leap forward in analytical capabilities and has opened up new possibilities in environmental and chemical analysis (Leendert et al., 2015).

To determine the identity of unknown compounds in mass spectrometry analysis, two common strategies are employed, depending on the available instrumentation. These strategies include:

i-Structural information gained from tandem MS (MS2) experiments: In these experiments, the compound of interest is subjected to fragmentation, and the resulting fragmentation patterns are analyzed to provide structural insights, aiding in the identification of the unknown compound.

ii-Highly accurate molecular mass measurements: Another approach involves precise measurement of the molecular mass of the compound. This information can be obtained using instruments capable of accurate mass determination, which helps confirm the presence of the compound and provides clues about its elemental composition.

The utilization of MS^n spectra greatly facilitates the elucidation of the fragmentation mechanism for unidentified species and enhances the level of certainty in assigning a specific structure. This multi-stage fragmentation technique has demonstrated itself to be one of the most potent methods for investigating suspected or unknown transformation products. The precise determination of accurate mass, as provided by Time-of-Flight (ToF) instruments, enables the acquisition of specific information pertaining to a particular molecule. This capability facilitates nearly indisputable confirmation of the compound's identity (Kosjek & Heath, 2008).

2.4. Fate of Pharmaceutical Compounds

Pharmaceutical compounds (PCs) are typical designed molecules for possessing chemical stability; however, they go through various physico-chemical and biotic transformations (Khetan & Collins, 2007). In recent years, there has been an increased understanding of the significance of not only PCs themselves but also the metabolites that arise from their structural modifications within the human body, treated animal organisms and the transformation products (Rahman et al., 2007). Transformation products refer to the compounds formed as a result of molecular structural changes that occur after excretion, specifically within the environment. These changes involve various biotic and abiotic processes, including those found in wastewater and drinking water treatment plants or photo transformation in the environment such as molecular degradation.

Consequently, a comprehensive understanding of pharmaceutical biodegradability, conjugation, deconjugation, metabolic pathways, persistence, and sorption is essential to predict their fate in the environment (Heberer, 2002a). Studies on pharmaceutical stability indicate that compounds with high stability tend to exhibit relatively long persistence under environmental conditions. On the other hand, pharmaceutical metabolites generated through processes such as oxidation, reduction, and hydrolysis can often be more prone to subsequent transformations, leading to diminished environmental stabilities .

Fate of pharmaceutical compounds can be classified in two parts.

2.4.1. Fate of Pharmaceutical Compounds Before Entering the Environment

Following administration, PCs undergo a series of processes in the body, including absorption, distribution, metabolism, and excretion. To ensure safe usage, most modern pharmaceuticals are designed to undergo metabolism in organs like the liver and kidney after achieving the desired pharmacological effects. Pharmaceuticals generally undergo enzymatic transformations within various body tissues, including the liver, intestine, kidney, and lung. The metabolism of pharmaceuticals by the body varies according to the type of pharmaceutical (Halling-Sørensen et al., 1998). The excess pharmaceutical molecules and xenobiotics substances are metabolized by the body as part of a detoxification and elimination process. The liver is primarily responsible for the majority of drug metabolism. During metabolism, drugs are converted into more polar metabolites at different levels within the body. This transformation reduces or eliminates the pharmaceutical activity of the original active substance (Silverman, 1992)(Silverman, 1992). Certain pharmacueticals undergo extensive metabolism before they are excreted, while others undergo moderate or poor metabolism. In contrast, substances like contrast media are excreted from the body without undergoing significant metabolism, remaining largely intact.

As a consequence of metabolism, the resulting metabolites exhibit distinct pharmacological and toxicological properties when compared to their parent PCs. Typically, it is postulated that the metabolism of pharmacological compounds leads to a reduction in toxicity. However, there are instances where the metabolic processes result in the generation of metabolites that possess enhanced toxicological activity compared to the parent compounds. (Lienert et al., 2007). In the organisms of humans and animals drug metabolism generally initiates with various biochemical reactions, such as oxidation, reduction, hydrolysis, hydroxylation and epoxidation which introduce or unmask functional groups (phase I transformation). Subsequently, highly polar endogenous molecules like glucuronic acid, sulfate, and amino acids bind to drugs or metabolites from phase I transformation, generating conjugates (phase II transformation). These conjugates are water-soluble and can be readily excreted in urine or bile, potentially leading to an environmental exposure. Phase I transformations result in the formation of metabolites that are often more reactive, and in some cases, more toxic than the parent drug molecule by the introduction of a functional group such as -OH, -SH, epoxide, -NH_2, or -COOH. This typically leads to a modest increase in hydrophobicity as well. In Phase II, conjugation reactions involve the attachment of a polar molecule readily available

in vivo to a susceptible functional group which generally leads to the formation of inactive substances. This results in the formation of O- and N-glucuronides, sulfates and acetate esters, carboxamides, and glutathionyl adducts. These conjugated metabolites exhibit greater hydrophilicity compared to the unconjugated form (Kalgutkar et al., 2002).

2.4.2. Fate of Pharmaceutical Compounds in the Environment

Biotic and abiotic factors influence the fate of pharmaceutical compounds in the environment. The attenuation of the pharmaceuticals in aquatic environment is primarily governed by biodegradation, photolytic degradation, and sorption processes where in addition to the parent active compounds and their metabolites, additional compounds can also be formed after the excretion of these compounds into the environment (Barra Caracciolo et al., 2015).

The primary fate processes of pharmaceutical compounds in different environmental compartments involve sorption (e.g., tetracyclines and quinolones) and bio-degradation. Various processes such as photolytic oxidation (e.g. quinolones and sulfonamides), hydrolysis (β -lactams) or biotic changes can also play a significant role in certain cases. When pharmaceuticals are released into the environment, they can undergo various reactions that result in partial or complete transformation and/or degradation of the parent compound. However, there are instances where total degradation is halted, leading to the generation of intermediates that may be even more stable than the parent compounds. These intermediates can exhibit higher toxicity compared to the parent compound and pose a greater potential risk to the environment and the human health, such as ibuprofen and its degradation products (Jan-Roblero & Cruz-Maya, 2023), and carbamazepine and its degradation products (Pohl et al., 2020).

Bacteria and fungi are the primary groups of microorganisms responsible for degrading organic compounds. Therefore, in waste water treatment plants, as well as in surface water, groundwater, and marine environments, bacterial populations are presumed to be primarily responsible for most biodegradation processes. For example, in a study under denitrification conditions in water/sediment batch reactors, sulfamethoxazole underwent biotransformation, leading to the formation of its nitro and desamino derivatives (Osorio et al., 2016). The presence of pharmaceuticals in aquatic environments serves as evidence of their incomplete degradation and elimination during waste water treatment (Kümmerer, 2009). Limited knowledge exists regarding the occurrence, fate, or activity of pharmaceutical metabolites in the environment.

2.4.3. Fate of Pharmaceutical Compunds in Drinking Water Treatment Plants

This topic has been extensively discussed in the subchapter "2.2. Removal of pharmaceutical compounds in drinking water treatment processes", providing detailed insights.

2.4.4. Effect of Physico-chemical Properties of Pharmaceutical Compounds

PCs exhibit significant variations in their physicochemical properties, resulting in diverse behavior patterns. Factors such as water solubility, hydrophobicity, volatility, and more contribute to their fate in aquatic environments. Dissociation constants (pK_a), solid-water distribution coefficients (log K_d), organic-carbon based sorption coefficients (log K_{oc}), octanol-water partition coefficients (log K_{ow}), and Henry's coefficient (K_H) play crucial roles in determining the fate of pharmaceuticals by influencing processes such as sorption, partitioning, hydrolysis, photodegradation, biodegradation and volatilisation (Lapworth et al., 2012b; Pal et al., 2010; Radjenovic et al., 2007; Yamamoto et al., 2009).

Pharmaceutical compounds commonly possess either basic or acidic functional groups, resulting in their ability to exist in neutral, ionic, or zwitterionic forms under environmental conditions. This multifaceted behavior further complicates their environmental dynamics and interactions.

2.5. Toxicity of the PCs and Their Degradation Products in Drinking Water Treatment Processes

Pharmaceuticals have been developed to target specific metabolic and molecular pathways or cellular/subcellular structures (receptors, channels, enzymes etc.) in humans and animals, however, they may have also unintended and undesired side effects, i.e. toxic effects. When released into the environment, pharmaceuticals may affect similar or identical target organs, tissues, cells or biomolecules in animals. Although the concentrations of these compounds in treated drinking water are typically deemed to be too low to pose a direct risk to human health, there is a rising apprehension regarding the formation of degradation or disinfection by-products during drinking water treatment. In certain instances, these DBPs have been found to exhibit higher levels of toxicity compared to the original pharmaceutical compounds, giving rise to additional concerns (Postigo & Richardson, 2014). For example, it has been observed that the toxicity of photodegradation products of naproxen is higher than toxicity of the parent molecule (Isidori et al., 2005). It is largely unknown that what type of products can be formed upon water treatment; i.e.

ozonation and chlorination from many pharmaceutical compounds as well as what type of and to what extent they may produce adverse effects in humans. Next to the short term (acute) toxicity, there are limited knowledge regarding the long-term toxic effects of repeated exposure to drinking water contaminated with these pharmaceutical residues and/or their degradation products (Kimura et al., 2004). Another important group of PCs in water sources is antibiotics which have the potential to exert toxicity on aquatic organisms and can contribute to the proliferation of antimicrobial resistance (AMR) even at low concentrations that are below the lethal or inhibitory thresholds. This phenomenon poses a huge risk to human health (Sanseverino et al., 2022).

From human health point of view, both acute and long-term effects should be investigated. Assuming that an average person drinks 2 liters of water a day, the amount of pharmaceuticals -or their degradation products- that can be taken in the form of residues is far below the amount before treatment process. However, it is important taking these residual amounts continuously lifelong and to take them in the form of a cocktail of pharmaceuticals. Here, it is possible that the active substances, their metabolites and the degradation products/disinfection by-products in the form of a cocktail of pharmaceuticals can have a synergistic effect with each other. In addition, this situation is more important in infants and children, as well as in adults (Balcı et al., 2010). Therefore, risk assessment of pharmaceutical molecules taken in drinking water should be carried out either through the parent pharmaceutical or the degradation products found in treated water. For the parent molecules, since they are licensed pharmaceuticals, relevant data exists in the literature. As for the degradation products, currently, there is insufficient data on toxicity, exposure, dose-response, kinetics, etc in the literature, making it challenging to conduct a proper assessment at the moment.

2.5.1. Toxicity of the PCs and Their Transformation Products in Drinking Water

PCs containing activated aromatic systems or electron-rich functional groups in their molecular structure can undergo rapid reactions with disinfectants employed in drinking water treatment, resulting in the formation of various degradation products (Postigo & Richardson, 2014).

Previous sections have discussed the successful elimination of pharmaceutical compounds found in raw drinking water sources through drinking water treatment processes, particularly through advanced oxidation techniques. However, the true significance of this achievement for human health relies on the non-toxic nature of the degradation products formed during the treatment process, or whether their toxicity is lower compared to the parent pharmaceutical molecules. This

section focuses on the cytotoxicity of the degradation products related to three selected model molecules carbamazepine (CBZ), clarithromycin (CLA), and sulfamethoxazole (SMX), in human liver and kidney cells *in vitro*.

When N-containing compounds undergo ozonation, they can produce hydroxylamine, nitroalkanes, and N-oxides, primarily originating from tertiary amines. Research indicates that N-containing compounds are more likely to generate toxic degradation (transformation) products during ozonation, including clarithromycin, roxithromycin, levofloxacin, and tetracycline (Hübner et al., 2015). Hence, it is crucial to consider the potential variations in cytotoxicity during the ozonation of organic contaminants, particularly those containing nitrogen atoms.

Number of scientific publications related to the human toxicity of the degradation or transformation products of PCs during drinking water treatment is extremely limited in the literature.

2.5.1.1. Carbamazepine. In a study, the dose-response of CBZ and its chlorination, chloramination and ozonation products generated non-biologically on the viability of CHO-K1 cells was illustrated (Han et al., 2018). Figure 2.11. shows the results of this assay on a log scale. Interestingly, all three disinfection treatments exhibited greater cytotoxicity compared to CBZ alone. The sequence of cytotoxicity, ranked from the highest to the lowest, was found to be ozonated, chloraminated, and chlorinated samples. This study strongly suggested that authentic water treatment processes may also induce the formation of such hazardous oxidation-chlorination or chloramination products that may cause harm in exposed humans via drinking water. Therefore, investigating the potential cytotoxic effect of these products in cells from human origin, i.e. HepG2 and HK-2 in this thesis is justified.

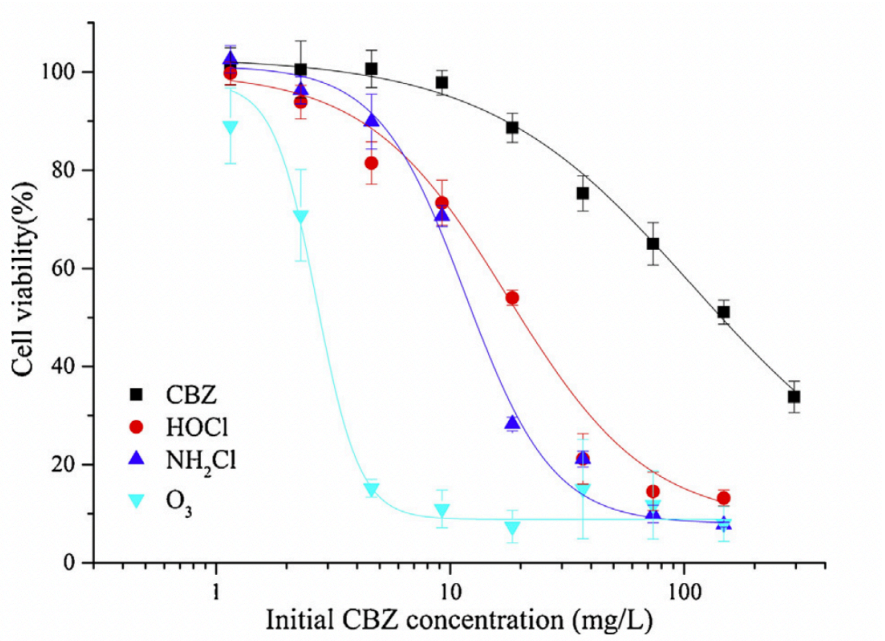


Figure 2. 11. The percentage of cell viability of CHO-K1 cells of Carbamazepine samples. The data presented are the mean values for triplicates±standard deviation.

2.5.1.2. Sulfamethoxazole. MTT assay results from the research of Yargeau and collegues in 2008 conclude that the degradation products of SMX does not adversely affect the proliferation of the HepG2 cells, hence do not induce cytotoxicity (Yargeau et al., 2008).

2.5.1.3. Clarithromycin. There is no published work regarding the (cyto)toxicity of clarithromycin degradation products formed in the drinking water treatment process.

2.6. Regulations

Regulatory point of view, the subject of water in Turkey is currently in a complex structure that involves the Ministry of Health, the Ministry of Agriculture and Forestry, the Ministry of Environment and Urbanization, and municipalities, leading to a confusion of responsibilities and authorities. The water legislation is also complex and lacks coherence in parallel with this structure. Therefore, in order to achieve effective, comprehensive, and sustainable management of water in terms of quantity, quality and safety based on a single authority, to regulate its legal nature through legislation, and to address new problems such as climate change, a draft "Water Law" has been prepared with the aim of complying with European Union legislation. It is believed that this law will be a significant step in achieving the comprehensive management of water that is needed in terms of both quantity and quality. On the other hand, the Turkish regulation on “Waters Intended

for Human Consumption", published in the Official Gazette with the number 25730 on February 17, 2005 (TDWR, 2005) , is not adopted with the recasted new European (EU) "Drinking Water Directive" entitled "On the Quality of Water Intended for Human Consumption" (EU Directive 2020/184, 2020). A watch list approach was also introduced in this new recasted EU "Drinking Water Directive" which aims at addressing emerging substances including pharmaceutical molecules of concern to the public or the scientific community on health grounds (Dettori et al., 2022)(Dettori et al., 2022).

The EU approaches the relationship between chemicals, environment, and public health as a whole, managing it through a comprehensive mechanism that encompasses strategy development and the establishment of limit values for compliance with pollutants. The European Council (EC) has approved conclusions regarding chemicals, providing political direction for the formulation of a sustainable EU chemicals policy strategy (Towards a Sustainable Chemicals Policy Strategy of the Union) (EU Conclusions 10713/19, 2019). Pharmaceuticals are one of the focus areas, and EC emphasizes the significance of expediting tangible and ambitious measures to mitigate the environmental risks associated with pharmaceuticals and their residues. This document highlights the crucial need to expedite concrete and ambitious measures aimed at reducing the environmental risk posed by pharmaceuticals and their residues. It also acknowledges that further research is necessary to gain a better understanding of the emerging impact on human health and the environment caused by pharmaceuticals and their residues. This means that significant changes are expected in the EU regulations concerning the residues of pharmaceutical compounds in waters and their relationship with public health in the coming years. During this transition, scientific studies will have an important role to play.

The main objective of EU water legislation is to safeguard human health and the environment by addressing the combined impacts of toxic and/or persistent pollutants. This objective is encompassed by the so called Water Framework Directive (EU Directive 2000/60/EC, 2000), along with its two subsidiary directives: the Ground Water Directive (EC Directive 2006/118/EC, 2006) and the Environmental Quality Standards Directive (EC Directive 2008/105). These directives specifically focus on the protection of groundwater and surface waters. They work in conjunction with other relevant water legislation, such as Directive (EU) 2020/184 (the Drinking Water Directive), Council Directive 91/271/EEC (the Urban Waste Water Treatment Directive), Directive 2008/56/EC (the Marine Strategy Framework Directive), Directive 2006/7/EC (the Bathing Water Directive), Directive 2007/60/EC (the Floods Directive), and Council Directive 91/676/EEC (the Nitrates Directive).

EU Water Framework Directive 2000/60/EC initially established a list of 33 priority substances to serve as control measures for a period of 20 years. In 2007, certain pharmaceuticals and personal care products such as diclofenac, carbamazepine, iopamidol, and musks were identified as potential future emerging priority candidates. Additionally, bisphenol A, phthalates, clofibric acid, ibuprofen, and triclosan were proposed as potential additions to this list. In 2018, the watch list was expanded to include compounds such as diclofenac, amoxicillin,erythromycin, venlafaxine, estrone, among others.

Although European and Turkish legislations are generally harmonized, this is a fundamental difference between Turkish and EU regulatory approaches. The backbone of European regulations consists of independent and scientific risk assessment and periodic monitoring studies. Following risk assessment, which evaluates risks in terms of both exposure and toxicity, the European Commission establishes regulations or directives to determine the limits that must be adhered to regarding pollutants.

3. MATERIALS AND METHODS

The experimental work consists of three phases namely; screening of pharmaceutical compounds (PCs) in before treatment (raw) and after treatment (treated) drinking water, simulation of the drinking water treatment process, the fate of PCs during drinking water treatment process, and testing cytotoxicity of the simulated after treatment water samples whether they may pose a health risk to human and ecological species in the environment.

3.1. Screening of PCs in Raw and Treated Drinking Water Samples

Screening of PCs study consists of sampling and testing.

3.1.1. Sampling

After receiving the official permit from ISKI (Istanbul Water and Sewerage Administration), 13 drinking water treatment plants located both in European and Asian sides of Istanbul were selected for the collection of raw water and treated water samples. Sample locations are listed below in Table 3.1. and Figure.3.1. To ensure sampling from the same water representativity, the sampling schedule was arranged considering the estimated hydraulic retention time of each drinking water treatment plant on the overall water treatment process capacity and corresponding daily mean quantities of treated water. Most of the tap water consumption (99%) was included in the sampling of raw and treated waters (ISKI Annual Report, 2022). In addition, tap water samples (13) were also collected from different regions of Istanbul . All samples were taken in the first half of March 2020. Samples were received into 1 L aluminum vessels which were prewashed with methanol and ultrapure water and kept refrigerated until they were transported to AGES-Austrian Agency for Food and Health laboratory in Linz, Austria where the analytical work was conducted in the second half of March 2020.

Table 3. 1. Sampling locations' details

Before Treatment (Raw)		After Treatment		Coordinates
Sample No.	**Location**	**Sample No.**	**Location**	
1	ÖMERLİ SU ARITMA MÜDÜRLÜĞÜ	2	ÖMERLİ, OSMANİYE - ORHANİYE - MURADİYE	N40°59'57.3828" E29°19'26.5476"
3	ÖMERLİ, EMİRLİ DRINKING WATER TREATMENT PLANT	4	ÖMERLİ, EMİRLİ	N40°59'55.3842" E29°19'51.6966"
5	İSAKÖY AĞVA PUMPING STATION	6	İSAKÖY AĞVA	N41° 6' 44.2152" E29° 49'35.2668"
9	KAĞITHANE WATER TREATMENT KAĞITHANE TERKOS	7	KAĞITHANE WATER TREATMENT-Y.Beyazıt Han	N41° 6' 22.6578" E28° 57' 49.8384"
		8	KAĞITHANE WATER TREATMENT-Ç.Mehmet Han	
10	CUMHURİYET WATER TREATMENT CUMHURİYET	12	CUMHURİYET WATER TREATMENT GENERAL OUTPUT	N41° 7' 25.593" E29° 16' 5.1918"
11	CUMHURİYET WATER TREATMENT ELMALI	13	CUMHURİYET ELMALI GENERAL OUTPUT	N41° 5' 1.4208" E29° 5' 43.767"
14	B.ÇEKMECE D.WATER TREAT.	19	B.ÇEKMECE GENERAL OUTPUT	N41° 3' 26.247" E28° 35' 24.8028"
15	B.ÇEKMECE İSKİ DANAMANDIRA PLANT	20	B.ÇEKMECE DANAMANDIRA GENERAL OUTPUT	N41° 19' 44.7312" E28° 13' 31.4826"
16	B.ÇEKMECE İSKİ YALIKÖY PACKAGE TREATMENT	21	B.ÇEKMECE YALIKÖY GENERAL OUTPUT	N41° 29' 33.1008" E28° 17' 15.4608"
17	B.ÇEKMECE İSKİ İHSANİYE PACKAGE TREATMENT	22	B.ÇEKMECE İHSANİYE GENERAL OUTPUT	N41° 15' 55.5942" E28° 21' 45.1794"
18	B.ÇEKMECE İSKİ HALLAÇLI PACKAGE TREATMENT	23	B.ÇEKMECE HALLAÇLI GENERAL OUTPUT	N41° 18' 0.7986" E28° 4' 58.4112"
24	İKİTELLİ FSMH	26	İKİTELLİ FSMH GENERAL OUTPUT	N41° 6' 23.0004" E28° 45' 54.6294"
25	İKİTELLİ TAŞOLUK TREATMENT PLANT	27	İKİTELLİ TAŞOLUK GENERAL OUTPUT	N41° 14' 9.9672" E28° 42' 1.2852"



Figure 3. 1. Sampling locations of water (before and after teatment) on the map

Table 3. 2. Sampling locations of tap water

Sample No.	Location	Adress
28	ÜMRANİYE	Elmalıkent Atatürk Cad.NO:36
29	KADIKÖY	Rasim Paşa Mah. Recaizade Sok No:29
30	ÜSKÜDAR	Halk Cad No:27
31	MALTEPE	Bağlarbaşı Mah.No:146
32	KARTAL	Atalar sispark karşısı
33	PENDİK	Sahil Balıkçılar Karşısı
34	SULTANBEYLİ	Hasan paşa cad.No:15
35	ÇEKMEKÖY	M.Akif Cad.No:29
36	BEYKOZ	Çiğdem mah.Karagözsırtı Cad.No:50
37	BAŞAKŞEHİR	Yan yol No:57
38	KAĞITHANE	Kemerburgaz Cad.No:45
39	SARIYER	F.S.M Mahallesi Evren paşa cad. No:62
40	BEŞİKTAŞ	Akgören sok No:8

3.1.2. Testing of Samples

Samples were analyzed for the selected antibiotics and other pharmaceutical compounds such as antiepileptics, analgesics, antihypertensives, and some other active compounds. The list of tested antibiotics and other pharmaceutical and active compounds is shown below in Table 3.3.

A total of 55 pharmaceuticals, both prescribed for human and veterinary purposes, were chosen based on their extensive usage, recognized long-lasting presence in the environment, and concerns by the scientific community. The selected pharmaceuticals consist of various antibiotic groups, including quinolones, lincosamides, diaminopyrimidines, sulfonamides, and tetracyclines.

Table 3.3. List of analyzed pharmaceutical compounds and their CAS numbers

Pharmaceutical Name	CAS No.	Pharmaceutical Name	CAS No.	Pharmaceutical Name	CAS No.
Acesulfam K	55589-62-3	Flumequine	42835-25-6	Sulfadiazine	68-35-9
Acetyl-sulfadimidine (=Acetyl-Sulfamethazine)	100-90-3	Gabapentin	60142-96-3	Sulfadimethoxine	122-11-2
Acetyl-Sulfamethoxazole	21312-10-7	Gabapentin D10	1126623-20-8	Sulfadimidine (= Sulfamethazine)	57-68-1
Amido-trizoic acid	117-96-4	Ioxaglin acid	5901764-64-0	Sulfadoxine	2447-57-6
Atrazine D5	163165-75-1	Irbesartan	138402-11-6	Sulfamerazine	127-79-7
Atrazine-desethyl D6	2733387-38-5	Josamycin	16846-24-5	Sulfamethoxazole	723-46-6
Atrazine-desisopropyl D5	1189961-78-1	Lamotrigine	84057-84-1	Sulfamethoxazole D4	1020719-86-1
Ciprofloxacin	85721-33-1	Lincomycin	154-21-2	Sulfamethoxypyridazine	80-35-3
Ciprofloxacin D8	1130050	Marbofloxacin	115550-35-1	Sulfamonomethoxine	1220-83-3
Clarithromycin	81103-11-9	Metformin-HCl	1115-70-4	Sulfaquinoxaline	59-40-5
Caffeine	58-08-2	Nalidixinic acid	389-08-2	Sulfathiazole	72-14-0
Danofloxacin	112398-08-0	Norfloxacin	70458-96-7	Sulfisoxazole	127-69-5
Danofloxacin D3	1217683-55-0	Oxolinic acid	14698-29-4	Telmisartan	144701-48-4
DEET	134-62-3	Paracetamol	103-90-2	Tramadol-HCl	36282-47-0
Difloxacin	91296-86-5	Propyphenazone	479-92-5	Trimethoprim	738-70-5
Enrofloxacin	93106-60-6	Roxithromycin	80214-83-1	Trimethoprim D3	1189923-38-3
Erythromycin	114-07-8	Sarafloxacin	98105-99-8	Tylosin	1401-69-0
Erythromycin C13 D3	959119-26-7	Spiramycin	8025-81-8	Valsartan	137862-53-4
Valsartan acid	164265-78-5				

3.1.3. Methods of Analysis

The analysis of pharmaceutical compounds (PCs) was conducted using an online solid-phase extraction (SPE)–high-performance liquid chromatography (HPLC)–high-resolution mass spectrometry (HRMS) method, commonly referred to as the online SPE-HPLC-HRMS method (Brueller et al., 2018; Inreiter et al., 2016). This method was originally described by (Khan et al., 2012) with adaptations documented by (Singer et al., 2009) and (Westrup et al., 2013). In summary, the testing setup consisted of a Thermo Scientific EQUAN online solid-phase extraction (SPE) system, which enabled high-volume sampling with an injection volume of 2.0 ml. Two Thermo Scientific Ultimate 300 Series UHPLC pumps, one for loading and the other for elution, were used in conjunction with a Thermo Scientific Q-Exactive mass spectrometer equipped with electrospray ion source (ESI), mass transitions were obtained in positive ESI mode.

The high-resolution accurate mass spectrometer was operated in full-scan and data-dependent-MS^2-mode. The limits of quantification (LOQs) ranged from 0.5 to 2.5 ng/L. The method operates on the following principle: C13 isotope-labeled internal standards are added to the water sample, which is then injected into the high-performance liquid chromatography system. The injection is carried out through online enrichment using a column switch. Chromatograms are recorded using medium-high-resolution mass spectrometers (LC-HRMS) in full-scan data-dependent MS^2 mode, allowing access to complete spectral information at all times. Additionally, an MS^2 spectrum is triggered when specific masses (exact mass of the analytes) are detected, providing confirmation of the analytes' identity. Quantification is achieved by extracting the mass trace from the full-scan spectrum.

3.1.3.1. Devices. LC-MS/MS consists of the following parts

-Thermo Ultimate 3000-Series HPLC pumps (analysis pump HPG-3400RS and enrichment pump LPG-3400SD)

-Thermo QExactive mass spectrometer with electro-spray ionization (heated ESI) with control and evaluation computer and Tracefinder software

-Thermo Open Accela Autosampler for EQUAN with integrated sample loop

-Column oven Thermo Scientific TCC-3000RS

-Separation column: Thermo Hypersil Gold (100 mm length, 2.1 mm ID, 1.9 µm)

-Separation column: Waters XSelect HSS T3 (150 mm length, 2.1 mm ID, 3.5 µm)

-Online enrichment column: Waters Oasis HLB (20 x 2.1, 15 µm)

3.1.3.2. Reference Solutions.

-Reference materials and internal standards: Certified ampoules (Trimethoprim D3, Sulfamethoxazole D4, Ciprofloxacin D8, Atrazine D5, Gabapentin D10, Diclofenac D4) from Ehrenstorfer, were used to prepare solutions at 100 mg/L concentration.

-Reference solutions for analytes (Mix macrolides): Certified mixing standard ampoules (Clarithromycin, Erythromycin, Josamycin, Roxithromycin, Lincomycin, Spiramycin, Tylosin-tartrate) from Neochema (Pharma Mix 7), were used at 50 mg/L

- Reference solutions for analytes (Mix Tetracyclines): Certified mixing standard ampoules (Chlortetracyclin-HCL, 4-Epitetracyclin-HCl, Oxytetracycline-HCl, Tetracycline-HCl, Doxycyclin-monohydrate) from Neochema (Pharma nix 5) were used at 50 mg/L

-Reference solutions for analytes (Mix sulfonamides with Trimethoprim): Certified mixing standard ampoules (Sulfadiazine, Sulfamethazine, Sulfadoxine, Sulfamethoxazole, Sulfathiazole, Acetylsulfamethoxazole, Sulfadimethoxine, Sulfamerazine, Sulfamethoxypyridazine, Sulfamonomethoxine, Sulfachinoxaline, Trimethoprim), from Neochema (Pharma Mix 12), were used at 50 mg/L

-Reference solutions for analytes (Mix Quinolones): Certified mixed standard ampoules (Ciprofloxacin, Danofloxacin, Enrofloxacin, Marbofloxacin, Norfloxacin, Difloxacin, Flumequine, Nalididixin acid, Oxolin acid, Sarafloxacin) from Ultra Scientific (CUS-15328), were used at 100 mg/L

- Reference solutions for analytes (Antibiotics and other PCs): Certified mixing standard ampoules(Acetyl-Sulfamethoxazole, Clarithromycin, Erythromycin, Anhydroerythromycin A, Lincomycin-HCl, Roxithromycin, Sulfamethazine (=Sulfadimidine), Sulfamethoxazole, Trimethoprim, Atenolol, Bezafibrat, Bisoprolol, Carbamazepine, Clofibrin acid, Diazepam, Diclofenac (free acid), Fenofibrat, Gabapentin, Ibuprofen, Ketoprofen, Metoprolol, Paracetamol, Propranolol-HCl, Sotalol-HCl) from Neochema (Pharma Mix 24), were used at 50 mg/L.

-Reference solutions for analytes (Mix wastewater indicators-1): Certified mixing standard ampoules (Carbamazepine, Tolyltriazole, 1H-Benzotriazole, Diclofenac, Ibuprofen,Triclosan, 17a-Ethynylestradiol) , from Neochema (Pharma Mix 7), were used at 50 mg/L

- Reference solutions for analytes (Mix wastewater indicators-2): Certified mixing standard ampoules (1H-Benzotriazole, Tolyltriazole, Carbamazepine, 10,11-Dihydrocarbamazepine, Metoprolol, Sotalol, Acesulfam, Sucralose), from Neochema (Pharma Mix 8), were used at 50 mg/L

-Reference solutions for analytes (Benzo- and Tolyltriazole): Certified individual standard ampoules (1H-Benzotriazole, Tolyltriazole) from Neochema, were used at 50 mg/L.

3.1.3.3. Chemicals/Reagents. Only reagents with the purity grade "for analysis" were used. Water means ultrapure water from the water treatment system (MilliQ).

-Acetone "For residue analysis"
-Ultrapure water “HPLC reagent” (Baker No. 4218)
-Ammonium formate
-Formic Acid LC/MS Grades
-Hydrochloric acid 1N (Titrisol, Merck)
-Acetonitrile for LC-MS (Merck)
-MilliQ water
-Eluent for HPLC
-Istanbul tap water (approx. 160 mg/l $CaCO_3$), membrane-filtered
-EDTA solution (Titriplex III, Merck) 0.50 mol/L

Two different mobile phases are required for gradient operation:
A: 0.10% formic acid (6.2.4) in water
B: 0.10% formic acid (6.2.4) in acetonitrile

The calibration was carried out by adding Istanbul tap water in order to take any matrix effects into account during the calibration. The freedom from blank values of the tap water used was checked for each analysis sequence, any blank value was quantified by standard addition and taken into account in the calibration.

3.1.3.4. Analysis. Two different procedures (neutral/acidic) were followed.

Sample preparation (Neutral)

10 mL of the filtered sample was filled directly into an autosampler vial and filled with 50 µl of the internal standard solution was added.

Sample preparation (Acidic)

Sample preparation to adjust the ionic strength and the pH value (for repeat measurements of positive results) 10 mL of the filtered sample were filled directly into an autosampler vial and

- 50 µL of the internal standard solution
- 10 µL formic acid
- 50 µL of the 4 M ammonium formate solution
- 100 µL EDTA solution added.

The buffering achieves an adjustment of the ionic strength and the pH value in the sample (pH: around 3.5 - 4.0), so that the influence of the sample composition (pH value, alkaline earth ions, etc.) on the recovery rates the analyte is largely compensated. The addition of EDTA serves to stabilize the samples. After optimization of the method and using the new column Waters XSelect HSS T3, the sample preparation only includes the "neutral" run.

3.1.4. High Performance Liquid Chromatography

The entire system (HPLC pumps, autosampler and mass spectrometer) was controlled via the Tracefinder software, the chromatographic conditions (flow, gradient, injection volume, etc.) are stored in the master method. Before each analysis sequence, the mobile phase suction hoses were on for approx. 5 minutes open flush valve and 1.0 mL flow to flush. The HPLC pump was then run in for about 5 minutes at a flow rate of 300 µL and a mixing ratio of 50/50. System pressure and any pressure fluctuations observed. Analysis settings for HPLC and EQUAN enrichment with the Hypersil Gold analysis column and XSelect HSS T3 analysis column are given below in Table 3.4. and Table 3.5. respectively.

Table 3. 4. Analysis settings for HPLC&EQUAN enrichment with the Hypersil Gold Column

LC- Column	Thermo Hypersil Gold (100x2.1mm, 1.9 mm)
Enrichment column	Waters Oasis HLB (20x 2.1 mm, 15mm)
Injection volume	2200 mL
Injection syringe	2.5 mL
Sample loop	2000 mL
Flow pump 1	300 mL/min (analysis column)
Flow pump 2	For enrichment or rinsing with enrichment columns: 1.5 mL/min
Gradient for Pump 1 and Pump 2	**Pump 1** Time %A %B ml/min 0 95 5 300 2.3 95 5 300 8.3 50 50 300 9.5 5 95 300 12.3 5 95 300 12.4 95 5 300 14.1 95 5 300 **Pump 2** Time %A %B ml/min 0 100 0 1500 1.8 100 0 1500 1.9 100 0 500 12.2 100 0 500 12.3 100 0 1500 14.1 100 0 1500
Mobile Phase A	0.10% Formic acid in water
Mobile Phase B	0.10% Formic acid in Acetonitrile
Loading Time	108 sec
Eluting Time	738 sec

Table 3. 5. Analysis settings HPLC and EQUAN enrichment with the XSelect HSS T3 column

LC- Column	Waters XSelect HSS T3 (150 x 2.1 mm, 3.5 µm)
Enrichment column	Waters Oasis HLB (20 x 2.1 mm, 15 µm)
Injection volume	2200 mL
Injection syringe	2.5 mL
Sample loop	2000 mL
Flow pump 1	300 mL/min (analysis column)
Flow pump 2	For enrichment or rinsing with enrichment columns : 1.5 mL/min
Gradient for Pump 1 and Pump 2	**Pump 1** Time %A %B ml/min 0 95 5 300 2.3 95 5 300 9.5 5 95 300 12.3 5 95 300 12.4 95 5 300 14.1 95 5 300 **Pump 2** Time %A %B ml/min 0 100 0 1500 1.8 100 0 1500 1.9 100 0 500 12.2 100 0 500 12.3 100 0 1500 14.1 100 0 1500
Mobile Phase A	0.10% Formic acid in water
Mobile Phase B	0.10% Formic acid in Acetonitrile
Loading Time	108 sec
Eluting Time	738 sec

3.1.5. Mass Spectrometric Detection

The mass spectrometer was put into operation according to the manufacturer's specifications and a mass calibration was carried out. The parameters of the QExactive mass spectrometer and the HPLC system were set by the master method in the Tracefinder software ("Aquisition" menu item). The analysis sequence was entered and the analysis data (calibration data, check of peak integration and MS2 spectra) was evaluated in the Tracefinder software. Device parameters of QExactive are given below in Table 3.6.

Table 3. 6. Device Parameters

MS Settings	Heated ESI Temperature: 350 ^{0}C Capillary Temperature: 350 ^{0}C Sheath Gas: 40 Aux Gas:10 Spray 3800
Tune	Tetracycline_350
Experiment	Fullscan with data dependent MS2
Mass resolution Fullscan	70.000 FWHM
Mass resolution MS2	17.500 FWHM

The measurements were carried out in FSddMS2 mode, i.e. a high-resolution full scan (mass resolution: 70.000 FWHM) was recorded over the entire retention area, with an MS2 spectrum (mass resolution: 17.500 FWHM) also being generated for preset masses from a certain threshold value. Table 3.7. below lists the analytes with the associated exact mass on which the quantification and triggering of the MS2 spectrum are based.

Table 3. 7. List of analytes with mass spectrometric details

Analyte	**Exact Mass**	**Ionisation**	**Neutral**	**Acidic**	**tMS2**
Acetyl-Sulfadimidine (=Acetyl-Sulfamethazine)	321.10159	Positive	Yes	Yes	
Acetyl-Sulfamethoxazole	296.06995	Positive	Yes	Yes	
Atrazine D5	221.13243	Positive	Yes	Yes	
Benzotriazole	120.05562	Positive	Yes		
Carbamazepine	237.10224	Positive	Yes		
Chlortetracycline	479.12157	Positive	Yes	Yes	Yes
Ciprofloxacin	332.14050	Positive	Yes	Yes	Yes
Ciprofloxacin D8	340.19071	Positive	Yes	Yes	
Clarithromycin	748.48417	Positive	Yes	Yes	
Danofloxacin	358.15615	Positive	Yes	Yes	Yes
Difloxacin	400.14672	Positive	Yes	Yes	
Doxycyclin	445.16054	Positive	Yes	Yes	Yes
Enrofloxacin	360.17180	Positive	Yes	Yes	
Erythromycin	734.46852	Positive	Yes		
Erythromycin C13 D3	738.49070	Positive	Yes		
Erythromycin-anhydro	716,45795	Positive	Yes	Yes	Yes
Fenofibrat	361.12066	Positive	Yes		
Flumequine	262.08740	Positive	Yes	Yes	
Gabapentin	172.13321	Positive	Yes		
Gabapentin D10	182.19652	Positive	Yes		
Josamycin	828.47400	Positive	Yes	Yes	
Lincomycin	407.22103	Positive	Yes	Yes	
Marbofloxacin	363.14631	Positive	Yes		Yes
Metoprolol	268.19072	Positive	Yes		
Nalidixinic acid	233.09207	Positive	Yes	Yes	

Analyte	Exact Mass	Ionisation	Neutral	Acidic	tMS2
Norfloxacin	320.14050	Positive	Yes	Yes	Yes
Oxolinic acid	262.07100	Positive	Yes	Yes	
Oxytetracycline	461.15546	Positive	Yes	Yes	Yes
Paracetamol	152.07115	Positive	Yes		
Propranolol	260.16505	Positive	Yes		
Roxithromycin	83753185	Positive	Yes	Yes	
Sarafloxacin	386.13107	Positive	Yes	Yes	Yes
Sulfadiazine	251.05972	Positive	Yes	Yes	
Spiramycine	843.52128	Positive	Yes	Yes	Yes
Sulfadimethoxine	311.08085	Positive	Yes	Yes	
Sulfadimidine=Sulfamethazine	279.09102	Positive	Yes	Yes	
Sulfadoxine	311.08085	Positive	Yes	Yes	
Sulfamerazine	265.07537	Positive	Yes	Yes	
Sulfamethoxazole	254.05939	Positive	Yes	Yes	
Sulfamethoxazole D4	258.08450	Positive	Yes	Yes	
Sulfamethoxypyridazine	281.07029	Positive	Yes	Yes	
Sulfamonomethoxine	281.07029	Positive	Yes	Yes	
Sulfaquinoxaline	301.07537	Positive	Yes	Yes	
Sulfathiazole	256.02089	Positive	Yes	Yes	
Sulfisoxazole	268.07504	Positive	Yes	Yes	
Tetracycline	445.16054	Positive	Yes	Yes	Yes
Tolyltriazole	134.07127	Positive	Yes		
Trimethoprim	291.14517	Positive	Yes	Yes	
Trimethoprim D3	294.16400	Positive	Yes	Yes	
Tylosin	916.52643	Positive	Yes	Yes	
Acesulfam K	161.98665	Positive	Yes		

To secure the results, a tMS2 run was also carried out for the tetracyclines and other selected quinolones (e.g. marbofloxacin, ciprofloxacin) in addition to the FSddMS2 run. With this measurement method, mass transitions can be measured – similar to a conventional triple quad device – which means that the sensitivity of the analytes mentioned can be increased. The sample preparation follows the same scheme as described above.

3.1.6. Calibration

The test method was calibrated under the defined chromatographic conditions using multi-component mixtures over the entire process, taking into account the following specifications:

- The working range of the measuring system is to be determined by injecting at least 5 reference solutions of different concentrations.
- The calibration range is to be selected in such a way that the real concentrations are covered (1 ng/L to 100 ng/L).

- The concentration of the lowest calibration standard must be above the determined limit of quantification.
- Each peak should be registered with at least 12 data points
- With each series of examinations, calibration is carried out with several – over the calibration range distributed - to recreate concentration levels in the work area.

The validity of the calibration function is checked by analyzing the control standard. The calibration data was evaluated using the Tracefinder software of the mass spectrometer.

3.1.3.5. Evaluation and Quantification. The analysis results were calculated by the Tracefinder software of the QExactive mass spectrometer based on the valid calibration function. According to the specifications of explained in the EU Directive on the performance of analytical methods and the interpretation of results (EU Directive 2002/657, 2002), 3 identification points are required for an absolutely unambiguous identification of substances. The hybrid mass spectrometer QExactive allows production spectra (MSMS spectra) to be generated, which were carried out depending on the information in the HR full scan data (data dependent acquisition), i.e. an unequivocal identification is given with this analysis technique if
- the precursor ion (MH+) is extracted with high resolution (2 identification points)
- at least one daughter ion is detected with high resolution (>10,000 FWHM) (2.5 IPs)
The analytes were quantified using the exact mass trace extracted from the HR full scan data, taking into account suitable internal standards in the Tracefinder software.
Positive results (> limit of quantification) were confirmed using standard addition methods or by simply spiking and determining the recovery rates in order to compensate for possible matrix influences. Likewise, the ionic strength and the pH value in the sample were adjusted by buffering (pH: around 3.5 - 4.0), so that the influence of the sample composition (pH value, alkaline earth ions, etc.) on the Recovery rates of the analytes were largely compensated. To confirm positive results, buffered samples therefore were measured or tMS2 experiments were also carried out for the selected substances in order to increase sensitivity.

3.2. Simulation of the Drinking Water Treatment Process

In the above study it was found that the drinking water treatment process has a substantial effect on the elimination of various pharmaceutical compounds. From a toxicological perspective, two main aspects emerge here: firstly, the extent of degradation of pharmaceutical molecules, and secondly, whether the resulting degradation products exhibit similar or different toxic effects on the

environment and human health compared to the parent molecule. Therefore, the aim of this study is to determine the extent of degradation and the formation of degradation products of three selected representative pharmaceutical molecules, namely carbamazepine, clarithromycin, and sulfamethoxazole, through a simulated three-step treatment process. Additionally, the study aims to investigate whether the raw (before-treatment) water collected from a specific region in Istanbul exhibits any qualitative and/or quantitative differences in *in vitro* cytotoxicity, specifically through a basic *in vitro* cytotoxicity test, done in before and after treatment samples. Since the before treatment (raw water) sample contains a wide variety of different chemical substances, this sample also needs to be tested for its cytotoxicity potential. Pure commercial forms of the three compounds were separately dissolved in distilled water, spiked to the before treatment sample and subjected to the simulation of the drinking water treatment process in the lab scale. The effects of the treated samples were then compared to the effects of the untreated samples. This approach was adopted to focus on the situation of these three PCs, allowing for a comprehensive understanding of their behavior, considering the presence of numerous other chemicals in the raw water sample. To address these questions, the treatment process was simulated in the laboratory and applied to the solutions of reference pharmaceutical compounds at the average concentrations found in the environmental water samples before treatment (20 ng/L). These simulations were conducted with three representing pharmaceutical compounds namely carbamazepine, clarithromycin, and sulfamethoxazole in both before and after treatment water samples obtained from the same raw water sources in Istanbul. There are several reasons for selecting these three PCs for simulation experiments. Firstly, despite being present in high concentrations in raw drinking water, all three compounds are completely removed during the treatment process. Moreover, CLA, CBZ, and SMX are three of the 5 most frequently quantified PCs in raw water samples. Secondly, CLA and SMX are included in the EU Watch list due to their high acute toxicities, making them potential emerging contaminants that are likely to be regulated in the future. CBZ and SMX (List 1), CLA (List 2) are classified in the Global Water Research Coalition Report (GWRC, 2008). One of the other important selection criteria that needs to be mentioned is the availability of isotopically labeled internal standards to be used in the analyses.

The simulations were also conducted in distilled water. The reason for preparing the standard solutions in distilled water is to determine any potential toxicity after treatment originates from either the parent pharmaceutical active compounds or its degradation products. There are many other contaminants in before treatment water, and these even generate higher amounts of degradation products upon treatment. Any cytotoxic effect of these highly contaminated samples cannot be attributed only to the dissolved active compound and/or its potential degradation products

upon simulated treatment. The same uncertainty is also valid for the positive identification of their degradation products by mass spectrometric analyses. So, simulation experiments provided materials to explore:

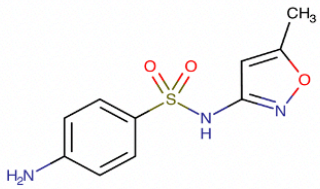
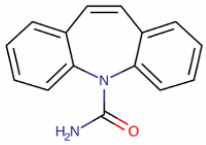
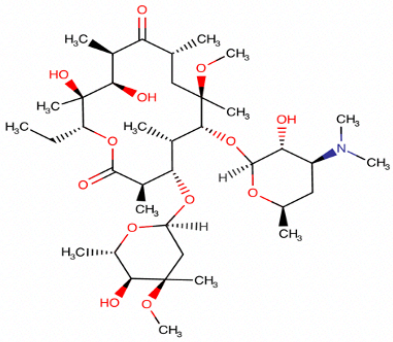
- Characterization of the degradation process
- Cytotoxicity assessment of the after treatment water samples

3.2.1. Experiment

Simulation using scaled-down jar tests consisting ozonation, coagulation, flocculation, sedimentation and chlorination practices were carried out by using the same chemicals and similar process conditions that are in place at ISKI (confirmed by the directorate of ISKI). Based on the 100% removability data in the first phase of the study, three different molecules (Carbamazepine:CBZ, Sulfamethoxazole: SMX, Clarithromycin:CLA) were selected as the model molecules for further steps of the study. These molecules were spiked in distilled water and before treatment water at their maximum concentration range found in the first phase of the study: CBZ=20 ng/L; SMX=20 ng/L; CLA= 20ng/L. Each test solution containing three molecules was after treatment separately in the same process, and then the after treatment water samples were lyophilized for further measurements.

Chemical structures and some important physico chemical properties related the environmental behaviour of the selected molecules are given in Table 3.8.

Table 3. 8. Chemical structures and physico-chemical properties of the tested PCs

Compound	Molecular Structure	Molecular Weight* g/mol	pKa*	Water solubility* g/100 ml	Henry's law constant* atm.m3/mol
Sulfamethaxazole		236,3	1,85	0,61	$6,42x10^{-13}$
Carbamazepine		253,3	13,9	0.12	$1,08x10^{-10}$
Clarithromycine		748	8,99	1,69	$3,37x10^{-33}$

* : https://pubchem.ncbi.nlm.nih.gov, https://go.drugbank.com/, Environmental Science SRC PhysProp Database, 2006, Yalkowsky et al., 2016

3.2.1.1. Chemicals.

-Iron (III) chloride (40% m/m), Iski Technical Grade

-Sodium hypochlorite (4.42% m/m), Iski Technical Grade

-Ozone gas (by means of ozone generator)

-Carbamazepine reference standard, API, Lek Pharmaceuticals

-Clarithromycin reference standard, Sigma Aldrich

-Sulfamethoxazole reference standard, Sigma Aldrich

3.2.1.2. Simulation Process**.** Based on the real practice in the ISKI treatment process, four major steps were included in the simulation of the drinking water treatment namely; ozonation, coagulation&flocculation, filtration and chlorination consecutively. Simulations were performed with before treatment and distilled water samples at the Environmental Sciences Institute-Boğaziçi University. The reason for having distilled water in the simulation is as explained above, simply to avoid the effect of many other contaminants and organic matter in before treatment water in the positive identification of the degradation products, as well as interference of potential cytotoxicity of the dissolved pharmaceutical compounds. As starting solutions before treatment water samples and distilled water samples were spiked with CBZ, CLA and SMX at 20 ng/L concentration. Experimental details are given below in Table 3.9.

Table 3. 9. Experimental details of the simulation experiment

Step	**Concentration**	**Duration**
Ozonation	1-2 mg/L	3 minutes
Coagulation&Flocculation with $FeCl_3$	50 mg/L	100 rpm 2 minutes 30 rpm 20 minutes Waiting 20 minutes
Filtration	-	-
Chlorination	3 mg/L	

Ozonation.

Water samples were subjected to ozonation in 0.5 L glass flasks. This was achieved by bubbling ozone by means of air stone diffusers through the water samples, aiming to replicate real ISKI waterworks conditions. By adjusting the flow rate of the bubbling process, specific ozone doses ranging from 1 to 2 mg/L were introduced into the water samples during the 3 minutes delivery time.

Coagulation-Flocculation.

For coagulation&flocculation experiments conducted at the laboratory scale, a batch procedure known as the "Jar test" was utilized. The experimental conditions, stirring velocity, and reaction times, were selected based on parameters obtained from ISKI plants. The laboratory setup involved glass beakers with a volume of 0.5 L, equipped with a stirrer. During fast stirring a 40% iron(III) chloride solution was added to 0.25 L of ozonated water samples from the above step. After stirring for 3 minutes at 300 rpm, the pH was adjusted to 7.5 by adding a 1 mol/L solution of $Ca(OH)_2$.

Subsequently, the formation of microflocs was facilitated by gently stirring the mixture for 2 minutes at a speed of 100 rpm, then the samples were kept undisturbed for the flocs to settle down.

Filtration.

Samples were filtered by vacuum filtration through the Sartorius glass fiber prefilters (0.3mm).

Chlorination.

Chlorination was carried out by adding 3 mg/L sodium hypochlorite solution to the filtered samples from the previous step.

Lyophilisation.

After the above mentioned treatment steps, in order to increase the concentration of the degradation products, water samples were obtained in powder form upon lyophilization in a freeze dryer (Labconco, FreeZone Freeze Dryer) for 24 hours at −50°C under vacuum (0.15 mbar). This part was conducted at the Sanyal Research Group/Chemistry Department-Boğaziçi University.

3.3. Extent of Degradation

The aim of this part of the study is to determine the extent of degradation and the number of degradation products formed for the selected representative pharmaceutical molecules, namely carbamazepine, clarithromycin, and sulfamethoxazole, after a three-step treatment process.

In studies focusing on chemical characterization, degradation products, and similar aspects, it is common to work with concentrations of the studied substances that are hundreds or thousands of times higher than those observed in the environment. However, when investigating the potential effects of these substances in biological systems, a realistic approach is to work with concentrations that are within the range of those observed in the environment, typically only a few times higher. Therefore, the chemical degradation was investigated at these realistic, low concentrations. Consequently, the low concentration of the parent molecules resulted in low concentrations of potential degradation products. For the characterization of the degradation products an UHPLC coupled to Ultra-High- definition Quadrupole Time-Of-Flight Mass Spectrometer LC/QTOF-MS was used. Analyzes were performed on the Agilent 1260 series HPLC system and the attached Agilent 6540 High Resolution Mass Spectrometer (Agilent Technologies, Inc., CA, USA). The MS system was used in the double spray Agilent Jet Stream Electrospray ionization technique. Analyses were performed both in negative and positive ion modes. MS operating mode was 2 GHz Extended

Dynamic Range. Agilent Poroshell SB-C18 (3.0 mm x 100 mm x 2.7 µm) column was used for chromatographic separation. HPLC and MS parameters are detailed in Table 3.10.

Table 3. 10. Specifications of LC/QTOF-MS

Column	Agilent Poroshell SB-C18 3.0 mm x 100 mm x 2.7 µm
Column Temperature	35 ◦C
Injection volume	20 µL
Analysis duration	22 min
Mobile Phase A	0.1% formic acid (v/v)
Mobile Phase B	Acetonitrile
Flow rate	0.6 mL/min
MS	Agilent 6540 LC-MS Q-TOF
Ionisation mode	Negative/positive
Drying gas Temp.	325 ^{0}C
Drying gas flow, N_2	10 L/min
Nebulizer	40 psi
Sheath gas Temp	375 ^{0}C
Sheat gas flow, N_2	11 L/min
Capillary Voltage	3000 V
Nozzle Voltage	500 V
Mass range	30-1700 amu
Reference ions	112.98558, 966.00072, 922.0098

The "total ion (TIC)" and "extracted ion (EIC)" chromatograms of the peaks that were not present in the pharmaceutical solutions before treatment, as well as the mass spectra of each peak, were obtained to determine whether the main pharmaceuticals molecules partially remained after the treatment and the number of fragmentation products generated from each molecule.

3.3.1. Sample Preparation

The lyophilized samples were dissolved with 2.0 mL ultrapure water and then homogenized. 0.5 mL of the solution was taken and diluted with 0.5 mL of distilled water and analyzed in the LC-MS system. 1.0 mL of acetonitrile containing 0.1% formic acid was added to the remaining solution and vortexed. 1.0 mL of the solution was taken and analyzed in the LC-MS system.

3.3.2. Calibration

Standard solutions containing 3 active PCs were prepared as a mix and a calibration curve was done before treatmentn. 5-point calibration was performed in the range of 100 ppt-100 ppb.

The 100 ppb concentration was excluded because it was out of linearity. Therefore, 100 ppt-500 ppt-1 ppb-10 ppb calibration points were used in the calibration.

3.4. Cytotoxicity Assessment of Drinking Water

The purpose of this step is to demonstrate whether the degradation products are not toxic or less toxic, or even more toxic. Lyophilized samples from the simulation experiments have been tested for their cytotoxicity profile at different concentrations. Cytotoxicity assays are employed to assess the potential toxicity of compounds at cellular level, typically by evaluating factors such as cell damage or cell viability, cell count, or cell proliferation following a predetermined duration of exposure. Since cytotoxicity is a comprehensive concept as it includes all toxic effects that contribute to cell death, cytotoxicity studies constitute the initial biological testing step in determining the potential toxicity of the chemicals in biological systems. To be able to measure cell damage, viable cell quantification can be performed through various staining methods; MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a very efficient one to avoid additional washing steps that could prolong processing time and introduce sample variability. When cells die, they lose the ability to convert MTT into formazan, thus color formation serves as a useful and convenient marker of viable cells (Vinken & Rogiers, 2015).

3.4.1. Method Principle

MTT assay was developed by Mosmann in 1983 that the potential of utilizing a color reaction was used as an indicator of viable cell count, leveraging the capabilities of multiwell scanning spectrophotometers that offer precise measurements (Mosmann, 1983). MTT assay is based on the total cellular metabolic activity of cells by measuring MTT into an insoluble formazan product via the action of dehydrogenase enzymes located within the intact mitochondria of all living cells. This enzymatic reaction occurs exclusively in viable cells and serves as a reliable indicator of their metabolic activity. As only viable cells are able to produce formazan crystal by reducing MTT using mitochondrial dehydrogenase enzyme, the assay is considered as a very sensitive assay to express cellular respiration, cell viability, and cytotoxicity (Mersch-Sundermann et al., 2004).

Another important point in the MTT assay is the selection of the cell lines. Human hepatoma HepG2 cells representing human liver and HK-2 cells representing human kidney have been intensively used in toxicity and genotoxicity evaluation of chemicals. The liver and kidneys are the organs with the highest perfusion in the body, and therefore, chemicals that enter the body through oral route reach their highest concentrations in these organs and exhibit their toxic effects most frequently. Hence, liver and kidney cell cultures were selected in the experimental part of the study.

3.4.2. Cell Culture Procedure

Before routine cell culture procedures, the UV light of the biosafety cabinet was turned on and the cabinet was sterilized for at least 25-30 minutes, then the interior was wiped with 70% ethanol. All kinds of materials to be introduced into the cabinet were also wiped with a napkin sprayed with 70% ethanol solution beforehand, thus avoiding the introduction of contaminated material into the cabinet. At the same time, any liquid that would come into contact with the cells was presterilized by filtering from an appropriate mesh-size syringe type filter and was heated in a water bath at 37°C beforehand to bring it to physiological temperature.

The potential cytotoxicity of both parent test compounds and their degradation products after treatment were tested on two different cell types both from human origin; these are HepG2 cells that represent liver, and HK-2 cells represents kidney.

Information about HepG2 Cell Line:

Organism:	Homo sapiens (Human)
Cell Type:	Epithelium
Tissue:	Liver Cancer
Growth Characteristic:	Adherent

Information about HK-2 Cell Line:

Organism:	Homo sapiens (Human)
Cell Type:	Epithelium
Tissue:	Kidney Proximal Tubule
Growth Characteristic:	Adherent

3.4.2.1. Thawing Frozen Cells.

- The cells are stored at -80°C for storage up to 6 months and are moved to a liquid nitrogen tank for storage longer than 6 months.
- The frozen cell vial was opened, then immersed in a water bath at 37°C to thaw the content.
- Then, the vial was wiped with an ethanol-sprayed napkin and taken into the cabinet. The content of the vial was taken with a sterile 2 mL pipette and transferred into a 15 mL falcon containing 8 mL of complete medium, then centrifuged at 125x*g* for 5 minutes.
- After the centrifugation, the falcon tube was wiped with a 70% ethanol sprayed napkin. The upper phase containing DMSO was aspirated without delay.
- The cell pellet was suspended in 5 mL of complete medium and transferred to a 25 cm^3 flask.
- After the information of the cell line (medium, etc.) and the PCsage numbers are recorded

on the flasks; cells were checked under a microscope. Afterwards, the flask was placed in an incubator at 37°C (95% O_2: 5% CO_2) and left for the cells to adhere and grow.

3.4.2.2. Passaging of Cells.

- Cells were regularly checked under the microscope, cells that reached 80% density were PCsaged and cells were maintained.
- For the PCsage process, first of all, the old medium was aspirated and washed with 5 mL of PBS solution. Following the washing process, the PBS solution was also removed and 1 mL of Trypsin-EDTA solution was added to the cells. In order for the trypsinization process to take place, the flask was put back into the incubator and waited for 2 minutes.
- Afterward, the cells were examined under the microscope, and the processes were continued after it was observed that the cells stopped adhering and floated.
- To inactivate trypsin, 4 mL of complete medium was added to the flask and trypsin was inactivated. Then, the cells were diluted 1:3 and 1:5 in accordance with the number of cells required by the experimental procedure and transferred to flasks containing fresh medium.
- For 1:3 dilution, 1.67 mL of 5 mL of cell suspension was taken and transferred to a 25 cm flask containing 3.33 mL of complete medium.
- Cells were monitored regularly with a microscope and were PCsaged when necessary.

3.4.2.3. Counting Cells.

- Following the trypsinization process, 9 mL of complete medium was added to the cell suspension, and the cell suspension was transferred to a 15 mL flask and centrifuged at 125xg for 5 minutes.
- The medium was then removed, and the cell pellet was suspended in 1 mL of complete medium and 50 µL of the cell suspension was taken and mixed with 50 µL of trypan blue. In order to be able to count, the mixture was transferred with an automatic pipette to the Neubauer slide.
- The Neubauer slide was placed on the microscope and counted. Briefly, counting was done in 4 different frames as shown in the figure below.
- The yellow-colored cells that did not let the dye in were considered alive, and the cells that appeared in blue color that took the dye in were considered dead.
- And again, as indicated in the formula below, the number of cells in mL was calculated.

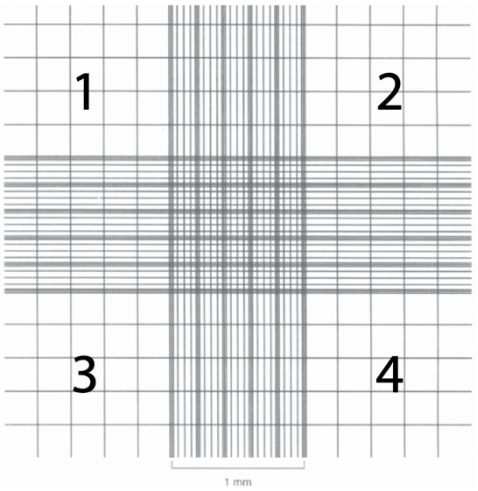


Figure 3. 2. Neubauer slide

Average Cell Count (ACC): (1+2+3+4)/4 (as number)
Dilution Factor: 2
Volume of the Counted Chamber: 10^{-4} mL
Cell Count: ACC x 2 x 10^4 /mL

3.4.2.4. Seeding Cells in 96-well Microplate. 100 µL (10.000 cells/100 µL) of the prepared cell suspension mixture was seeded into 60 wells. When seeding the 96-well microplate, cells were not seeded on the outer edges of the wells, instead 100 µL of sterile PBS solution was added to each well. After the seeding process was completed, the microplate was incubated in the incubator to allow the cells to attach for 24 hours.

3.4.2.5. Preparation of the Test Substances for Incubation. Cells were incubated with two different types of test substances: before and after treatment. In before treatment samples, stock solutions of each test substance were prepared at 1 mg/mL and then four dilutions were prepared to provide final concentrations of the substances in the incubation media between 0.02, 0.16, 0.2 and 2 µg/L. To accomplish this, 1 mg of each pharmaceutic substance was dissolved in 1 mL DMSO so main stock solutions were prepared. Then from each main stock solution 1 µL was taken, and added 999 µL to DMSO, so 1. intermediate solutions was prepared. Lastly, each 1. intermediate solutions were taken 400 µL and added to 600 µL, so 2. intermediate solutions were prepared. Then 5 µL of the second intermediate solutions were taken and added to 995 µL medium and solutions were obtained at a concentration of 2 µg/L (0.5% DMSO). Finally, a series of working solutions were prepared from 2 µg/L (0.5% DMSO) by serial dilutions in the medium.

In after treatment samples, 125 mL solutions of each test substance in distilled water (20 ng/L) were freeze-dried and transported to the laboratory of the Pharmaceutical Toxicology Department

of Ege University for further analyses. Although test substances in these solutions were completely degraded because of the treatment (as the following mass spectrometric analyses proved), authentic average environmental concentrations of the test substances were accounted, and each residue was dissolved in 50 µL DMSO (2500-fold concentrated). From these stock solutions, 80 µL was diluted to 1 mL with DMSO so 200-fold concentrated 1. intermediate stock was prepared. Lastly each 1. intermediate stock solutions were taken 5 µL and diluted to 1 mL with medium and 125 ng/L (0.5% DMSO) solutions were prepared. This was used to prepare a series of working solutions by serial dilutions in the medium. All tests were carried out in triplicates.

3.4.2.6. Adding Solutions to the Cells. At the end of 24 hours, the old medium in the wells was aspirated, and 100 µL of solutions of the medium control, Triton-X, Medium + Water samples were added to the relevant wells and removed to the incubator for another 24 hours.

3.4.2.7. Addition of MTT Solution and Termination of the Experiment:

- MTT solution was prepared at 5 mg/mL in PBS and sterilized by filtering through a 0.22 µm membrane filter in a laminar flow safety cabinet.
- It is wrapped with aluminum foil to prevent it from being affected by light until it is used, and at +4 ℃ for short-term storage and -20 for long-term storage.
- At the end of 24 hours, the media in the wells were aspirated and removed, then 100 µL of sterile PBS was added to each well, the wells were washed, and the PBS solutions were aspirated and removed.
- Then, 50 µL of maintenance medium (10 µL of MTT + 40 µL of complete medium) was added to each well and removed to the incubator for incubation for 4 hours.
- At the end of 4 hours, the medium in the wells was removed, and 150 µL of DMSO was added to each well to dissolve the formed formazan crystals, and the surface of the plate was covered with aluminum foil and left in the plate shaker for 2 minutes to dissolve the crystals.
- Finally, the absorbance value in each well was measured at 550 nm using a microplate reader and the viability of the control group was accepted as 100% and the viability of the other wells was calculated.

4. RESULTS AND DISCUSSION

The results of the experimental work consist of three parts namely, screening of pharmaceutical compounds (PCs) in 27 samples from 13 selected water sources of Istanbul including raw and treated water samples, degradability of the spiked PCs in the drinking water treatment process, and assessment of cytotoxicity of the treated drinking water linked to the degradation products of the PCs. Degradability and cytotoxicity studies were performed in the simulated water samples with spiking of three model substances.

4.1. Screening of PCs in Raw (before treatment) and Treated (after treatment) Drinking Water Samples

55 targeted PCs were tested in all water samples from 13 selected water collection sites of ISKI in Istanbul (13 before treatment, and 14 after treatment); 17 pharmaceutical compounds were quantified (Table 4.1.) (Figure 4.1.), 37 compounds were not detected (<LOD = 1 ng/L).

Table 4. 1. Concentrations of 18 PCs quantified in the samples collection sites of ISKI

	Concentration (ng/L) (Before-treatment)		Concentration (ng/L) (After-treatment)	
Compounds	Mean±sd	Max	Mean±sd	Max
1H-Benzotriazole	35.48±96.39	347.68	1.30±2.84	9.12
Acetyl-Sulfamethoxazole	1.22±2.36	6.53	0.56±0.89	2.3
Carbamazepine	2.95±6.03	20.89	0.00±0.00	<1
Clarithromycin	4.75±9.22	28.55	0.00±0.00	<1
DEET	4.51±14.98	54.19	0.40±0.99	2.84
Gabapentin	53.48±87.18	243.4	0.00±0.00	<1
Iopromid	4.46±12.40	43.53	2.36±6.52	22.81
Irbesartan	23.24±67.00	242.26	2.40±8.65	31.18
Lincomycin	2.19±4.45	12.65	0.00±0.00	<1
Propyphenazone	1.80±4.97	17.4	0.00±0.00	<1
Sulfadoxin	0.91±3.29	11.85	0.00±0.00	<1
Sulfamethoxazole	2.44±5.03	17.27	0.00±0.00	<1
Telmisartan	2.32±3.93	10.4	0.08±0.30	1.1
Tolyltriazole	33.74±65.73	223.05	1.73±6.24	22.51
Trimethoprim	0.17±0.32	0.84	0.00±0.00	<1
Valsartan	4.57±7.45	21.34	6.28±8.77	28.72
Valsartan acid	11.14±19.17	47.22	5.9±14.22	50.49

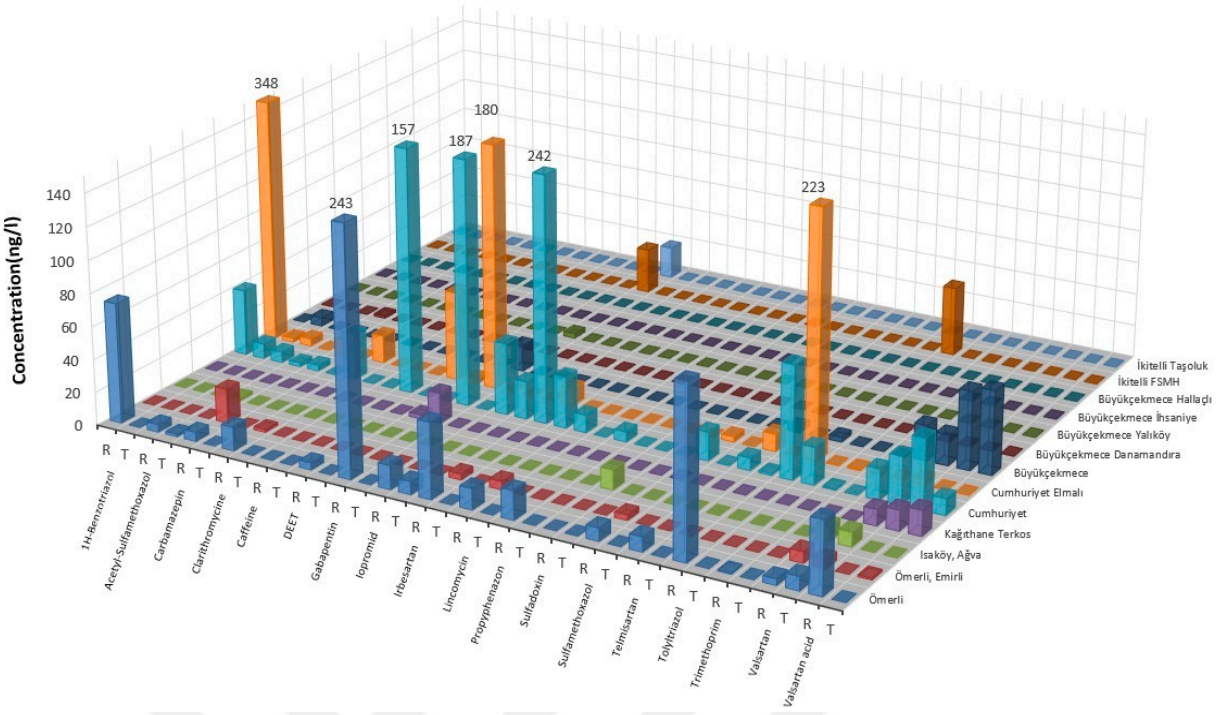


Figure 4. 1. Distribution of 17 PCs in different sampling locations for raw and treated water samples R: Raw; T: Treated

At least one or more PCs were determined in 77% of samples collected from water sources (raw water-before treatment) in 13 locations of ISKI plants. The top three locations in terms of the highest number of different PCs quantified in raw water samples are:

1. Ömerli (25% PCs)
2. Cumhuriyet (25% PCs)
3. Cumhuriyet-Elmalı (18% PCs)

The occurrence of various PCs at substantial concentration in the sampling locations in Ömerli region (Ömerli and Cumhuriyet) is highly likely to be linked to agricultural activities, nearby villages, water streams, and the overflow of wastewater treatment facilities during intense rainfall. On the other hand, no PCs were quantified in Büyükçekmece-Danamandıra, Büyükçekmece İhsaniye and Büyükçekmece Hallaçlı locations (33% of samples) where water is obtained from underground wells.

Taking into account all the raw water samples collected from various facilities of İSKİ (Istanbul Water and Sewerage Administration) and their corresponding test results, it has been observed that in 80% of the samples where pharmaceutical compounds were detected, these molecules have been completely removed with a removal efficiency exceeding 99%. This shows that the existing plants are doing a good job in terms of eliminating PCs from the water.

Cumhuriyet and Ömerli are the locations with the highest number of different PCs (44% and 22 respectively) found in the treated water (Table 4.2.).

Table 4. 2. Distribution of quantified PCs in the selected water sample locations

Location	Number of Quantified PCs	
	Raw Water	Treated Water
Ömerli	15	4
Ömerli, Emirli	6	2
İsaköy, Ağva	2	1
Kağıthane Terkos	2	1
Cumhuriyet	15	8
Cumhuriyet Elmalı	11	2
Büyükçekmece	5	0
Büyükçekmece Danamandıra	0	0
Büyükçekmece Yalıköy	1	0
Büyükçekmece İhsaniye	0	0
Büyükçekmece Hallaçlı	0	0
İkitelli FSMH	2	0
İkitelli Taşoluk	1	0

The most 5 frequently quantified PCs and their frequencies of quantification in raw water is given below. Except valsartan other PCs were not detected in treated water samples (Table 4.3.).

1. Gabapentin (14.2 %)
2. Valsartan (9.0%)
3. Sulfamethoxazole (7.1%)
4. Carbamazepine (7.1%)
5. Clarithromycin (7.1%)

Gabapentin as the most frequently quantified PC in raw water was detected at mean concentration of 53,48 ng/L and maximum concentration of 242,24 ng/L. Some of the analyzed pharmaceutical compounds were detected at concentrations of 3–50 ng/L in the drinking water samples.

Table 4. 3. Frequency of quantification of PCs in raw and treated water samples

Compounds	Frequency of quantification	
	Raw Water	Treated Water
1H-Benzotriazole	3	2
Acetyl-Sulfamethoxazole	3	2
Carbamazepine	4	0
Clarithromycin	4	0
DEET	2	1
Gabapentin	8	0
Iopromid	2	0
Irbesartan	4	1
Lincomycin	3	0
Propyphenazon	2	0
Sulfadoxine	1	0
Sulfamethoxazole	4	0
Telmisartan	4	0
Tolyltriazole	5	1
Trimethoprim	3	0
Valsartan	5	7
Valsartan acid	4	4

Therefore particular attention should be given to valsartan which was not removed during the drinking water treatment process. In the literature, different studies have reported contrasting results regarding the removal of valsartan during ozonation. Some studies indicate the successful removal of valsartan, while others suggest its resistance to ozonation (Diehle et al., 2019; Ibáñez et al., 2013). In studies showing the removal of valsartan, both the concentration of valsartan and ozone are observed to be significantly higher than the concentrations used in this thesis study.

In other studies, the antiepileptic pharmaceutical compound gabapentin was eliminated to less than 50% (Kovalova et al., 2013), whereas here in the ISKI plants it was removed completely (>99.9%).

According to the findings obtained from the analyses, it is generally observed that there is a higher concentration of valsartan acid in raw water samples compared to valsartan itself. This indicates the formation of the acid transformation derivative of valsartan during activated sludge treatment, which is in line with the literature data (Nödler et al., 2013).

Considering the overall experimental data given in Tables 4.4., 4.5., and 4.6. performance of existing drinking water treatment facilities of ISKI for the removal of the tested pharmacological

compounds from raw (before treatment) water seems to be quite efficient for most of the analyzed PCs.

Statistical point of view, significance analysis of the concentrations of contaminants between before treatment,after treatment water sample and tap water data sets, mean, median values, paired-t test, student t-test and p-values were calculated by using SPSS 23 (IBM, 2015); Table 4.7., Table 4.8., Table 4.9., and 4.10.. Although there is a clear and substantial difference between the before treatment and after treatment water samples points for most of the PCs (Table 4.7.), no significant difference was observed between these samples statistically (except Gabapentin). The reason for this ambiguity can be explained by the presence of so many absent (zero) results obtained in the measurements (median=0) and high standard deviation of the concentrations from site to site. Another supporting result was also obtained regarding the expected agreement related to the occurrence and the concentrations of the tested compounds between after treatment and tap water samples (Table 4.8.).

According to the experimental data obtained from this study, Carbamazepine, Clarithromycin, Sulfamethoxazole, Gabapentin, Lincomycin, Propyphenazone and Sulfadoxin were removed entirely in all sampling locations resulting in the formation of possible degradation products. This finding is in line with the literature data (Boleda et al., 2011; Simazaki et al., 2015). While 1H-Benzotriazole, Telmisartan, Irbesartan, and Tolyltriazole can be removed entirely at the Ömerli facilities, they remain to some extent at the Cumhuriyet and Büyükçekmece facilities. This can be attributed to the absence of ozonation processes in the drinking water treatment plants in these locations. Finding is inline with the literature, highly selective antihypertensive pharmaceuticals like Irbesartan and Telmisartan can be removed mostly by using ozonation and chlorination (Ladhari et al., 2021; Szabová et al., 2020). Iopromide was only partially removed (45%) in the raw water samples where iopromide is present. This finding is in line with the previous studies where it was shown that both ozonation and chlorination were not sufficiently effective for the removal of iopromide (Knopp et al., 2016; Wu et al., 2012). In this study the term “removal” implies elimination of parent compounds by different mechanisms, mainly degradation.

From a toxicological perspective, two main aspects emerge here: firstly, the extent of degradation of pharmaceutical molecules, and secondly, whether the resulting degradation products exhibit similar or different toxic effects on human health compared to the parent molecule.

Table 4. 4. Concentrations of measured PCs (ng/L) (< LOD)

Location	1H-Benzotriazole			Acetyl-Sulfamethoxazole			Carbamazepine			Clarithromycin			DEET		
	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal
Ömerli	73.00	<1	>99.9	4.74	1.71	64.0	5.76	<1	>99.9	14.44	<1	>99.9	4.39	<2	>99.9
Ömerli, Emirli	<1	<1	<1	<0.5	<0.5	<1	20.89	<1	>99.9	1.80	<1	>99.9	<2	<2	-
İsaköy, Ağva	<1	<1	<1	<0.5	1.67	<1	<1	<1	<1	<1	<1	-	<2	<2	-
Kağıthane Terkos	<1	<1	<1	<0.5	<0.5	<1	<1	<1	<1	<1	<1	-	<2	2.41	-
Cumhuriyet Hamsu	40.57	9.12	77.5	6.53	2.30	64.8	3.49	<1	>99.9	28.55	<1	>99.9	<2	<2	-
Cumhuriyet Elmalı	347.68	2.33	99.3	4.57	<0.5	>99.9	8.27	<1	>99.9	16.94	<1	>99.9	54.19	2.84	94.8
Büyükçekmece	<1	5.47	-	<0.5	<0.5	-	<1	<1	-	<1	<1	-	<2	<2	-
Büyükçekmece Danamandıra	<1	<1	-	<0.5	1,60	-	<1	<1	-	<1	<1	-	<2	<2	-
Büyükçekmece Yalıköy	<1	<1	-	<0.5	<0.5	-	<1	<1	-	<1	<1	-	<2	<2	-
Büyükçekmece İhsaniye	<1	<1	-	<0.5	<0.5	-	<1	<1	-	<1	<1	-	<2	<2	-
Büyükçekmece Hallaçlı	<1	<1	-	<0.5	<0.5	-	<1	<1	-	<1	<1	-	<2	<2	-
İkitelli FSMH	<1	<1	-	<0.5	<0.5	-	<1	<1	-	<1	<1	-	<2	<2	-
İkitelli Taşoluk	<1	<1	-	<0.5	<0.5	-	<1	<1	-	<1	<1	-	<2	<2	-

Table 4. 5. Concentrations of measured PCs (ng/L) (< LOD)

Location	Gabapentin			Iopromid			Irbesartan			Lincomycin			Propyphenazone			Sulfadoxine		
	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal
Ömerli	243.40	<1	>99.9	14.45	7.89	45.4	45.60	<2	>99.9	12.65	<1	>99.9	17.40	<1	>99.9	<1	<1	-
Ömerli, Emirli	<1	<1	-	<1	<1	-	3.63	<2	>99.9	5.12	<1	>99.9	<1	<1	0,0	<1	<1	-
İsaköy, Ağva	<1	<1	-	<1	<1	-	<2	<2	-	<1	<1	-	<1	<1	-	11.85	<1	>99.9
Kağıthane Terkos	18.51	<1	>99.9	<1	<1	-	<2	<2	-	<1	<1	-	<1	<1	-	<1	<1	-
Cumhuriyet Hamsu	186.71	<1	>99.9	43.53	22.81	47.6	242.3	31.18	87.1	10.66	<1	>99.9	6.01	<1	>99.9	<1	<1	-
Cumhuriyet Elmalı	180.34	<1	>99.9	<1	<1	-	10.59	<2	>99.9	<1	<1	-	<1	<1	-	<1	<1	-
Büyükçekmece	16.09	<1	>99.9	<1	<1	-	<2	<2	-	<1	<1	-	<1	<1	-	<1	<1	-
Büyükçekmece Danamandıra	<1 ng/l	<1	-	<1	<1	-	<2	<2	-	<1	<1	-	<1	<1	-	<1	<1	-
Büyükçekmece Yalıköy	2.83	<1	>99.9	<1	<1	-	<2	<2	-	<1	<1	-	<1	<1	-	<1	<1	-
Büyükçekmece İhsaniye	<1 ng/l	<1	-	<1	<1	-	<2	<2	-	<1	<1	-	<1	<1	-	<1	<1	-
Büyükçekmece Hallaçlı	<1 ng/l	<1	-	<1	<1	-	<2	<2	-	<1	<1	-	<1	<1	-	<1	<1	-
İkitelli FSMH	27.84	<1	>99.9	<1	<1	-	<2	<2	-	<1	<1	-	<1	<1	-	<1	<1	-
İkitelli Taşoluk	19.53	<1	>99.9	<1	<1	-	<2	<2	-	<1	<1	-	<1	<1	-	<1	<1	-

Table 4. 6. Concentrations of measured PCs (ng/L) (< LOD)

Location	Sulfamethoxazole			Telmisartan			Tolyltriazole			Trimethoprim			Valsartan			Valsartan acid		
	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal	Raw Water	Treated Water	% Removal
Ömerli	7.86	<1	>99.9	8.91	<1	>99.9	102.10	<1	>99.9	0.84	<0.5	>99.9	3.90	8.40	-115.1	43.11	<2	>99.9
Ömerli, Emirli	3.38	<1	>99.9	<1	<1	-	<1	<1	-	<0.5	<0.5	-	6.71	4.80	28.5	<2	1.09	-
İsaköy, Ağva	<1	<1	-	<1	<1	-	<1	<1	-	<0.5	<0.5	-	8.91	8.53	4.3	<2	<2	-
Kağıthane Terkos	<1	<1	-	<1	<1	-	<1	<1	-	<0.5	<0.5	-	<1 ng/l	9.58	-	12.21	15.15	-24.1
Cumhuriyet Hamsu	17.27	<1	>99.9	7.52	1.10	85.4	68.32	22,51	67.0	0,73	<0.5	>99.9	18.50	28.72	-55.2	42.23	9,94	76.5
Cumhuriyet Elmalı	3.17	<1	>99.9	10.40	<1	>99.9	223.05	<1	>99.9	0,64	<0.5	>99.9	<1	3.04	-	<2	<2	-
Büyükçekmece	<1	<1	-	3.37	<1	>99.9	2.77	<1	>99.9	<0.5	<0.5	-	21.34	18.53	13.2	47.22	50.49	-6.9
Büyükçekmece Danamandıra	<1	<1	-	<1	<1	-	<1	<1	-	<0.5	<0.5	-	<1	<1	-	<2	<2	-
Büyükçekmece Yalıköy	<1	<1	-	<1	<1	-	<1	<1	-	<0.5	<0.5	-	<1	<1	-	<2	<2	-
Büyükçekmece İhsaniye	<1	<1	-	<1	<1	-	<1	<1	-	<0.5	<0.5	-	<1	<1	-	<2	<2	-
Büyükçekmece Hallaçlı	<1	<1	-	<1	<1	-	<1	<1	-	<0.5	<0.5	-	<1	<1	-	<2	<2	-
İkitelli FSMH	<1	<1	-	<1	<1	-	42.42	<1	>99.9	<0.5	<0.5	-	<1	<1	-	<2	<2	-
İkitelli Taşoluk	<1	<1	-	<1	<1	-	0,00	<1	-	<0.5	<0.5	-	<1	<1	-	<2	<2	-

Table 4. 7. Significance analysis of concentrations of PCs between before-treatment and after-treatment water samples

Compounds	Before treatment		After treatment		t**	p
	Mean±sd (ng/l)	Median (min-max)	Mean±sd (ng/l)	Median (min-max)		
1H-Benzotriazole	35.48±96.39	0 (0-347.68)	1.30±2.84	0 (0-9.12)	1.285	0.223
Acetyl-Sulfamethoxazole	1.22±2.36	0 (0-6.53)	0.56±0.89	0 (0-2.3)	1.192	0.256
Carbamazepine	2.95±6.03	0 (0-20.89)	0.00±0.00	0 (0-0)	1.766	0.103
Clarithromycin	4.75±9.22	0 (0-28.55)	0.00±0.00	0 (0-0)	1.856	0.088
DEET	4.51±14.98	0 (0-54.19)	0.40±0.99	0 (0-2.84)	1.037	0.320
Gabapentin	53.48±87.18	16,09(0-243)	0.00±0.00	0 (0-0)	2.212	**0.047***
Iopromid	4.46±12.40	0 (0-43.53)	2.36±6.52	0 (0-22.81)	1.287	0.223
Irbesartan	23.24±67.00	0 (0-242.26)	2.40±8.65	0 (0-31.18)	1.284	0.224
Lincomycin	2.19±4.45	0 (0-12.65)	0.00±0.00	0 (0-0)	1.771	0.102
Propyphenazone	1.80±4.97	0 (0-17.4)	0.00±0.00	0 (0-0)	1.306	0.216
Sulfadoxin	0.91±3.29	0 (0-11.85)	0.00±0.00	0 (0-0)	1.000	0.337
Sulfamethoxazole	2.44±5.03	0 (0-17.27)	0.00±0.00	0 (0-0)	1.745	0.107
Telmisartan	2.32±3.93	0 (0-10.4)	0.08±0.30	0 (0-1.1)	2.114	0.056
Tolyltriazole	33.74±65.73	0 (0-223.05)	1.73±6.24	0 (0-22.51)	1.775	0.101
Trimethoprim	0.17±0.32	0 (0-0.84)	0.00±0.00	0 (0-0)	1.882	0.084
Valsartan	4.57±7.45	0 (0-21.34)	6.28±8.77	3.04(0-28.7)	1.512	0.156
Valsartan acid	11.14±19.17	0 (0-47.22)	5.9±14.22	0 (0-50.49)	1.292	0.221

**:p<0,05, **:paired t test*

Table 4. 8. Significance analysis of concentrations of PCs between before-treatment and tap water samples

Compound	Before treatment		Tap		t**	p
	Mean±sd (ng/l)	Median (min-max)	Mean±sd (ng/l)	Median (min-max)		
1H-Benzotriazole	35.48±96.39	0 (0-347.68)	2.24±5.82	0 (0-19.48)	1.241	0.238
Acetyl-Sulfamethoxazole	1.22±2.36	0 (0-6.53)	0.47±0.90	0 (0-2.24)	1.066	0.303
Carbamazepine	2.95±6.03	0 (0-20.89)	0.00±0.00	0 (0-0)	1.766	0.103
Clarithromycin	4.75±9.22	0 (0-28.55)	0.00±0.00	0 (0-0)	1.856	0.088
DEET	4.51±14.98	0 (0-54.19)	0.00±0.00	0 (0-0)	1.085	0.299
Gabapentin	53.48±87.18	16.09(0-243)	0.00±0.00	0 (0-0)	2.212	**0.047***
Iopromid	4.46±12.40	0 (0-43.53)	4.83±4.69	7.72 (0-9.96)	-0.101	0.920
Irbesartan	23.24±67	0 (0-242.26)	0.37±1.34	0 (0-4.85)	1.230	0.242
Lincomycin	2.19±4.45	0 (0-12.65)	0.00±0.00	0 (0-0)	1.771	0.102
Propyphenazone	1.80±4.97	0 (0-17.4)	0.00±0.00	0 (0-0)	1.306	0.216
Sulfadoxin	0.91±3.29	0 (0-11.85)	0.00±0.00	0 (0-0)	1.000	0.337
Sulfamethoxazole	2.44±5.03	0 (0-17.27)	0.00±0.00	0 (0-0)	1.745	0.107
Telmisartan	2.32±3.93	0 (0-10.4)	0.09±0.31	0 (0-1.13)	2.045	0.063
Tolyltriazole	33.74±65.73	0 (0-223.05)	4.03±6.35	0 (0-18.56)	1.622	0.130
Trimethoprim	0.17±0.32	0 (0-0.84)	0.00±0.00	0 (0-0)	1.882	0.084
Valsartan	4.57±7.45	0 (0-21.34)	8.30±8.36	5.55(0-25.4)	-1.203	0.241
Valsartan acid	11.14±19.17	0 (0-47.22)	8.58±7.89	12.48(0-22.2)	0.445	0.662

**:p<0.05, **:student t test*

Table 4. 9. Mean values and medians of removal percentages of the PCs between before-treatment and after-treatment water samples

Compounds	mean±sd (ng/l)	Median (min-max)
1H-Benzotriazole	21.29±40.80	0 (0-100)
Acetyl-Sulfamethoxazole	17.60±34.48	0 (0-100)
Carbamazepine	30.77±48.04	0 (0-100)
Clarithromycin	30.77±48.04	0 (0-100)
DEET	14.98±36.59	0 (0-100)
Gabapentin	61.54±50.64	100 (0-100)
Iopromid	7.15±17.47	0 (0-47.6)
Irbesartan	29.78±46.60	0 (0-100)
Lincomycin	23.08±43.85	0 (0-100)
Propyphenazone	15.38±37.55	0 (0-100)
Sulfadoxine	7.69±27.74	0 (0-100)
Sulfamethoxazole	30.77±48.04	0 (0-100)
Telmisartan	29.65±46.43	0 (0-100)
Tolyltriazole	35.92±48.06	0 (0-100)
Trimethoprim	23.08±43.85	0 (0-100)
Valsartan	-9.56±36.64	0 (-115.1-28.5)
Valsartan acid	11.19±35.18	0 (-24.1-100)

Table 4. 10. Significance analysis of concentrations of PCs between after treatment and tap water samples

	After treatment		Tap water		t*	p
	Mean±sd	Median (min-max)	Mean±sd	Median (min-max)		
1H-Benzotriazole	1.30±2.84	0 (0-9.12)	2.24±5.82	0 (0-19.48)	-0.52	0.608
Acetyl-Sulfamethoxazole	0.56±0.89	0 (0-2.3)	0.47±0.90	0 (0-2.24)	0.249	0.806
Carbamazepine	0.00±0.00	0 (0-0)	0.00±0.00	0 (0-0)	-	-
Clarithromycin	0.00±0.00	0 (0-0)	0.00±0.00	0 (0-0)	-	-
DEET	0.40±0.99	0 (0-2.84)	0.00±0.00	0 (0-0)	1.471	0.167
Gabapentin	0.00±0.00	0 (0-0)	0.00±0.00	0 (0-0)	-	-
Iopromid	2.36±6.52	0 (0-22.81)	4.83±4.69	7.72 (0-9.96)	-1.109	0.278
Irbesartan	2.40±8.65	0 (0-31.18)	0.37±1.34	0 (0-4.85)	0.835	0.412
Lincomycin	0.00±0.00	0 (0-0)	0.00±0.00	0 (0-0)	-	-
Propyphenazone	0.00±0.00	0 (0-0)	0.00±0.00	0 (0-0)	-	-
Sulfadoxine	0.00±0.00	0 (0-0)	0.00±0.00	0 (0-0)	-	-
Sulfamethoxazole	0.00±0.00	0 (0-0)	0.00±0.00	0 (0-0)	-	-
Telmisartan	0.08±0.30	0 (0-1.1)	0.09±0.31	0 (0-1.13)	-0.02	0.984
Tolyltriazole	1.73±6.24	0 (0-22.51)	4.03±6.35	0 (0-18.56)	-0.931	0.361
Trimethoprim	0.00±0.00	0 (0-0)	0.00±0.00	0 (0-0)	-	-
Valsartan	6.28±8.77	3.04 (0-28.72)	8.30±8.36	5.55 (0-25.39)	-0.604	0.552
Valsartan acid	5.9±14.22	0 (0-50.49)	8.58±7.89	12.48 (0-22.17)	-0.595	0.558

*:*Student t test*

4.2. Extent of Degradation

The aim of this part of the study is to determine the extent of degradation and the number of degradation products formed for the selected representative pharmaceutical molecules, namely carbamazepine (CBZ), clarithromycin (CLA), and sulfamethoxazole (SMX) in the simulation of the three-step treatment process. After treatment, the "Total Ion Chromatogram-TIC" and "Extracted Ion Chromatogram-EIC", as well as the mass spectra of the peaks that were not present in the before-treatment PC solutions, were obtained to determine whether the parent molecule partially remained or not, and how many degradation products were formed from each molecule upon the treatment process.

Figure 4.2. shows chromatograms of CLA, CBZ, and SMX at a concentration of 100 ppb in distilled water (DW) before treatment. The TIC analysis in ESI+ mode for clarithromycin, carbamazepine, and sulfamethoxazole is shown in (A). The EIC for the CBZ precursor ion ($[M+H]^+$) at 6.031 minutes is shown in (B), while the EIC for the CLA precursor ion ($[M+H]^+$) at 7.713 minutes is shown in (C), and the EIC for the SMX precursor ion ($[M+H]^+$) at 8.4 minutes is shown in (D).

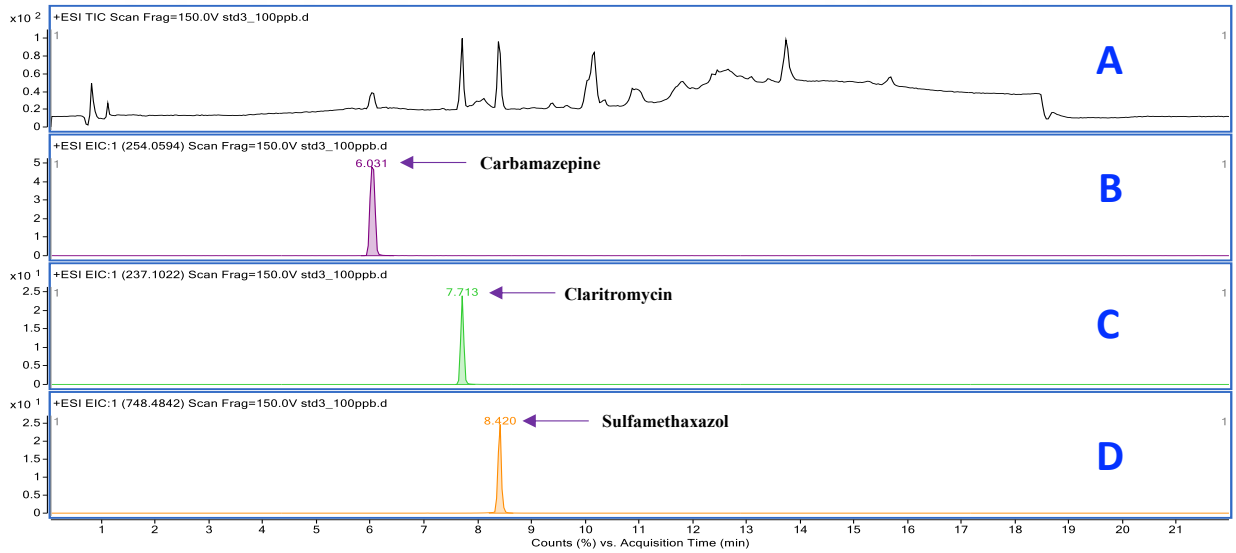


Figure 4. 2. Chromatograms of CBZ, CLA and SMX at a concentration of 100 ppb in distilled water (DW) before-treatment

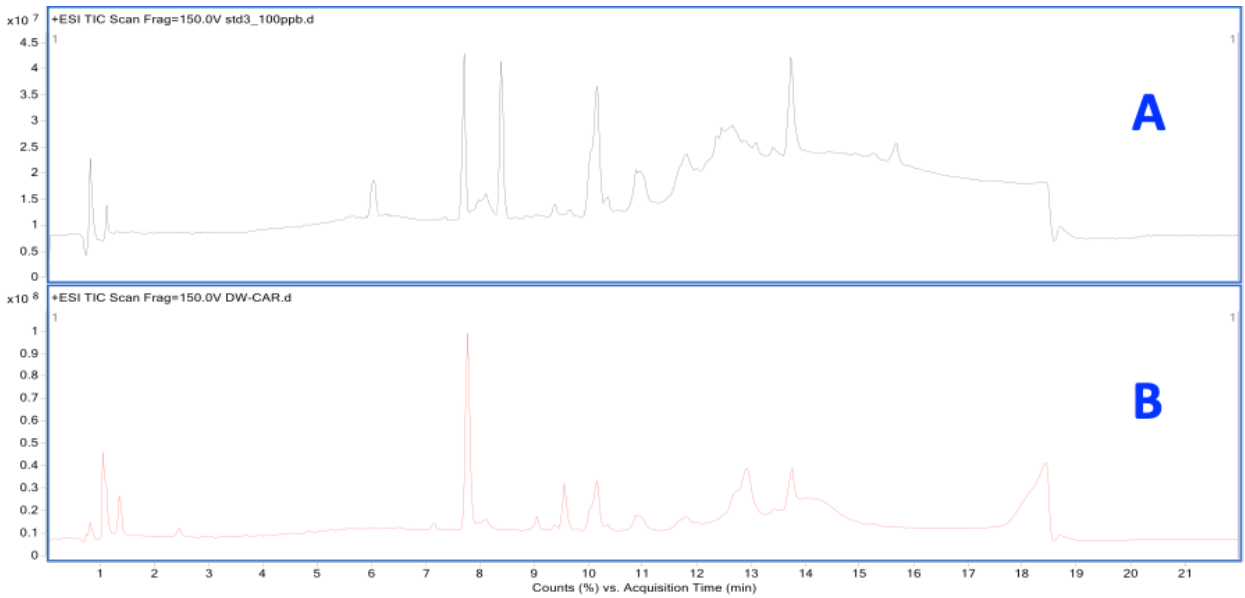


Figure 4. 3. (A) Chromatograms of CBZ, CLA, SMX standards before-treatment; (B) chromatogram of the CBZ after-treatment

Figure 4.3. shows the chromatograms of all three PCs at a concentration of 20 ng/L foe each before treatment (A), and chromatogram of CBZ after the treatment (B). It was observed that CBZ is completely degraded upon the treatment (B).

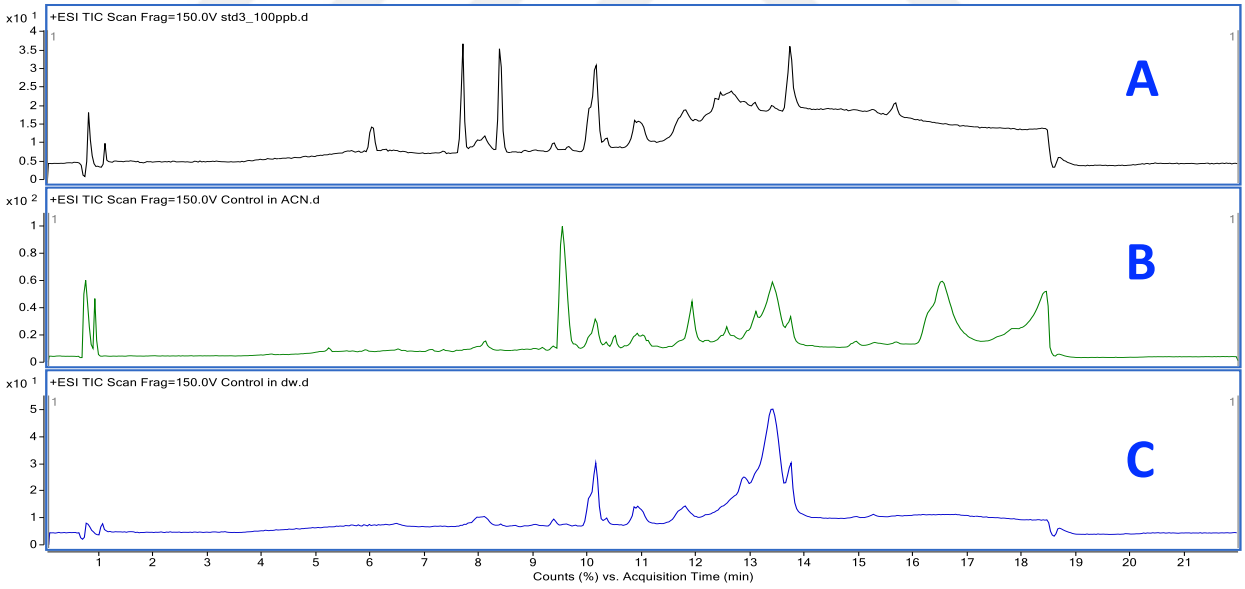


Figure 4. 4. (A) Chromatograms of CBZ, CLA, and SMX standards before-treatment; (B) chromatogram of ACN (blank) used as solvent, (C) chromatogram of DW (blank) used as solvent before-treatment

Figure 4.4. represents the chromatograms of the standards (A), blank acetonitrile used as component of the mobile phase (B), and distilled water (C) used as solvent and component of the mobile phase. As clearly seen , the extra peaks in (A), which do not belong to other three PCs, came from the solvents ACN and DW.

The overlaid chromatograms shown in the Figure 4.5.below were obtained by aligning the chromatograms of Before-Treatment (BT); After-Treatment (AT); and control samples, consisting of Acetonitrile (ACN) and Distilled Water (DW). The black arrow indicates the before treatment peak of carbamazepine, which completely disappears after the treatment. The peaks indicated by red arrows solely represent the peaks associated with Carbamazepine after the treatment. These peaks are believed to be the degradation products of Carbamazepine resulting from the simulation of the drinking water treatment process. Identification of these products was not attempted since they have already been identified in the literature.

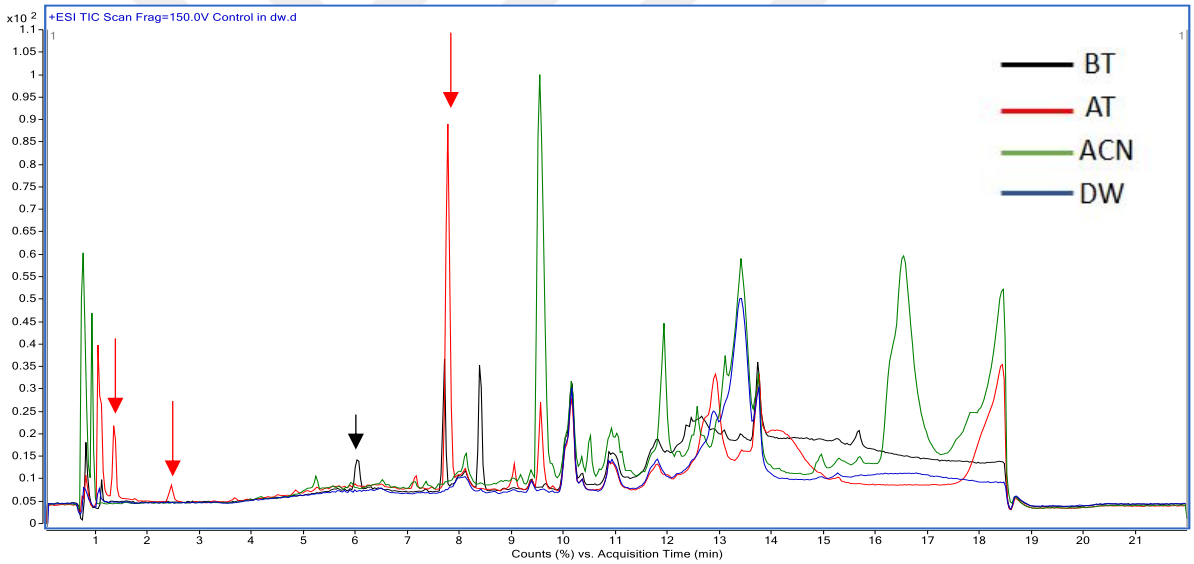


Figure 4. 5. Overlaid chromatograms of CBZ before-treatment and after-treatment together with ACN and DW as background controls.

When comparing the two chromatograms in Figure 4.6., the molecular ion peak at m/z 237.1022 is clearly observed in (A) while it is absent in (B), indicating complete degradation of Carbamazepine after the treatment. There is a 10,000-fold difference in intensity between the y-scales of the two chromatograms; the intensity in (A) is 10^{2}, whereas in (B), it is 10^{-2}.

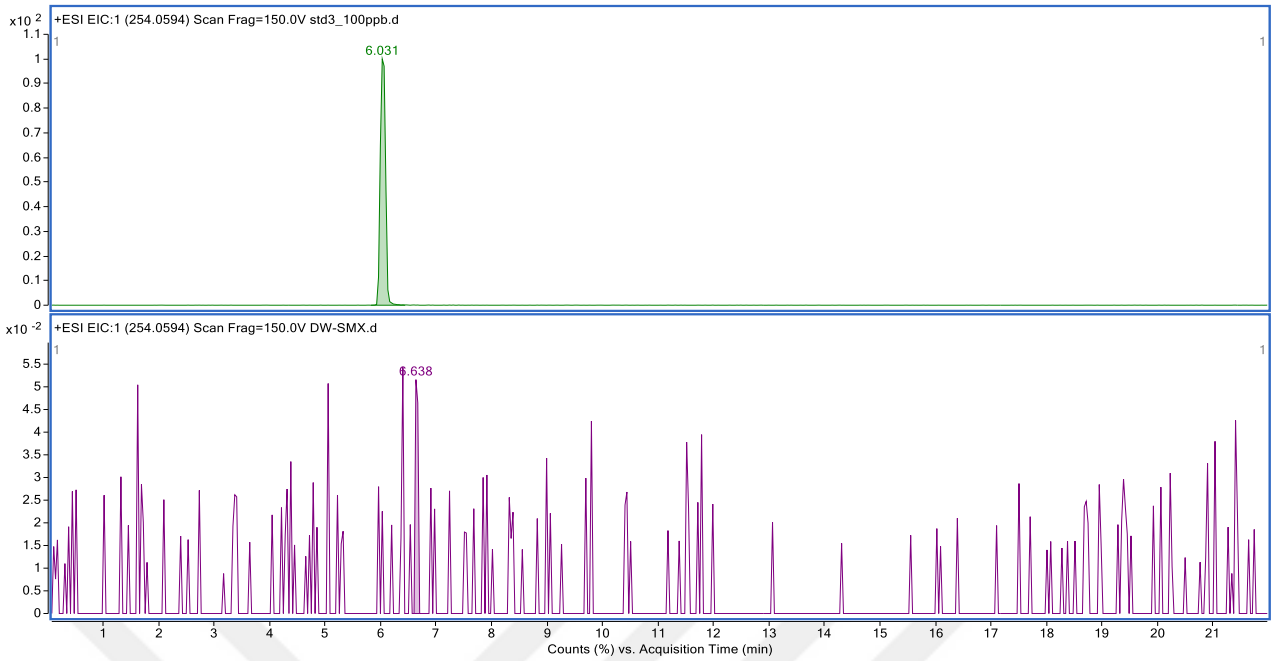


Figure 4. 6. EIC chromatograms of CBZ solutions, (A) before-treatment, (B) after-treatment

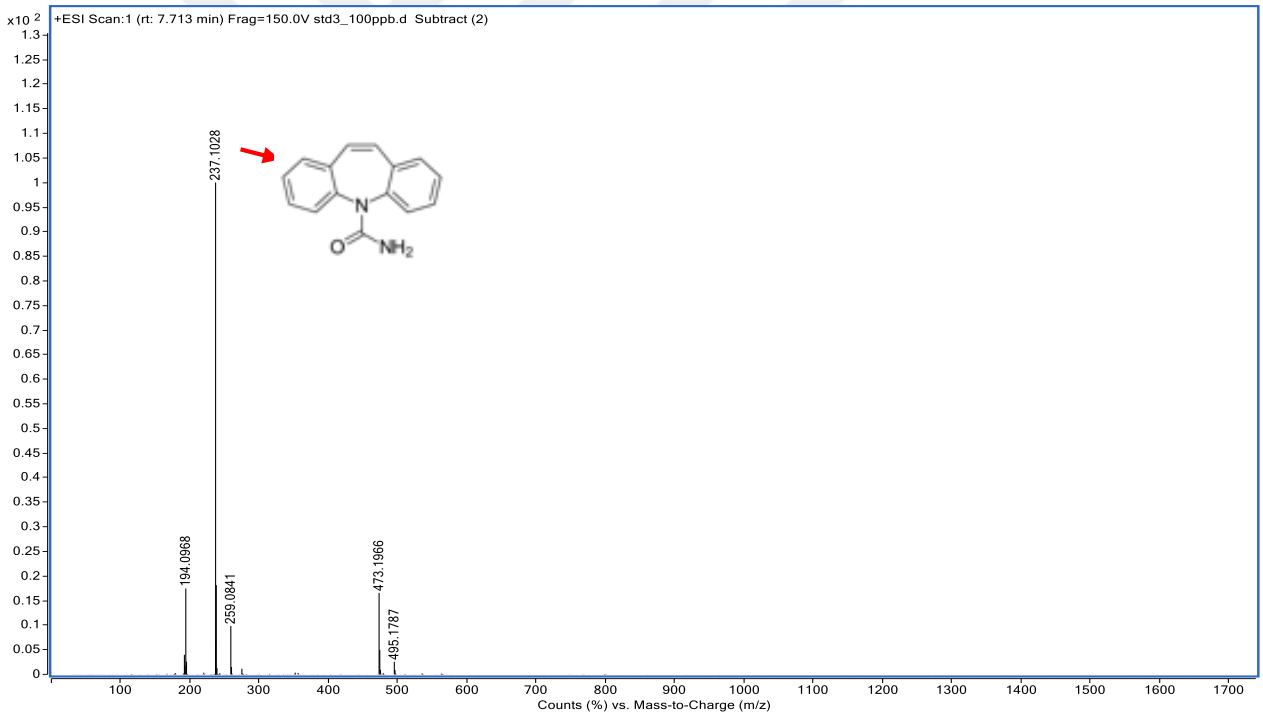


Figure 4. 7. Mass spectrum and molecular ion of CBZ molecule (before-treatment)

In Figure 4.8., upon examining the mass spectrum after-treatment, the absence of the molecular ion at m/z 237.1027 suggests that the treatment process has fragmented the main CBZ molecule.

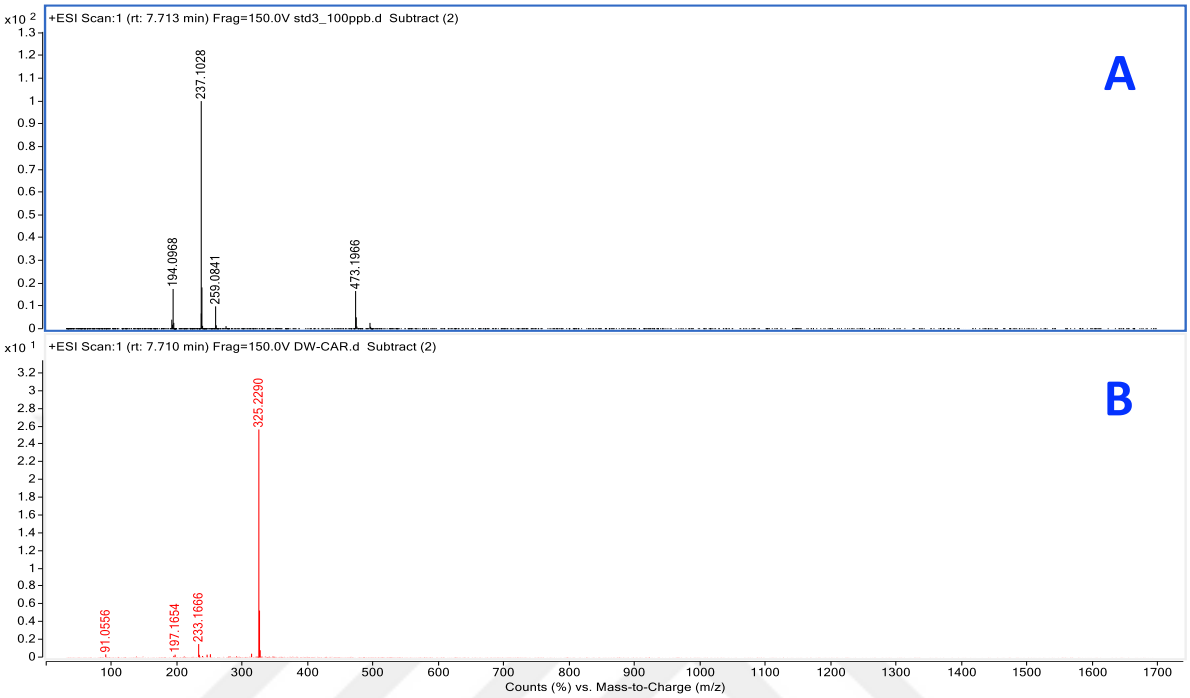


Figure 4. 8. Mass spectra of CBZ, (A) before-treatment, and (B) after-treatment

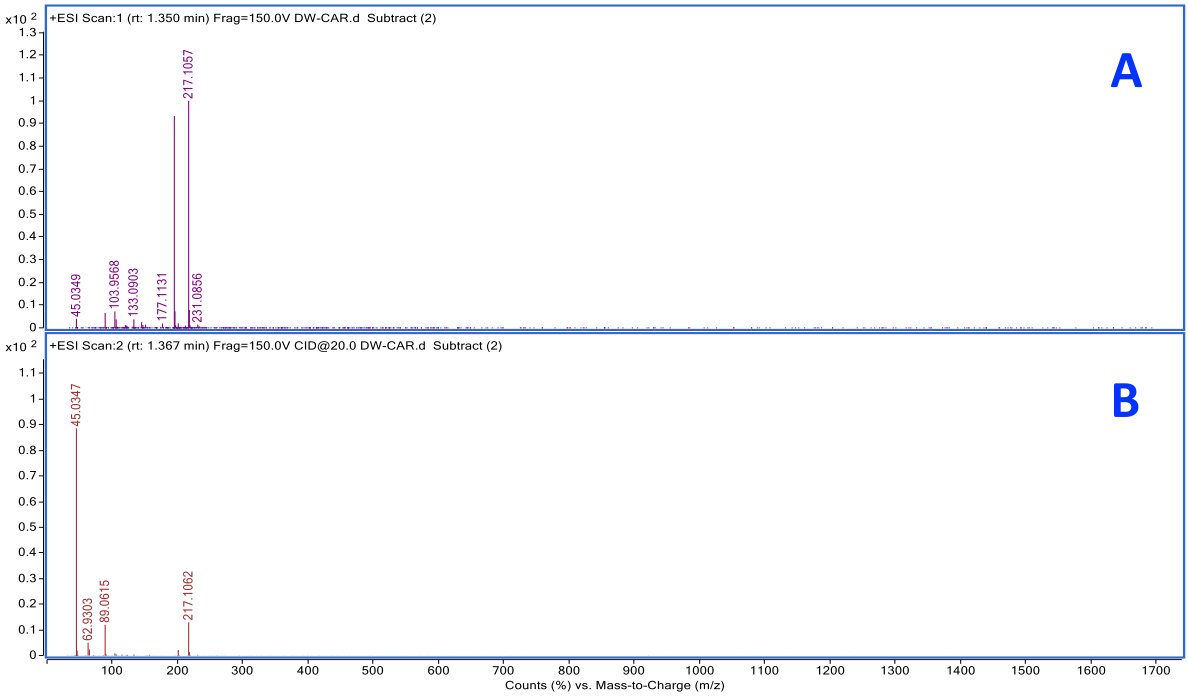


Figure 4. 9. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=1.350 belonging to the CBZ degradation product (after-treatment)

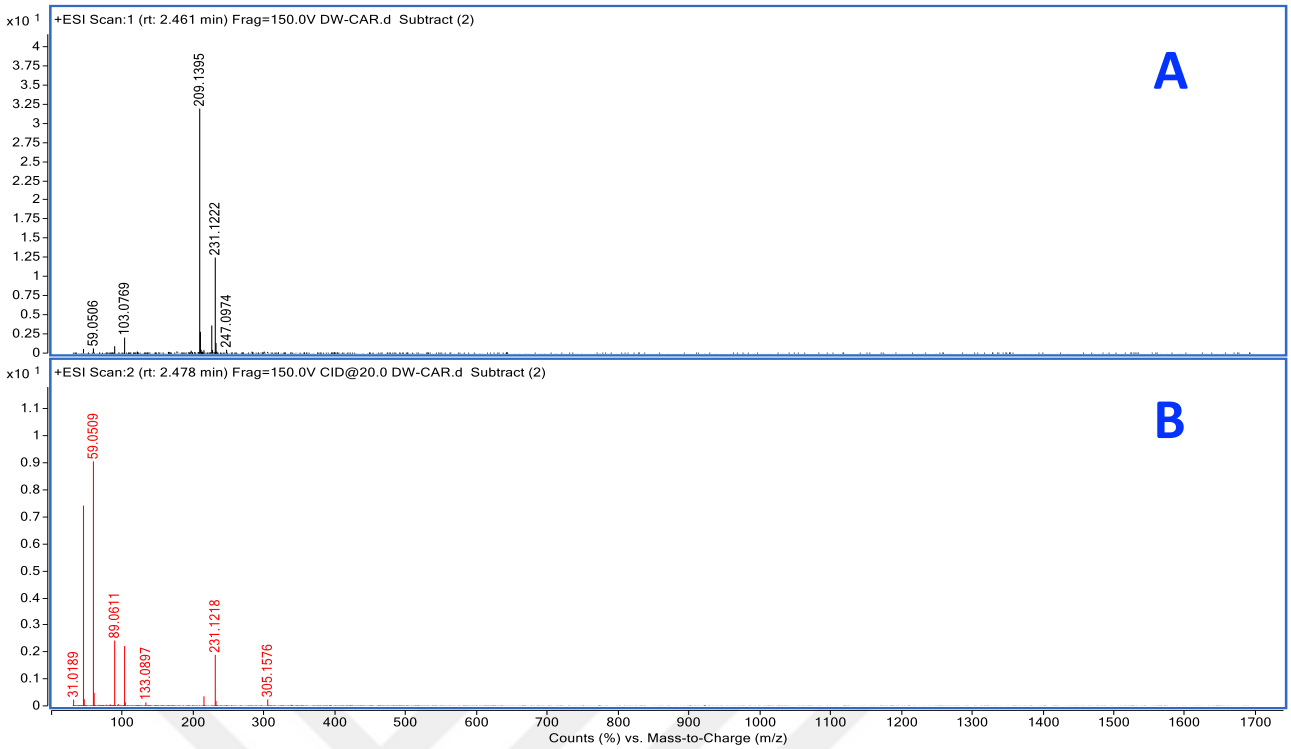


Figure 4. 10. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=2.461 belonging to the CBZ degradation product (after-treatment)

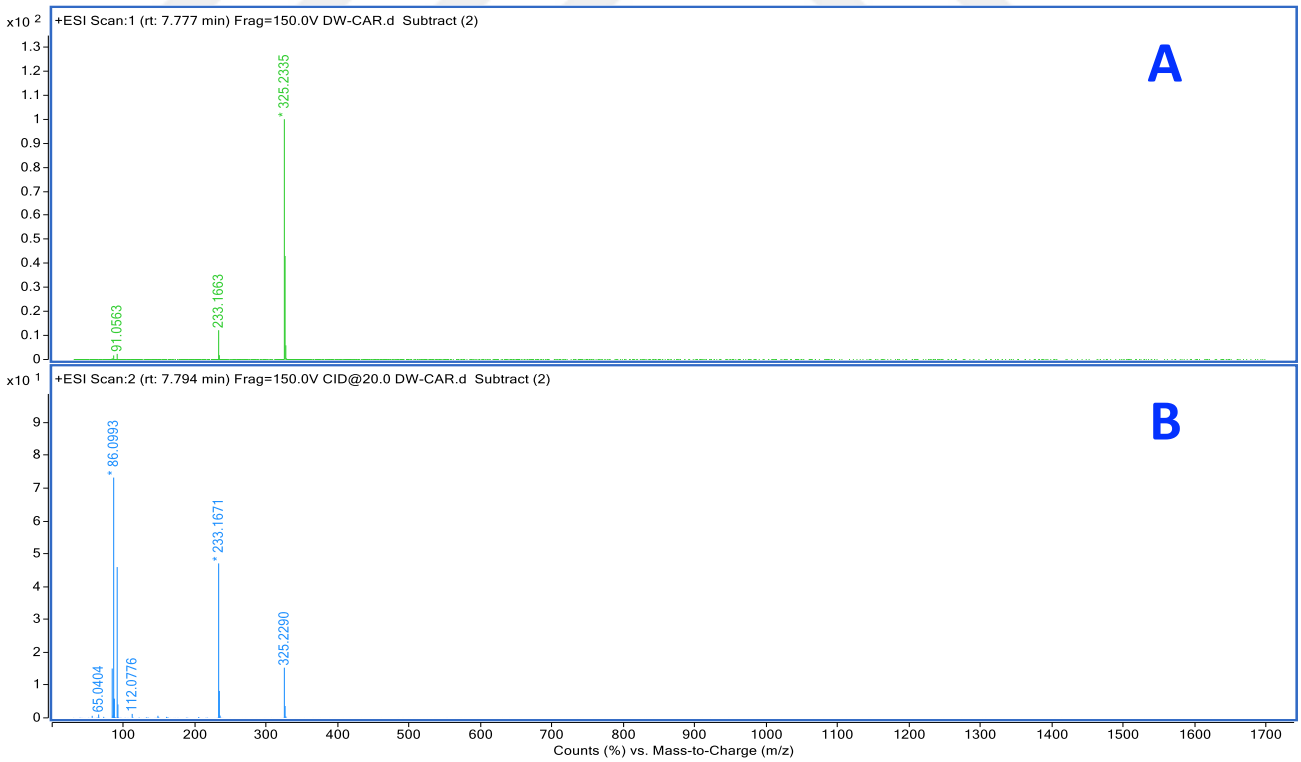


Figure 4. 11. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=7.777 belonging to the CBZ degradation product (after-treatment)

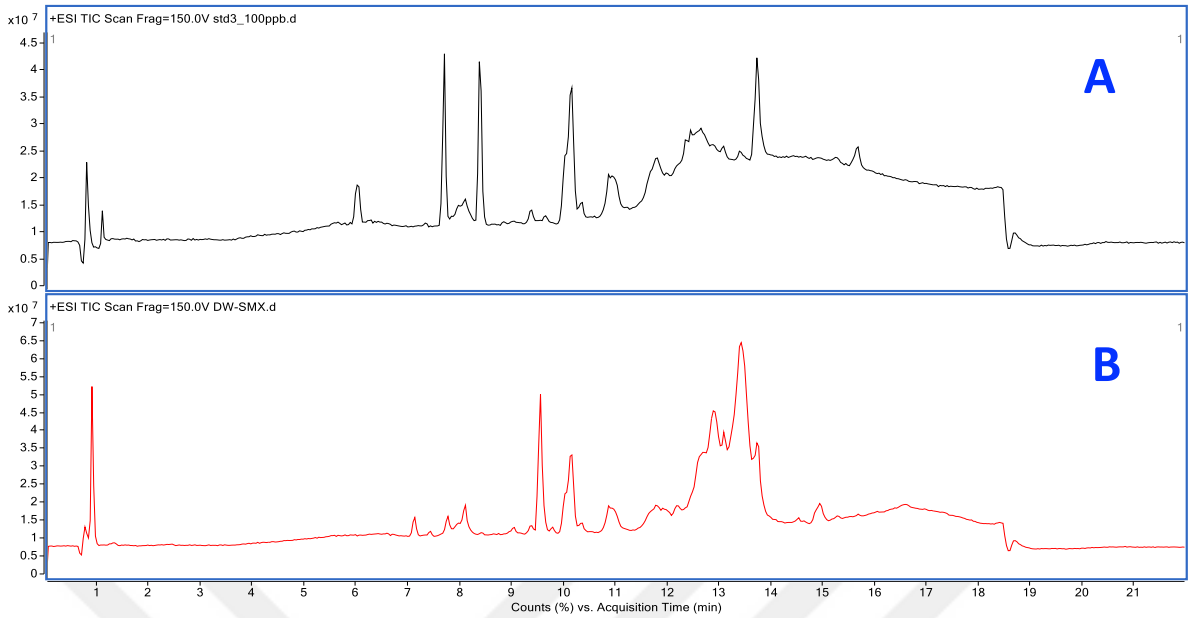


Figure 4. 12. (A) Chromatogram of three standards (CBZ, CLA, SMX) before-treatment; (B): chromatogram of the SMX after-treatment

When comparing the two chromatograms in Figure 4.13., it is evident that the molecular ion peak at m/z 254.0594 is clearly observed in (A) while it is absent in (B), indicating complete degradation of SMX after the treatment. There is a 10,000-fold difference in intensity between the y-scales of the two chromatograms; the intensity in (A) is 10^2, whereas in (B), it is 10^{-2}.

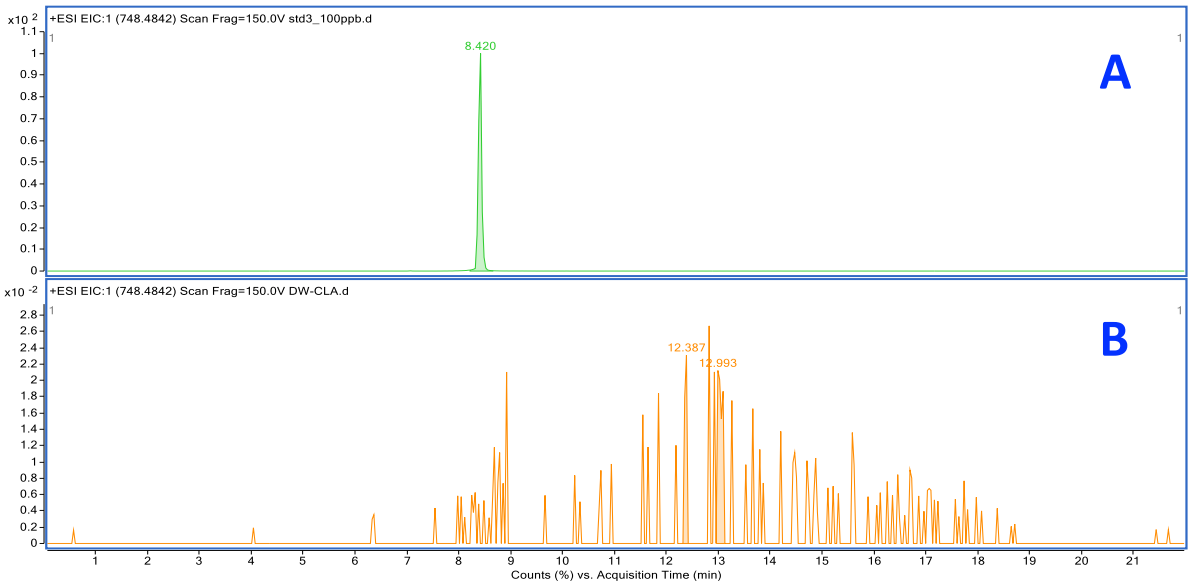


Figure 4. 13. EIC chromatograms of SMX solutions, (A) before-treatment; and (B) after-treatment

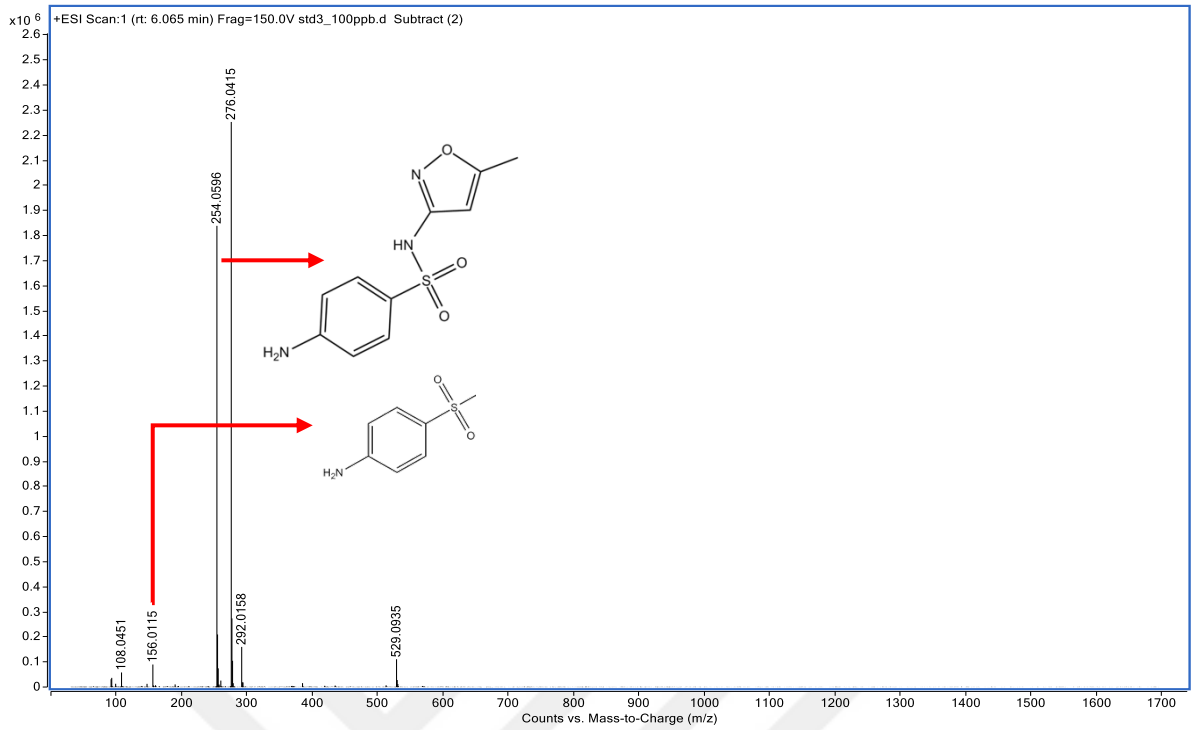


Figure 4. 14. Mass spectrum and molecular ion of SMX molecule and one its fragments (before-treatment)

In Figure 4.15, upon examining the mass spectrum after-treatment, the absence of the molecular ion at m/z 254.0594 suggests that the treatment process has fragmented the main Sulfamethoxazole molecule.

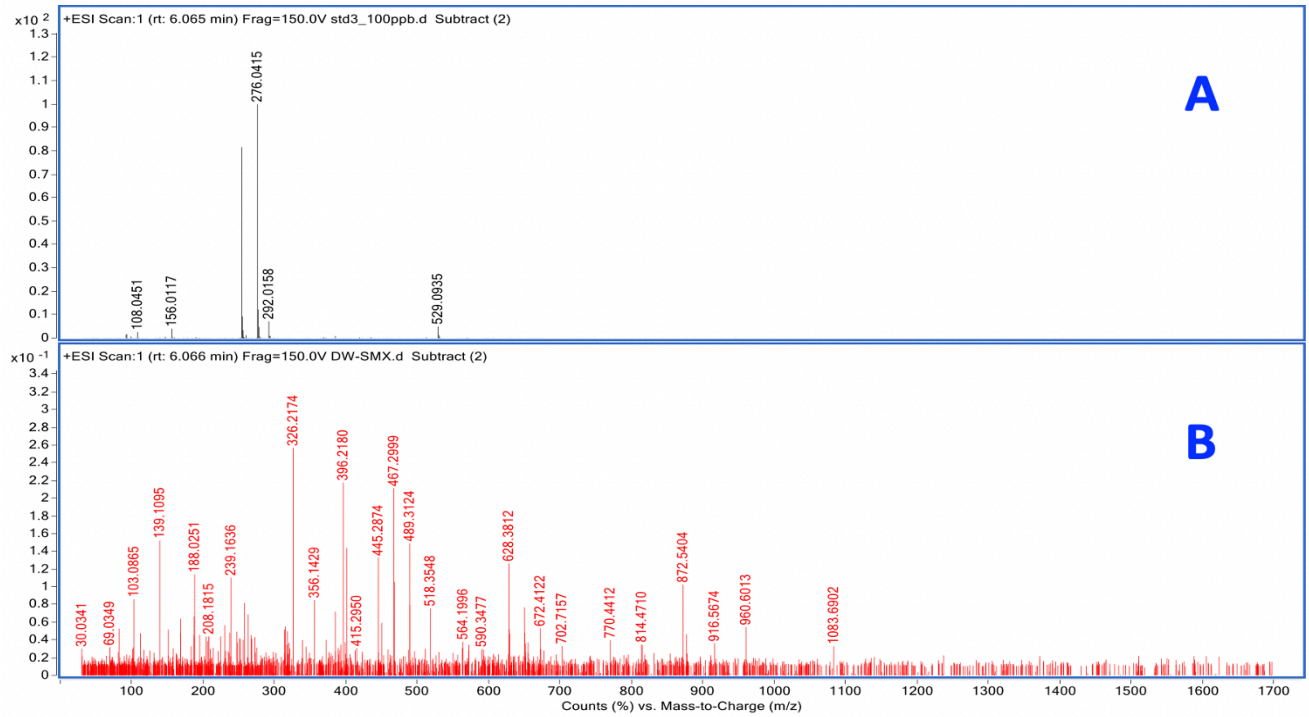


Figure 4. 15. Mass spectra of SMX, (A) before-treatment, and (B) after-treatment

The overlaid chromatograms shown in Figure 4.16. below were obtained by aligning the chromatograms of Before-Treatment (BT); After-Treatment (AT); and control samples, consisting of Acetonitrile (ACN) and Distilled Water (DW). The peaks indicated by red arrows solely represent the peaks associated with sulfamethoxazole after the treatment. These peaks are believed to be degradation products of Sulfamethoxazole resulting from the applied treatment process.

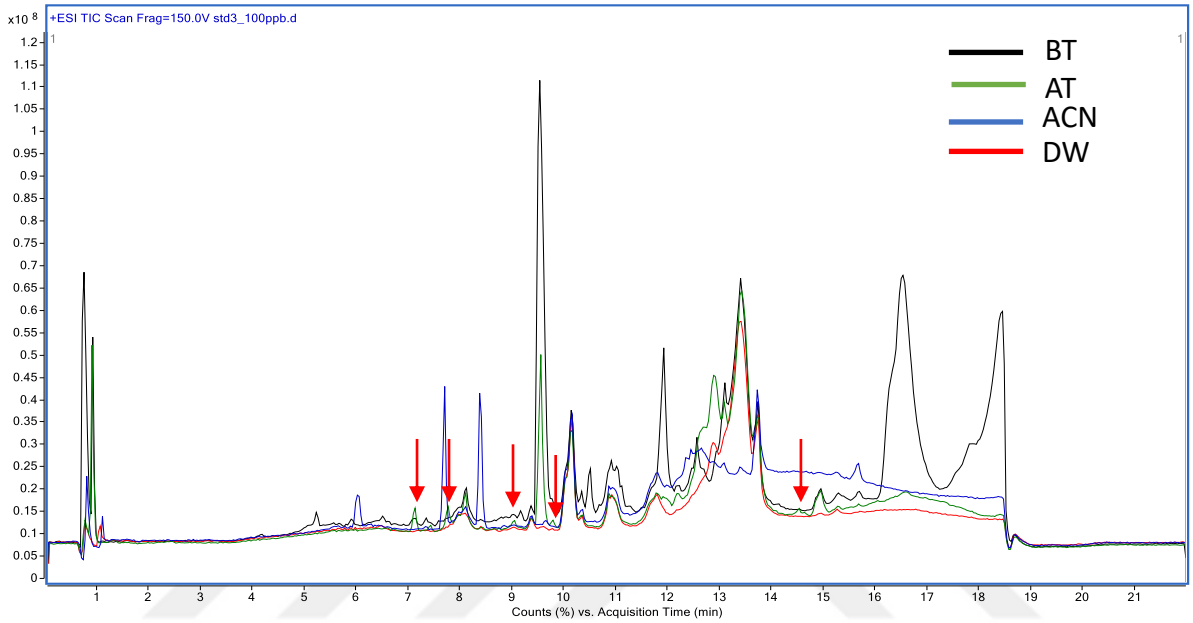


Figure 4. 16. Chromatograms of SMX before-treatment and after-treatment together with ACN and DW as control.

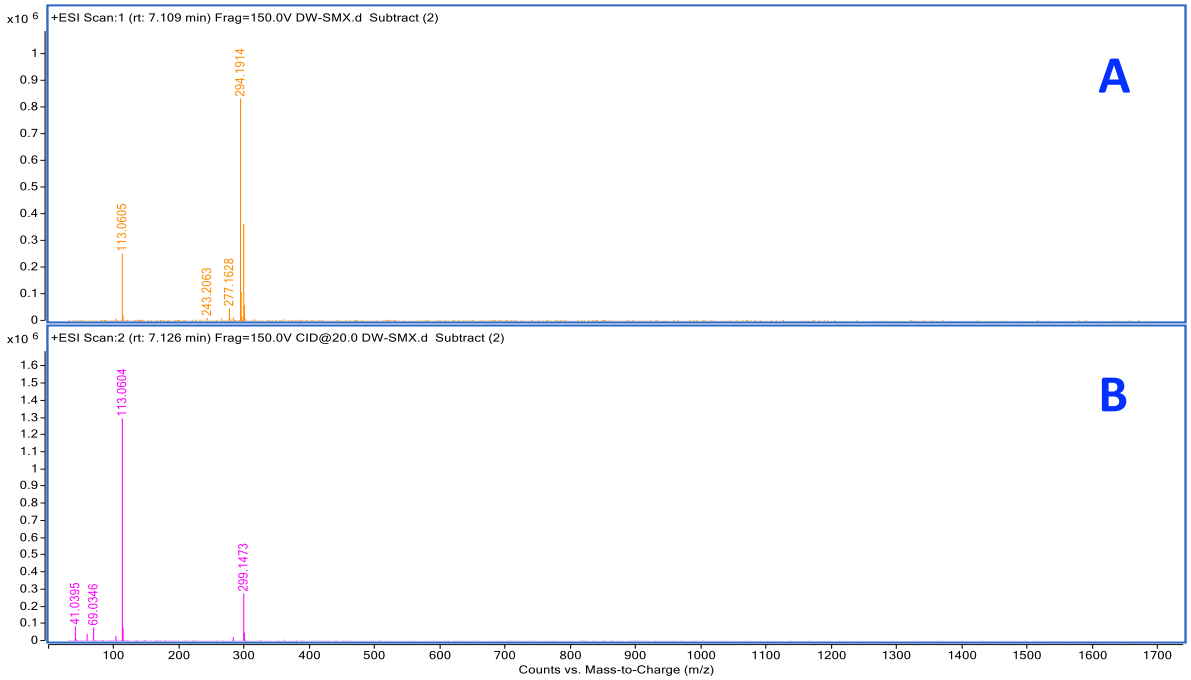


Figure 4. 17. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=7.109 belonging to the SMX degradation products (after-treatment)

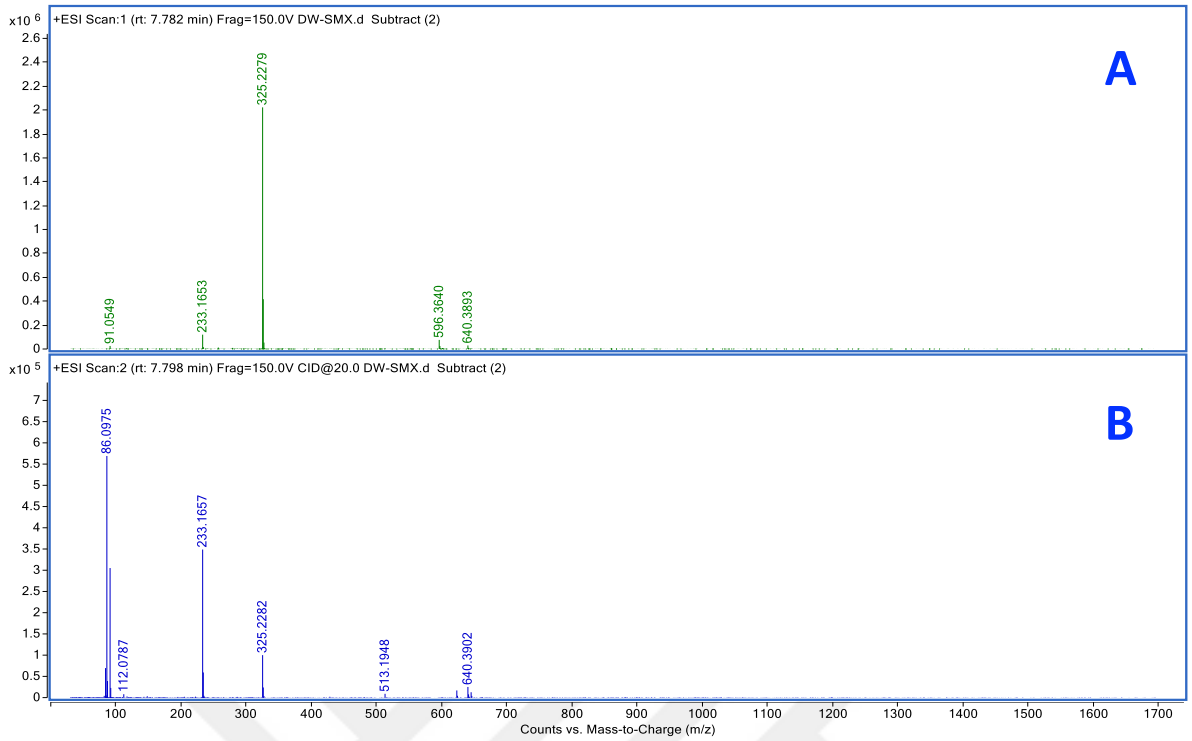


Figure 4. 18. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=7.782 belonging to the SMX degradation products (after-treatment)

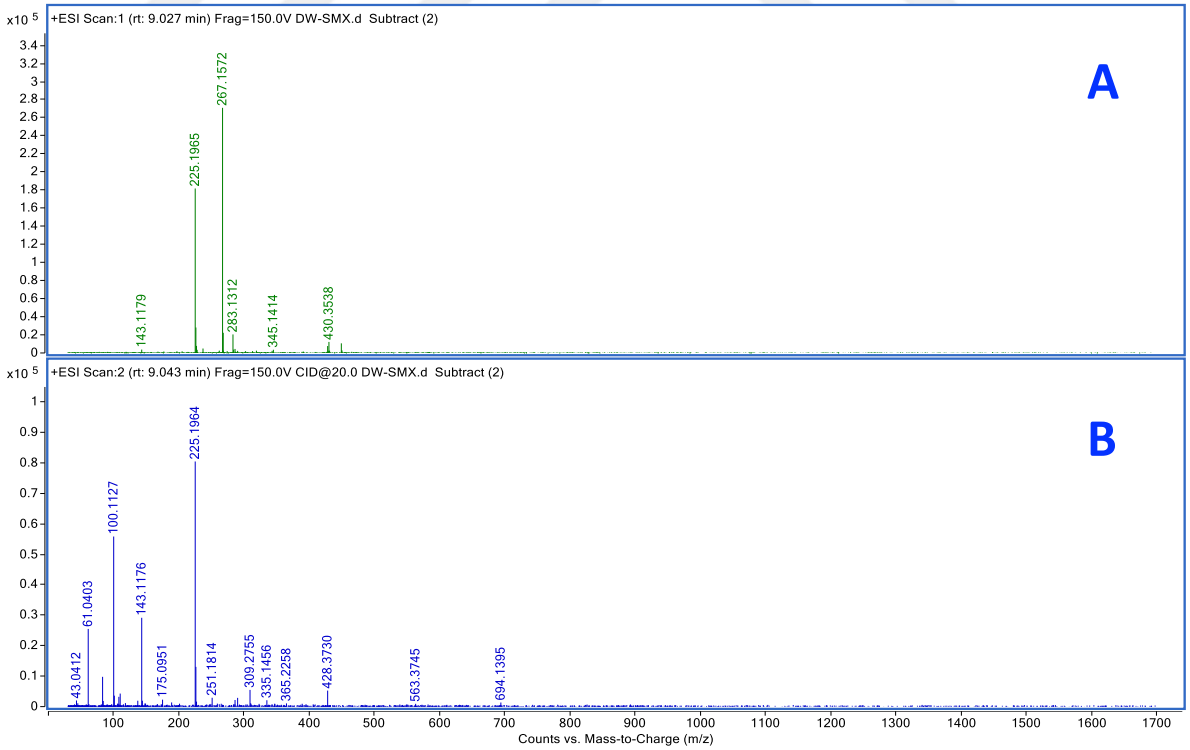


Figure 4. 19. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=9.027 belonging to the SMX degradation products (after-treatment)

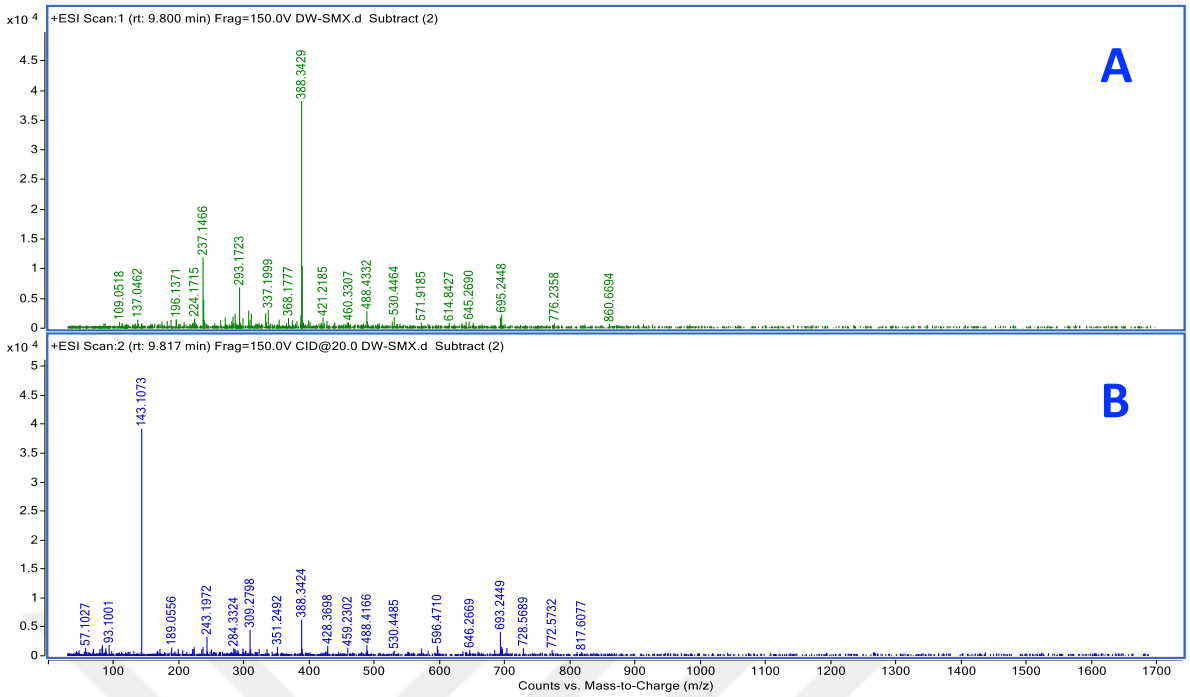


Figure 4. 20. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=9.8 belonging to the SMX degradation products (after-treatment)

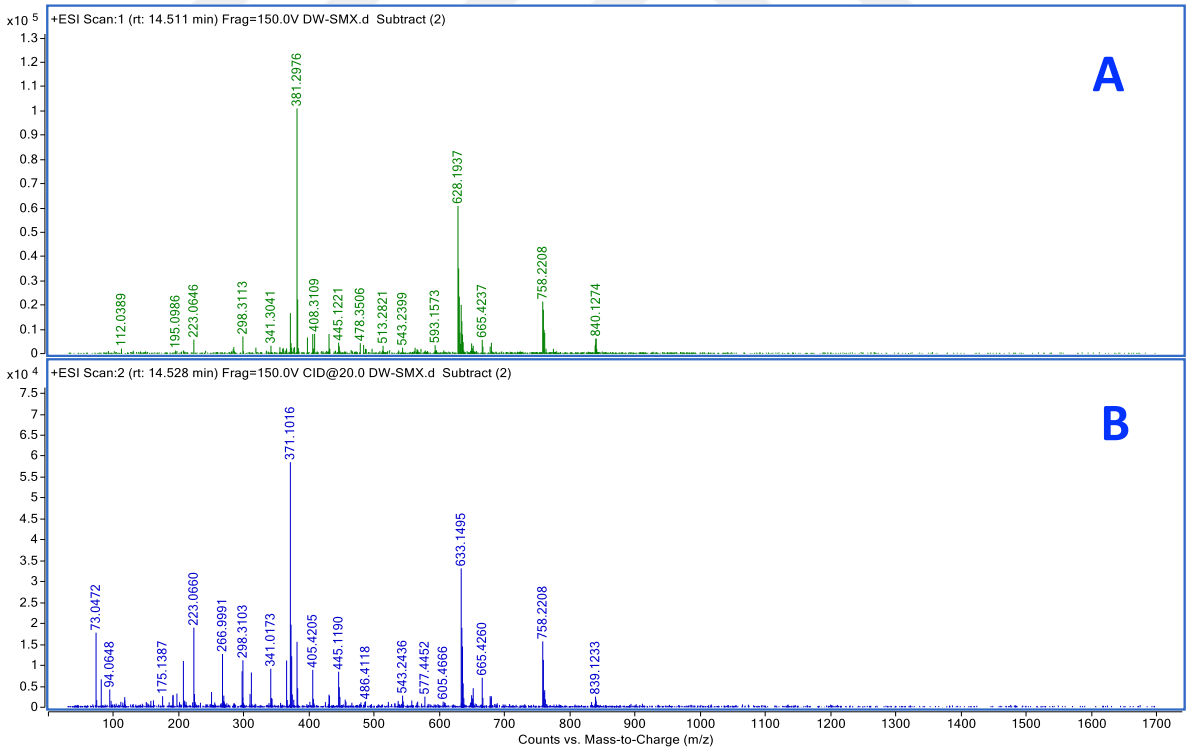


Figure 4. 21. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=14.5 belonging to the SMX degradation products (after-treatment)

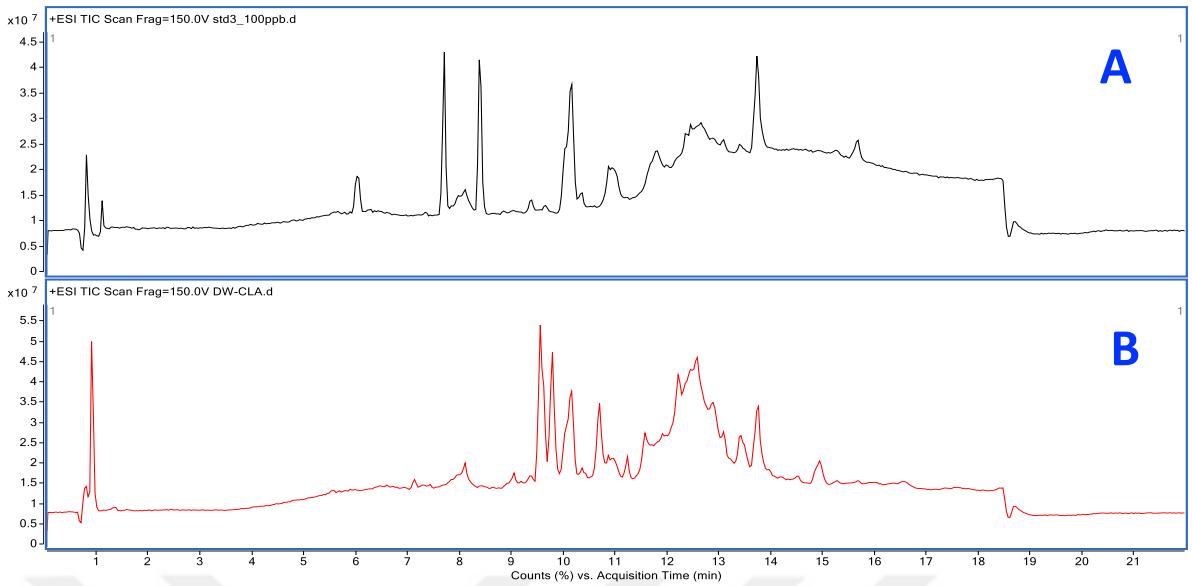


Figure 4. 22. (A) Chromatogram of CBZ, CLA, SMX standards before-treatment; (B): chromatogram of the CLA after-treatment

When comparing the two chromatograms in Figure 4.23, it is evident that the molecular ion peak at m/z 748.4842 is clearly observed in (A) while it is absent in (B), indicating complete degradation of Clarithromycin after the treatment. There is a 10,000-fold difference in intensity between the y-scales of the two chromatograms; the intensity in (A) is 10^2, whereas in (B), it is 10^{-2}.

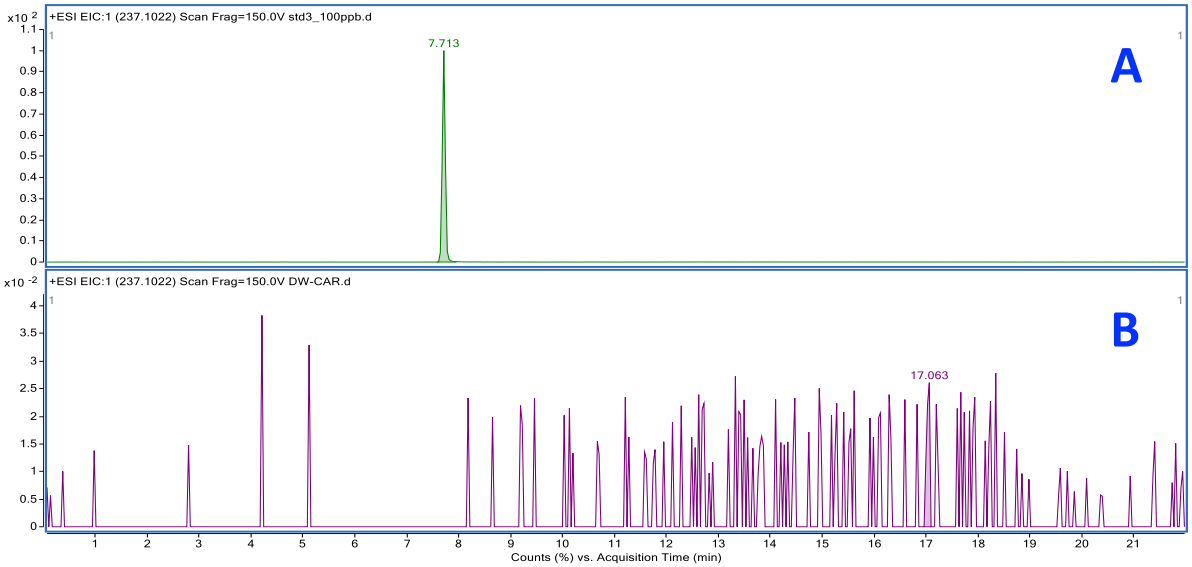


Figure 4. 23. EIC chromatograms of CLA solutions, (A) before-treatment; and (B) after-treatment

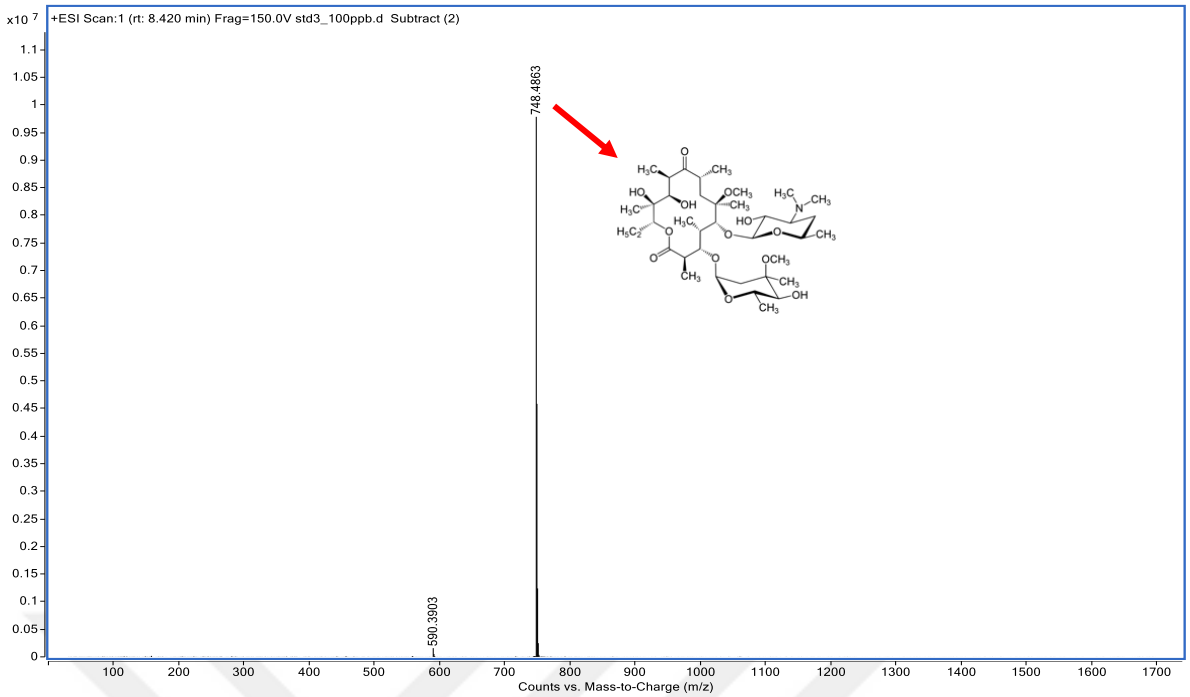


Figure 4. 24. Mass spectrum and molecular ion of CLA molecule (before-treatment)

In Figure 4.25, upon examining the mass spectrum after-treatment, the absence of the molecular ion at m/z 748.4842 suggests that the treatment process has fragmented the main Clarithromycin molecule.

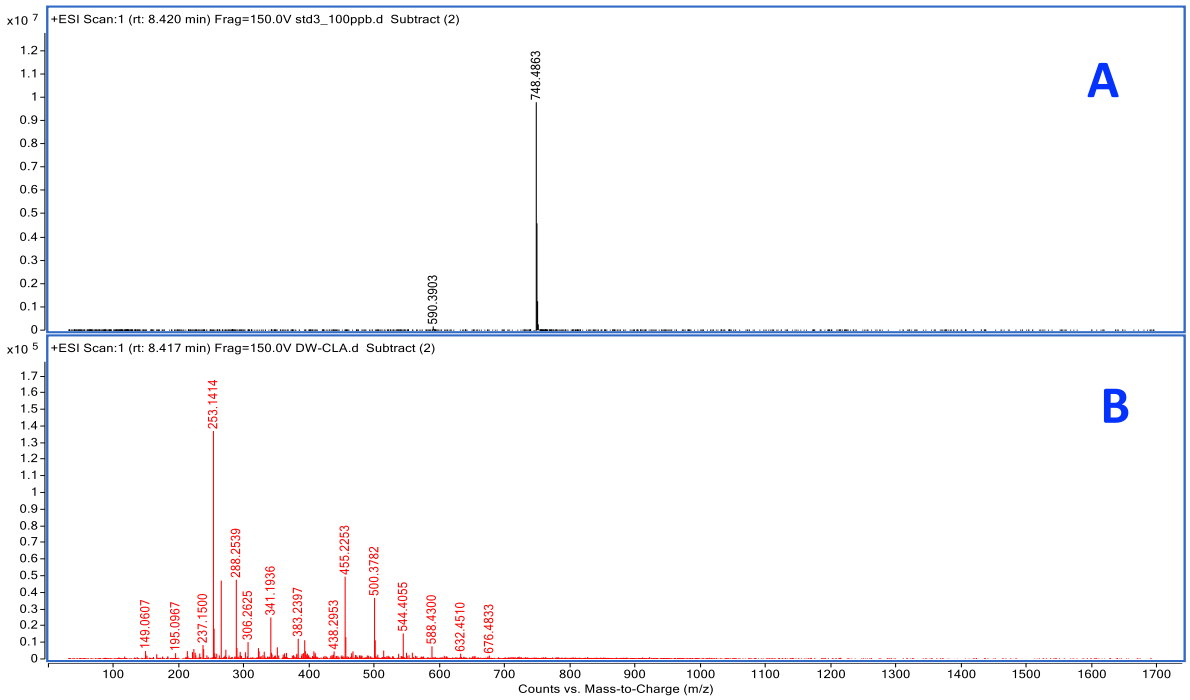


Figure 4. 25. Mass spectra of CLA, (A) before treatment, and (B) after-treatment

The overlaid chromatograms shown in the Figure 4.26 below were obtained by aligning the chromatograms of Before-Treatment (BT); After-Treatment (AT); and control samples, consisting

of Acetonitrile (ACN) and Distilled Water (DW). The peaks indicated by red arrows solely represent the peaks associated with CLA after the treatment. These peaks are believed to be degradation products of CLA resulting from the applied treatment process.

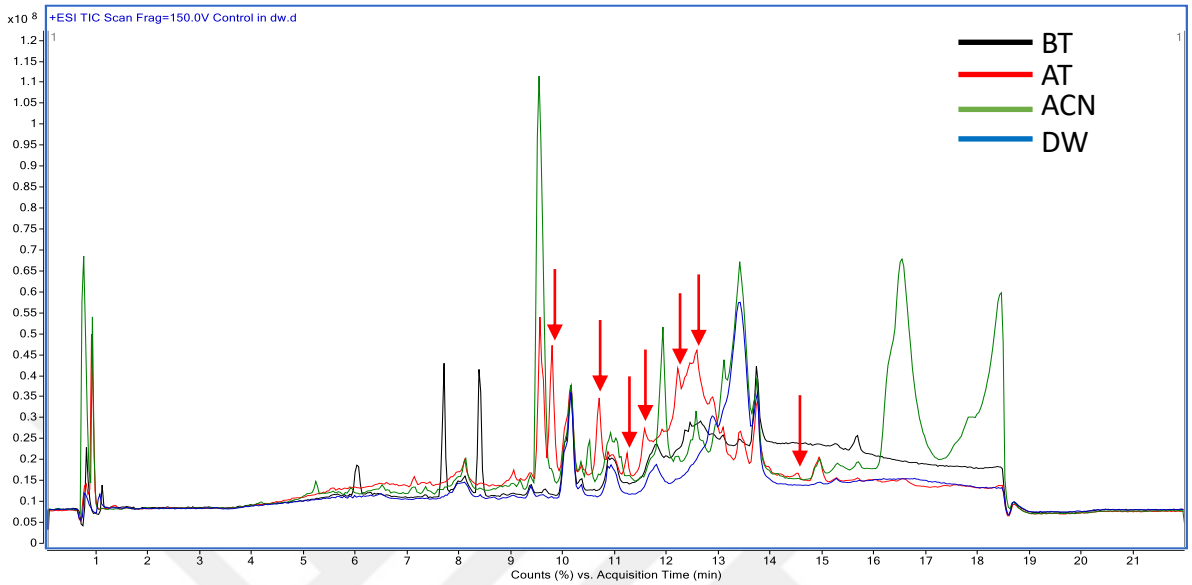


Figure 4. 26. Chromatograms of CLA before-treatment/ after-treatment with ACN and DW

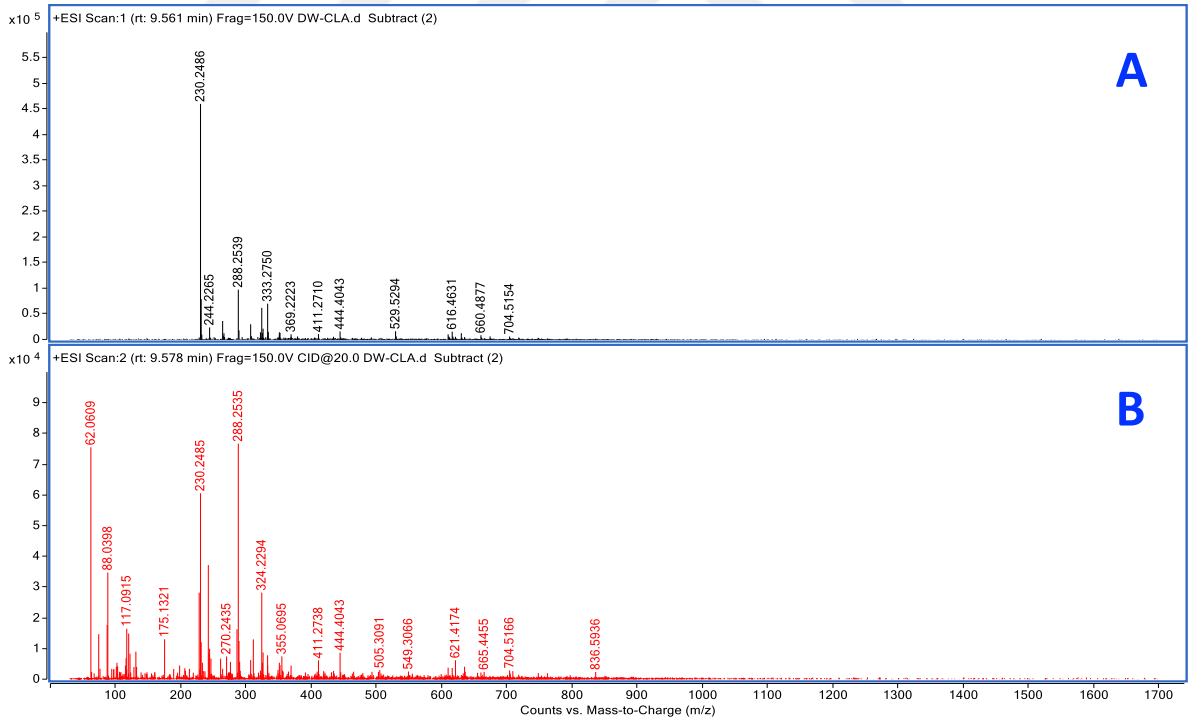


Figure 4. 27. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=9.561 belonging to the CLA degradation products (after-treatment)

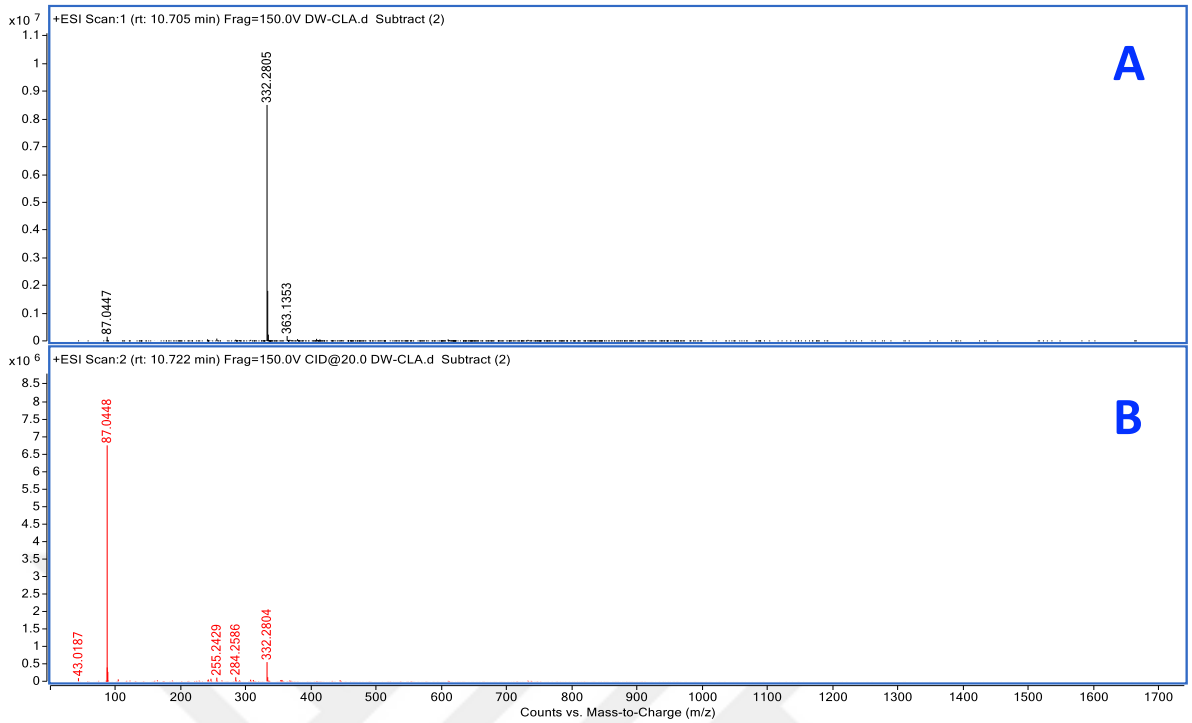


Figure 4. 28. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=10.705 belonging to the CLA degradation products (after-treatment)

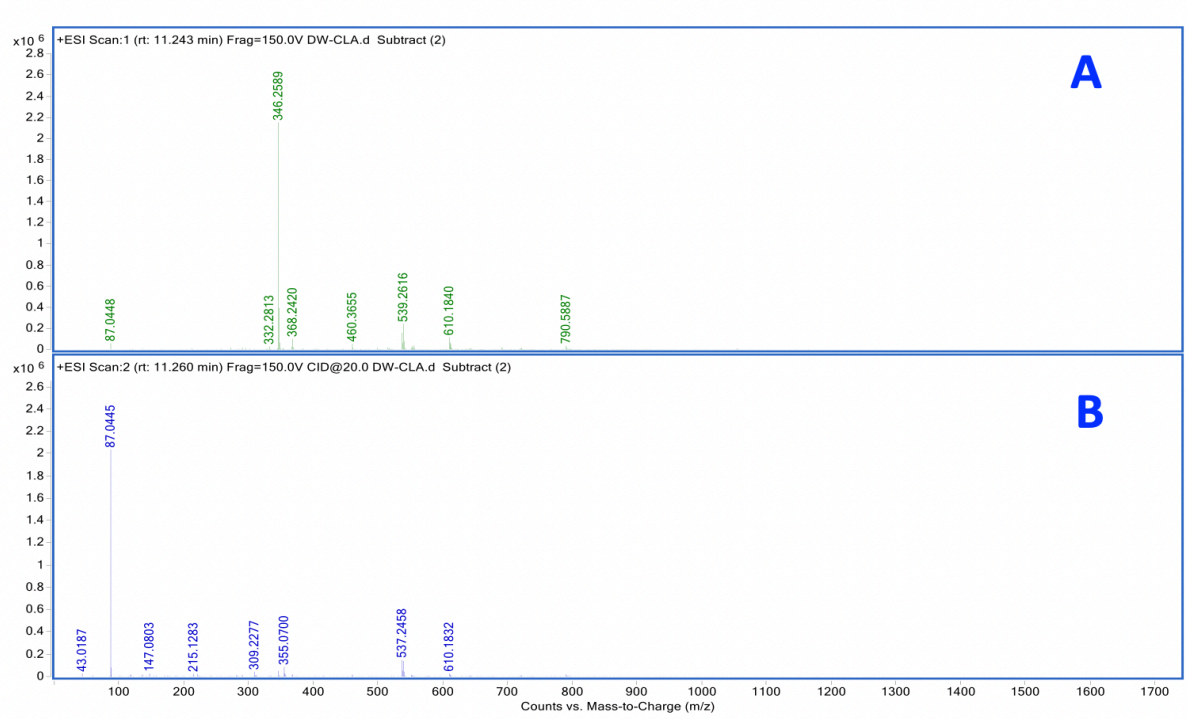


Figure 4. 29. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=11.243 belonging to the CLA degradation products (after-treatment)

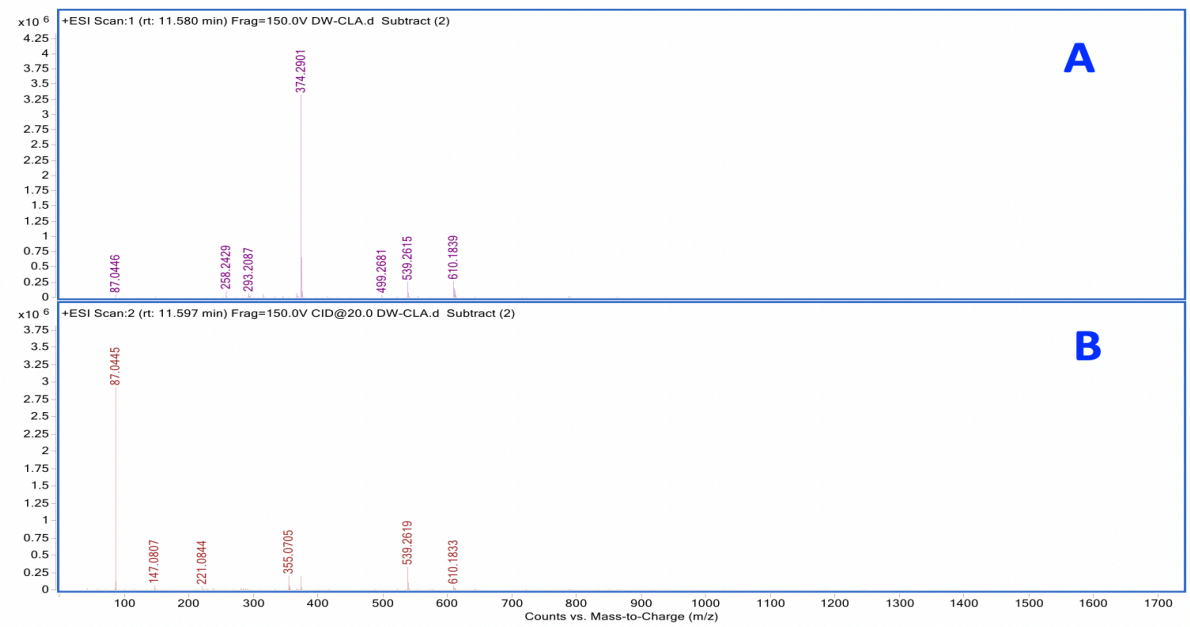


Figure 4. 30. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=11.58 belonging to the CLA degradation products (after-treatment)

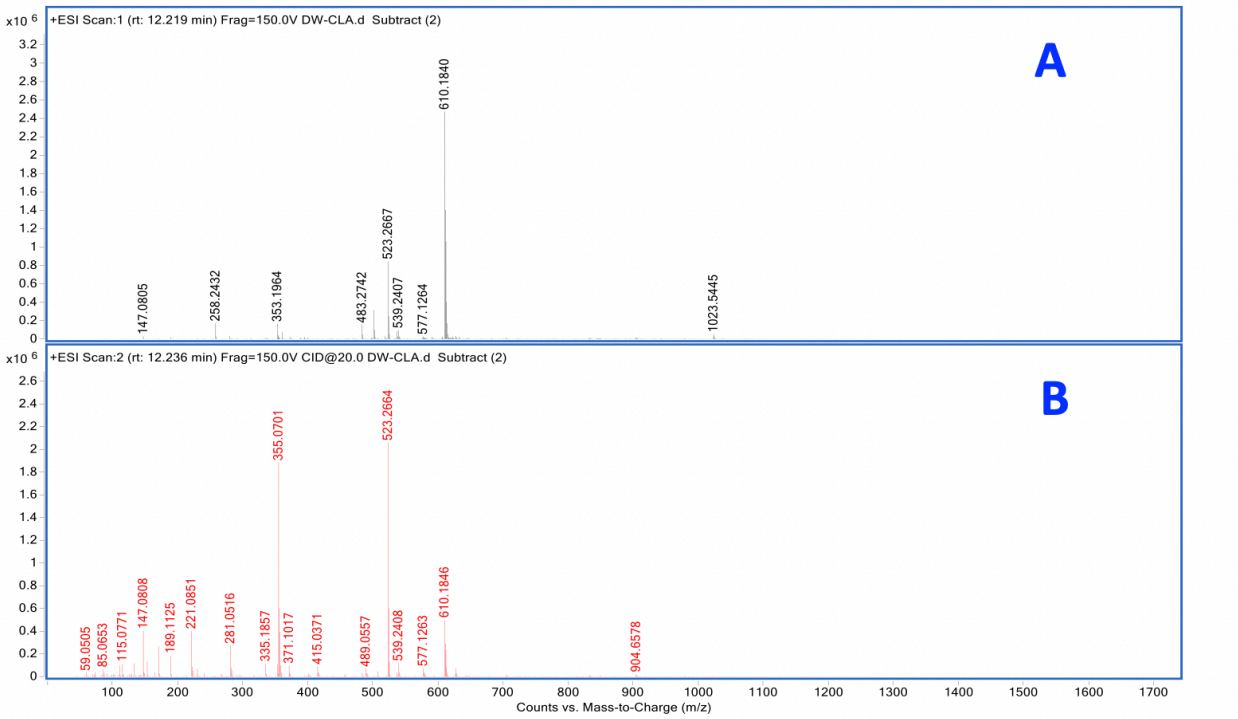


Figure 4. 31. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=12.21 belonging to the CLA degradation products (after-treatment)

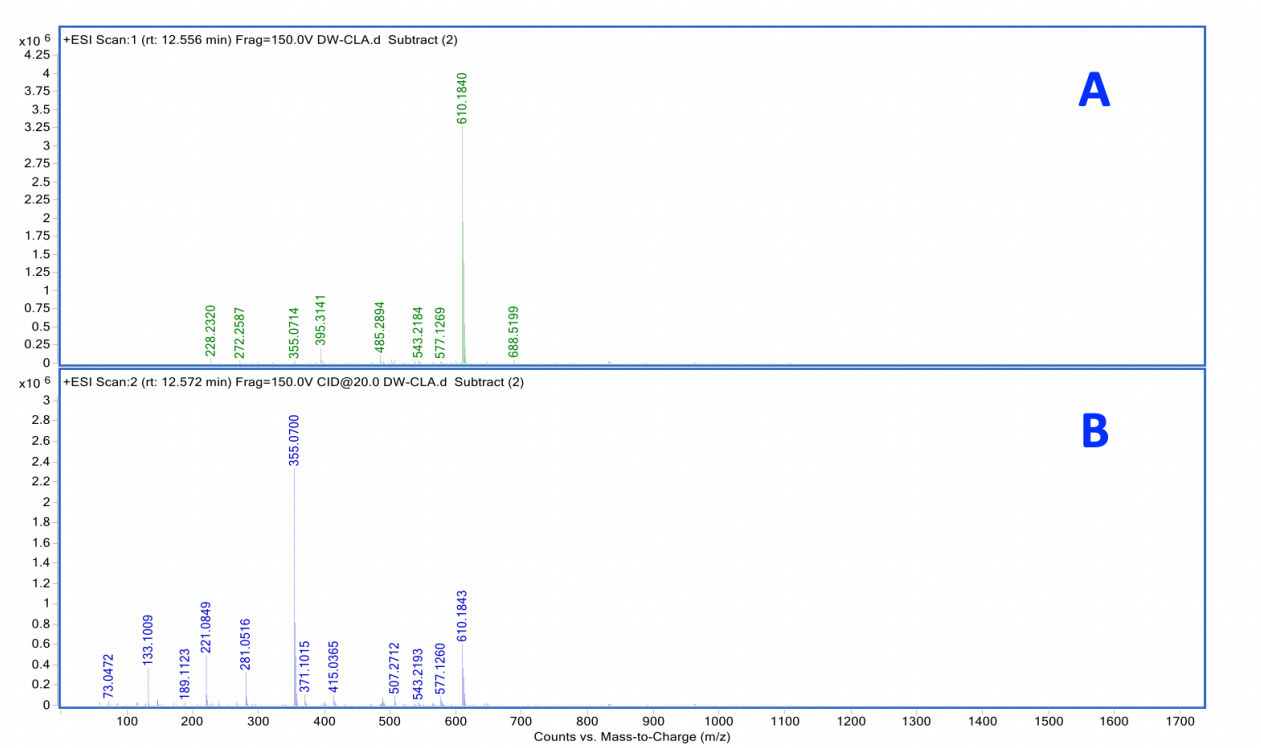


Figure 4. 32. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=12.556 belonging to the CLA degradation products (after-treatment)

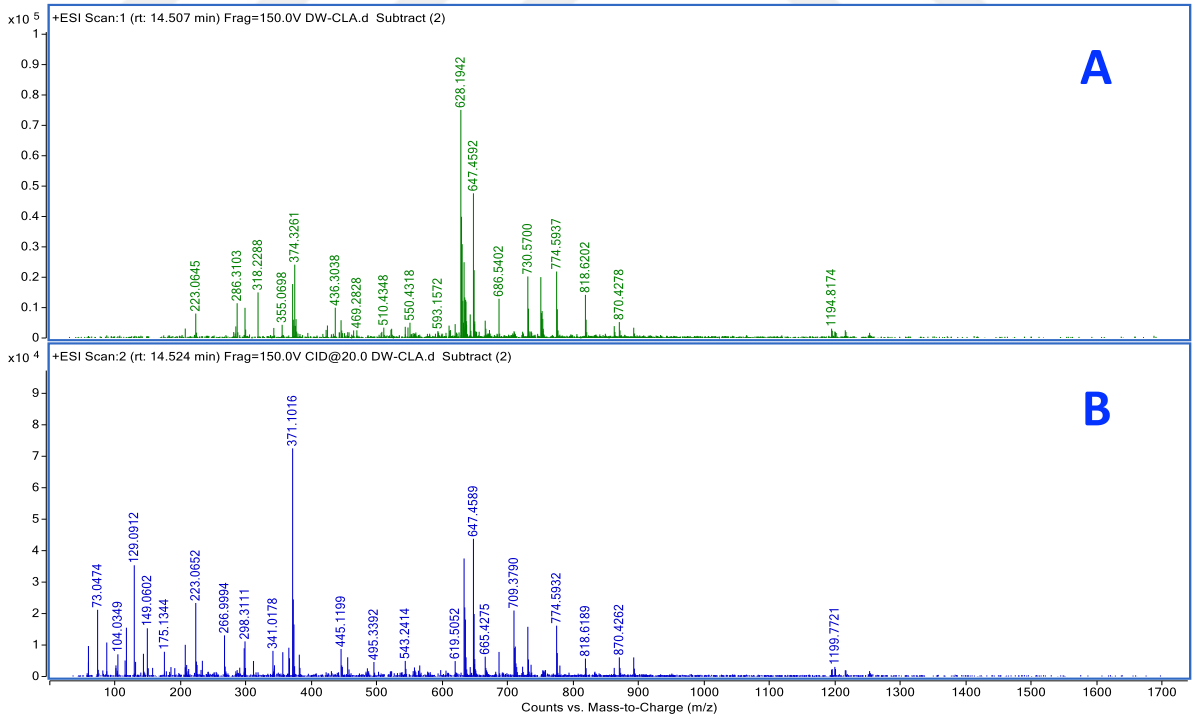


Figure 4. 33. Mass spectra of the scan 1 (A) and scan 2 (B) for the peak at Rt=14.507 belonging to the CLA degradation products (after-treatment)

The main result obtained from this part of the study is the demonstration that all three tested

PCs were completely degraded into other organic transformation products after treatment at an average concentration of 20 ng/L, which is the measured environmental concentration in the first part of the experimental study. The interpretation, including the relevant literature knowledge, regarding the potential chemical structures to which the pharmaceutical molecules may have transformed, is provided as follows.

The sulfamethoxazole metabolites identified in studies conducted by (Gómez-Ramos et al., 2011) and some other researchers were not identified in the data obtained from this study. An important reason for this is our intention to simulate the actual conditions to which individuals consuming drinking water are exposed. Therefore, in our treatment simulation, we included precipitation, ozonation, and chlorination, while Gomes-Ramos et al (2011). Only performed ozonation, and the chemical structures that could form with chlorination were not included. Furthermore, the ozonation process in our simulation was conducted for a shorter duration (3 minutes - representing the situation in ISKI) compared to Gomes-Ramos et al.'s study (7 minutes). Additionally, while Gomes-Ramos et al. ozonated sulfamethoxazole at a much higher concentration of 38 mg/L, whereas our simulation treated SMX at a concentration of 20 ng/L (representing the real environmental concentration). There is a difference of 1.9 million-fold between these concentrations. There are also differences in other parameters of the analysis method. All these differences may have contributed to the lack of similar results. In another study conducted by (Han et al., 2018) on CBZ, a separate ozonation study was performed, and the concentration used in their study was higher than the concentrations used in our simulation study. The absence of the peaks identified in this study regarding the degradation of CBZ in the literature could be attributed to these differences in experimental conditions.

4.3. Cytotoxicity Assessment of Drinking Water

The aim of this step is to see whether the potential cytotoxicity of the process-induced degradation products is different compared to the parent molecule. Lyophilized samples from the simulation experiments were tested for their cytotoxicity profile at different concentrations.

4.3.1. Results

Potential cytotoxicity of the three test substances, namely Carbamazepine (CBZ), Clarithromycin (CLA) and Sulfamethoxazole (SMX), were investigated before and after the water

treatment process. Water treatment process is composed of flocculation, ozonation and chlorination, respectively, and the major aim is removing the solid material and potentially pathogen bacteria to provide safe potable water. Ozonation and chlorination are chemically harsh processes that may cause the generation of various products with some electrophilic nature. Electrophilicity provides chemical reactivity to molecules, they react with nucleophilic chemicals instantly. In case such electrophilic molecules are formed in biological systems, they react with nucleophilic sites in the endogenous molecules such as proteins, carbohydrates, or DNA. Electrophilicity varies over a wide range, such molecules can react with nucleophiles instantly in less than a second, or they can travel from one biological site to another for some time and react at the final site. In this sense, highly reactive electrophiles that may be formed upon water treatment may be neutralized before reaching humans via drinking water and some may reach without reacting, or their reactivity may increase in cells via enzymatic pathways.

In literature, cytotoxic potential of CBZ, CLA or SMX has been studied at extremely higher concentrations compared to therapeutic levels or environmental levels (Han et al., 2018). This is usually done to determine the hazard of test compounds; what is the nature of their toxicity? Then this targeted toxicity is checked at the realistic concentrations whether it occurs or not. In this thesis, we tested cytotoxicity of CBZ, CLA and SMX at their environmental concentrations that we measured in Istanbul's selected drinking water sources with an average of 20 ng/L in the raw water, before treatment. We also used human-origin cells to test cytotoxicity, these are HepG2 cells from human liver and HK-2 cells from human kidneys. Available cytotoxicity data in literature regarding the test substances were usually provided by using cells from other species such as CHO cells from Chinese hamster to show the general potential toxicity (Han et al., 2018; Lau et al., 2022). However, our aim in this thesis is to test cytotoxicity of three representative molecules in water before- and after-treatment on cells from human origin. Most of the studies about the toxicity/cytotoxicity in literature focus on the impact of water treatment techniques per se (Barceló et al., 2020; Hübner et al., 2015; Sharma et al., 2018), rather than the tap water at the end of the treatment which is consumed by humans. Again, in order to observe the real situation, our experimental design focus on the completely treated water after flocculation, ozonation and chlorination. By this way, including human cells, actual concentrations and involvement of the final treated water, extrapolation of the cytotoxicity results to human exposure to pharmaceuticals via drinking water will be relatively easier. These pharmaceuticals, namely CBZ, CLA and SMX were chosen for potential cytotoxicity as representative environmental pollutants because of several reasons:

First, they are from different pharmacological classes and therefore represent a wide range of environmental pharmaceuticals. Second, CLA and SMX are antibiotics, and they potentially have adverse effects also on environmental species from monocellular to multicellular organisms. These adversities comprise direct cytotoxic effect to multicellular animals, or monocellular beneficial organisms. Third, these antibiotics may contact pathogenic bacteria and viruses in the environment at low concentrations which may cause developing antibiotic resistance against these and similar chemical structures. The bad consequence is that such antibiotics may be inefficient in clinics when they are used in patients to cure infections. Fourth, different than this expected adversity, these PCs may cause different/unexpected toxic effects in aquatic, terrestrial or avian species (Bilal et al., 2020). From the holistic view, each affected organism may negatively affect other species including humans, either directly or indirectly.

Toxicity is a very broad term, and there are many different types of toxicities such as acute or chronic (single dose and repeated dose for several decades) when the duration and frequency of exposure are considered, genotoxic, carcinogenic, teratogenic etc. when special toxicities are considered, and neurotoxic, cardiotoxic, hepatotoxic, nephrotoxic, myelotoxic etc. when target organ or tissue is considered. Cytotoxicity of chemicals is the first step of toxicity tests done with biological material. Apparently, it shows the essential cellular adversity regardless of special or target organ toxicity, although such rough extrapolations can be made depending on the origin of the cell such as liver (HepG2 cells etc.) or kidney (HK-2 cells etc.) like in this thesis. Cytotoxicity tests generally provide a signal that the test compound is likely to cause an adverse effect in the organism, and it should be tested with more specific tests to reveal the toxicity target at subcellular, biochemical and organ level. By testing the raw water samples before- and after the treatment, we basically showed (1) if this initial toxicity is diminished, increased, or remains the same, and (2) if there is a need for further toxicity investigation. Our present findings below warrant further research on the toxicity profile of degradation products of the three test pharmaceuticals, especially in the tap water.

Figure 4.34. represents the concentration dependency of cytotoxicity of CBZ, CLA and SMX on HepG2 cells before treatment, i.e. the parent molecules that were dissolved in distilled water. We aimed to test the cytotoxic potential of only the test compound, therefore we dissolved each PC in distilled water. Raw water already contains numerous pollutants as our data in this thesis also indicated above. If raw water is used to dissolve test compound, it is not possible to differentiate whether any cytotoxic effect belongs to the test compound or any other pollutant in raw water. Medium controls (without any test substance) at all graphs were shown as a continuous dashed line

at the level of 100%. The lowest concentration of the substances is 20 ng/L (the 1st white bars), which is equal to the average environmental concentrations measured in the first part of the experimental study. The following concentrations are 8, 10 and 100-fold higher, respectively (light grey, dark grey and black columns, respectively).

This concentration escalation was done to determine:

a) cytotoxicity in case of same pollutants somehow are concentrated in wastewater or drinking water in the environment, and,

b) whether any potential cytotoxic effect follows a dose dependency.

Triton-X was used as positive control, its surfactant property causes cell death and proves whether the cell culture model works properly or not. As seen, the expected cytotoxicity was observed around 90 % cell death at 171 µM of Triton X.

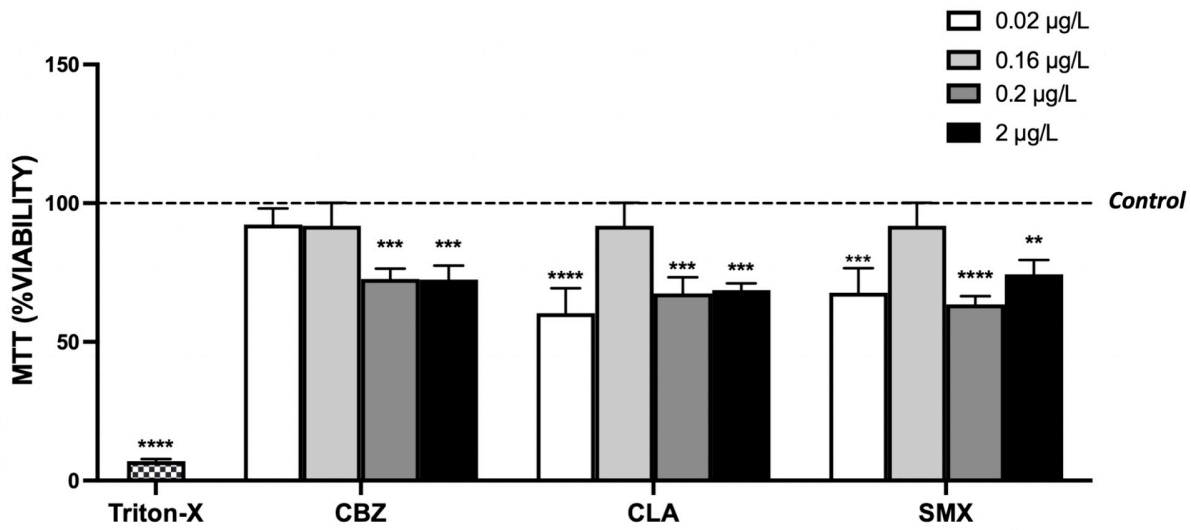


Figure 4. 34. Concentration-dependent cytotoxicity of the test substances spiked into distilled water on HepG2 cells by MTT test before treatment. Triton-X (171 µM) was used as a positive control. CBZ, CLA and SMX. *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 compared to the medium control (dashed line in the graphic)

CBZ before treatment did not cause cytotoxicity at 0.02 and 0.16 µg/L, while the following 0.2 and 2 µg/L concentrations resulted in around 29% cytotoxicity. Interestingly CLA caused 41% cytotoxicity at the lowest concentration, while there was no cytotoxicity at 8-fold higher concentration. Then, cytotoxicity is back with 33% at higher concentrations. A very similar pattern was observed with SMX in Figure 4.34. Such unexpected phenomena have been seen with some other chemicals in literature, although mostly with endocrine disruptors and not frequently. Such

dose-response relationships differ from the linearity and are explained with a special term as nonmonotonic dose-response relationship (Vandenberg et al., 2012).

A similar cytotoxicity test was performed with human kidney cells, HK-2. CBZ behaved differently in these cells, there was no cytotoxicity at any concentrations, hence, 0.16 µg/L caused a marginal increase in viable cell number by 16%. CLA caused cytotoxicity at only the lowest 0.02 µg/L with 23%, and higher concentrations were not effective. SMX exerted 21% cytotoxicity only at the highest concentration, 2 µg/L (Fig. 4.35.). Compared to HepG2 cells, HK-2 cells seem more resistant to toxicity of the test substances before treatment.

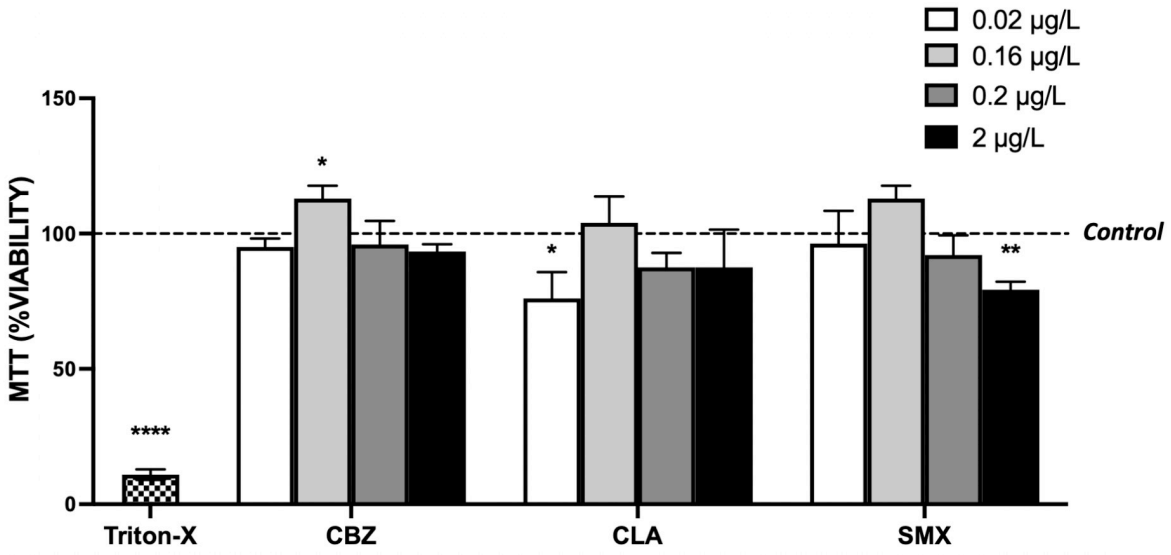


Figure 4. 35. Concentration-dependent cytotoxicity of the test substances spiked into distilled water on HK-2 cells before-treatment by MTT test. Triton-X (171 µM) was used as a positive control. CBZ, CLA and SMX . *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 compared to the medium control (dashed line in the graphic)

Next to the cytotoxicity profile of the test substances before treatment, we conducted similar experiments to determine the cytotoxicity profile of the test substances after the simulation of the drinking water treatment process, only the difference was the concentration. Treated samples were freeze-dried first, and then dissolved in distilled water to provide the parent molecules' their average environmental concentrations (AEC), as well as 10-fold higher concentrations. As our LC tandem MS analyses proved, parent test substances were completely degraded to several chemically unknown transformation products for each PC. This shows that the cytotoxicity observed in after-treatment samples will be free of the effect of the parent substance on one hand and will represent the sum of the cytotoxic effects of the degradation products on the other hand.

Figure 4.36. represents the cytotoxicity of the test substances spiked into distilled water in HepG2 cells at AEC (white column) and 10-fold higher concentrations (black columns). CBZ and CLA did not cause cytotoxicity at both concentrations, while SMX caused 24% and 37%, respectively.

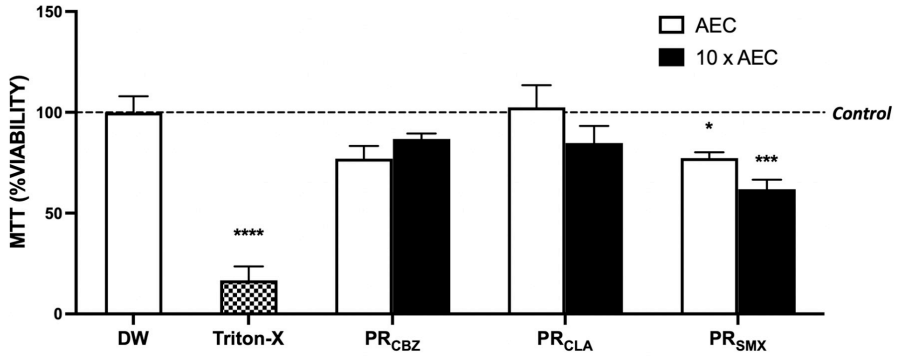


Figure 4. 36. Concentration-dependent cytotoxicity of the test substances spiked into distilled water on HepG2 cells after-treatment by MTT test. Triton-X (171 µM) was used as positive control. AEC (Average Environmental Concentration which is 20 ng/L), PR_{CBZ}, PR_{CLA} and PR_{SMX}, degradation products of each test substance after treatment, CBZ, CLA, and SMX. *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 compared to medium control (dashed line in the graphic)

Figure 4.37. is the counterpart of the same experiment in HK-2 human kidney cells. The cytotoxicity of the degradation products, i.e. after treatment samples was pronounced in these cells as both concentrations of degradation products of CBZ and lower concentration of CLA exerted statistically significant cytotoxicity with 38% (p<0,0001), 28% (p<0.001) and 18% (p<0,05) , respectively. A significant toxicity with 25% (p<0,01) and 23% (p<0,01) was also observed for the degradation products of SMX at both concentrations.

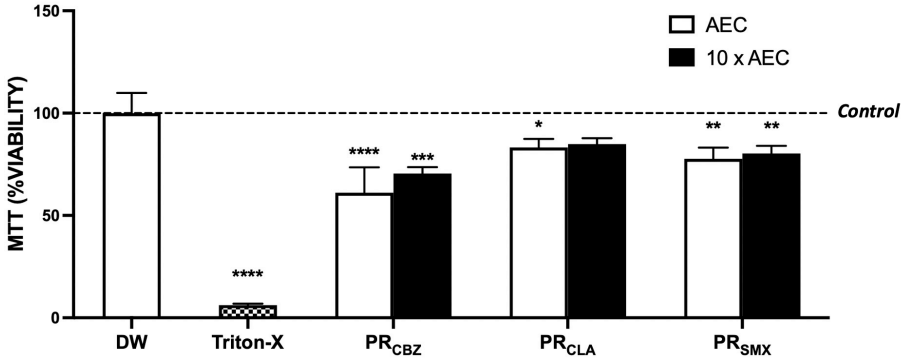


Figure 4. 37. Concentration-dependent cytotoxicity of the test substances spiked into distilled water on HK-2 cells after-treatment by MTT test. Triton-X (171 µM) was used as a positive control. AEC (Average Environmental Concentration), PR_{CBZ}, PR_{CLA} and PR_{SMX}, degradation products of each test substance after treatment. CBZ , CLA and SMX. *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 compared to the medium control (dashed line in the graphic)

Following cytotoxicity testing of the 3 test substances (before- and after-treatment) spiked into distilled water, they were also spiked into raw water that were sampled Ömerli Plant. These samples spiked into raw water are treated as the former samples spiked into distilled water (Figures 4.34.-4.37.) with flocculation, ozonation and chlorination. The difference between these samples was the samples from raw water intrinsically contain numerous environmental contaminants including probably the test substances we spiked additionally too. In this case, after treatment samples theoretically contain diverse degradation products of also differentt parent chemicals, as well as extremely stable parent chemicals to ozonation and chlorination. The aim here was to see how the cytotoxicity of this mixture differs compared to the cytotoxicity of samples spiked only with one of the test substances. Figure 4.38 summarizes these comparisons between the cytotoxicity of each PC before- and after the treatment in the same diagram for both HepG2 and HK-2 cells. After-treatment samples were tested only at the two concentrations of the parent molecules, at AEC and 10-fold higher AEC. These concentrations correspond to the first and the third column of Figures 4.34. and 4.36.

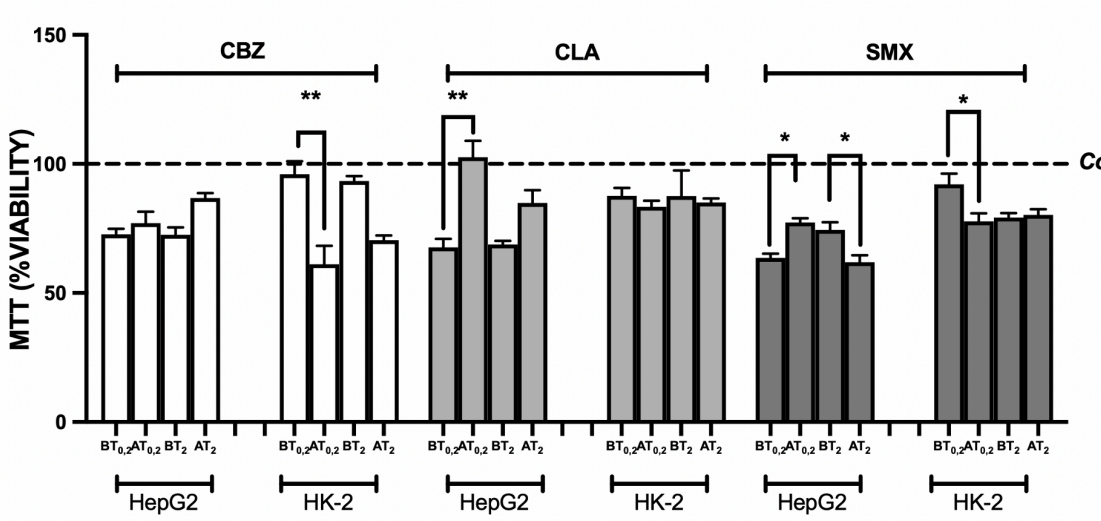


Figure 4. 38. The cytotoxicity of the test substances spiked into distilled water before- and after treatment on HepG2 & HK-2 cells by MTT test. AEC=0,2 µg/L; 10xAEC=2 µg/L CBZ, CLA and SMX, parent molecules; BT: Before-treatment; AT: After-treatment. *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 compared to the before-treatment.

CBZ cytotoxicity is increased significantly at AEC in HK-2 cells after-treatment compared to before-treatment. When the concentration increased 10-fold (200 ng/mL), then the increased cytotoxicity remains still higher in HK-2 cells, but not as significantly as at AEC (Figure 4.38.).

Treatment of CLA caused a significant increase in cell viability at AEC (20 ng/mL) concentration in HepG2 cells ($p<0,01$), while it did not affect the cytotoxicity of CLA in HK-2 cells. There was a tendency to increase in cell viability in the presence of 10-fold (200 ng/L) CLA in HepG2 cells, however, this was not significant due to relatively higher standard deviations. For SMX, in the same manner with CLA, the treatment caused significant increase in cell viability at AEC (20 ng/mL) concentration, but a significant increase ($p<0,05$) in cytotoxicity at 10-fold (200 ng/ml) in HepG2 cells. Treatment of SMX caused a significant increase ($p<0,05$) in cytotoxicity at AEC (20 ng/L) in HK-2 cells as well (Figure 4.38.).

Figure 4.39 and 4.40 represent cytotoxicity of these raw water-spiked samples after treatment in HepG2 cells and HK-2 cells, respectively. To exclude the matrix effect (all contaminants in raw water without spiking), non-spiked raw water sample was also treated and tested in cells. The first white columns represent this sample. In HepG2 cells, there was no statistically significant cytotoxicity of raw water after-treatment (Fig. 4.39., the 1st white column), while it caused a significant cytotoxicity in HK-2 cells (Fig. 4.40., the 1st white column). However, none of the test substances caused cytotoxicity at the AEC, as well as 10-fold higher concentrations in both cell types compared to medium control that represented in the graph by the dashed line. There was a tendency to decrease in viable cell number at the higher concentrations of CBZ and SMX in HepG2 cells, but these were statistically not significant because of the relatively high standard deviation of at least three independent experiments (Figure 4.39.).

On the contrary, CBZ caused a marginal but significant increase in cell viability at both concentrations compared to the medium control in HK-2 cells (Figure 4.40.). When comparing the cytotoxicity of the degradation products of the substances to the cytotoxicity of treated raw water (RW+T), all products at both concentrations caused significant decreases (Figure 4.40.). This is also interesting because the presence of each test substance seems to prevent the cytotoxicity of the treated raw water. This can be explained by the instant reaction of electrophilic substances that are formed in the treatment process from the spiked molecules with the nucleophilic substances (dissolved or suspended organic biological molecules such as proteins, amino acids, etc.) which are intrinsically present in the raw water. This finding is perfectly in line with the literature. In a comprehensive review, it is mentioned that most degradation products have been identified in controlled laboratory experiments, but not in real drinking water (Postigo & Richardson, 2014). However, it remains unknown why same does not occur for the potentially reactive degradation products of intrinsically present contaminants in raw water. The most plausible explanation may be the sampling time difference; the first raw water samples collected for analysis of pharmaceutics in

March 2020, while the present raw water sample for the cytotoxicity was collected in March 2023. Chronological changes in the contaminant composition of raw water are very likely, and this may have caused the difference in cytotoxicity.

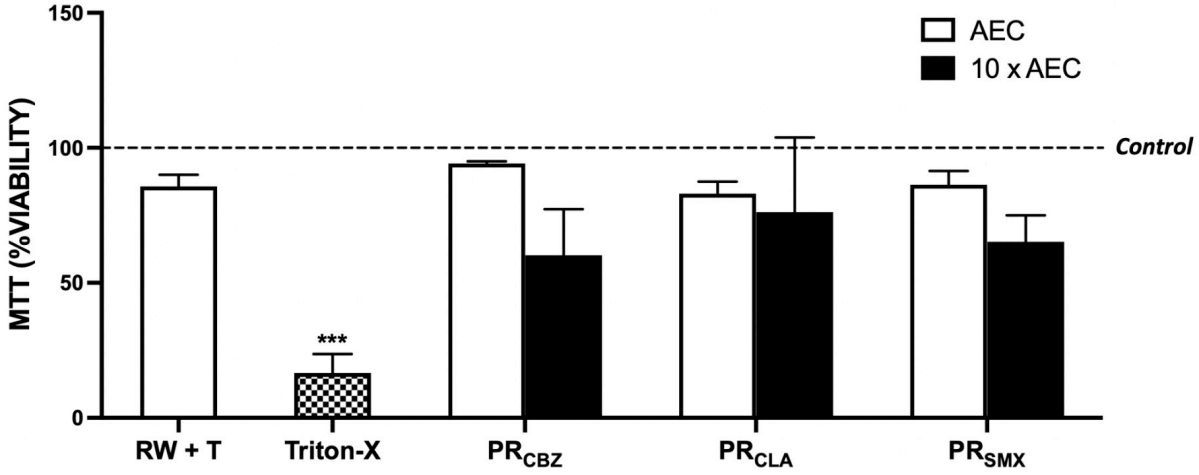


Figure 4. 39. Concentration-dependent cytotoxicity of the test substances spiked into raw water on HepG2 cells after treatment by MTT test. Triton-X (171 µM) was used as positive control. AEC (Average Environmental Concentration which is 20 ng/L), RW+T (Raw Water + Treatment), PR_{CBZ}, PR_{CLA} and PR_{SMX}, degradation products of each test substance after treatment. ***p<0.001 compared to the medium control (dashed line in the graphic)

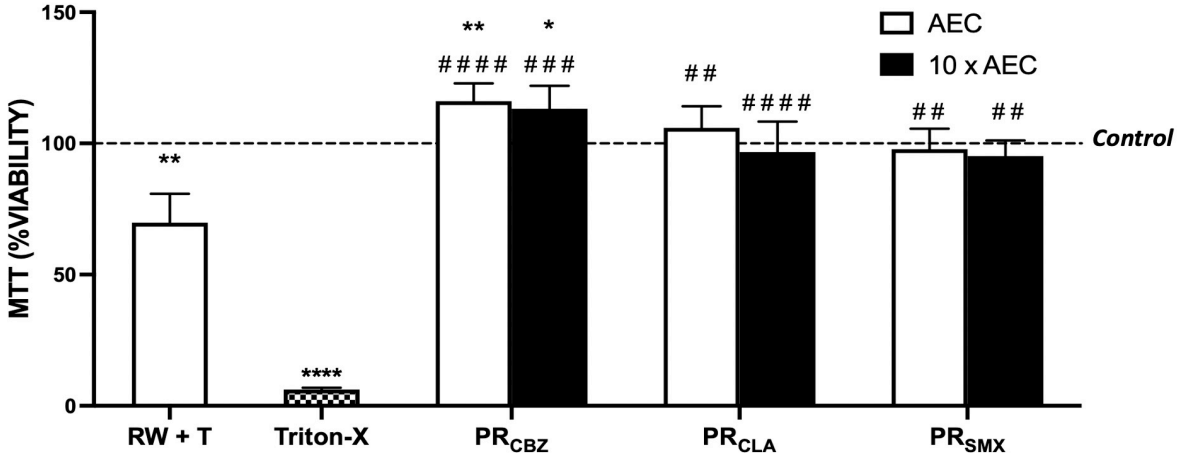


Figure 4. 40. Concentration-dependent cytotoxicity of the test substances spiked into raw water on HK-2 cells after-treatment by MTT test. Triton-X (171 µM) was used as positive control. AEC (Average Environmental Concentration), RW+T (Raw Water + Treatment), PR_{CBZ}, PR_{CLA} and PR_{SMX}, degradation products of each test substance after treatment. *p<0.05 and **p<0.01, compared to the medium control (dashed line in the graphic), and #p<0.05, ##p<0.01, ###p<0.001, and ####p<0.0001 compared to RW+T.

Lastly, we also compared cytotoxicity of raw water before and after the treatment in both HepG2 cells and HK-2 cells as it is and at 10-fold higher concentrations (Figs. 4.41. and 4.42.). In addition, cytotoxicity of Istanbul tap water was also tested in cells as it is, and 10-fold concentrated by freeze drying and redissolved to see whether toxicity is changed at the other end of the piping system, and whether concentration is somehow increased.

In this experimental setting, raw water samples did not cause cytotoxicity in HepG2 cells as it is and 10-fold higher concentrations before and after the treatment in HepG2 cells (Fig. 4.41.). This was in well accordance with the former result with RW+T (Fig. 4.39.), where RW+T did also not cause cytotoxicity in HepG2 cells. Tap water was marginally cytotoxic as it is, while this was disappeared when it is concentrated 10-fold by freeze drying and redissolving (Fig. 4.41).

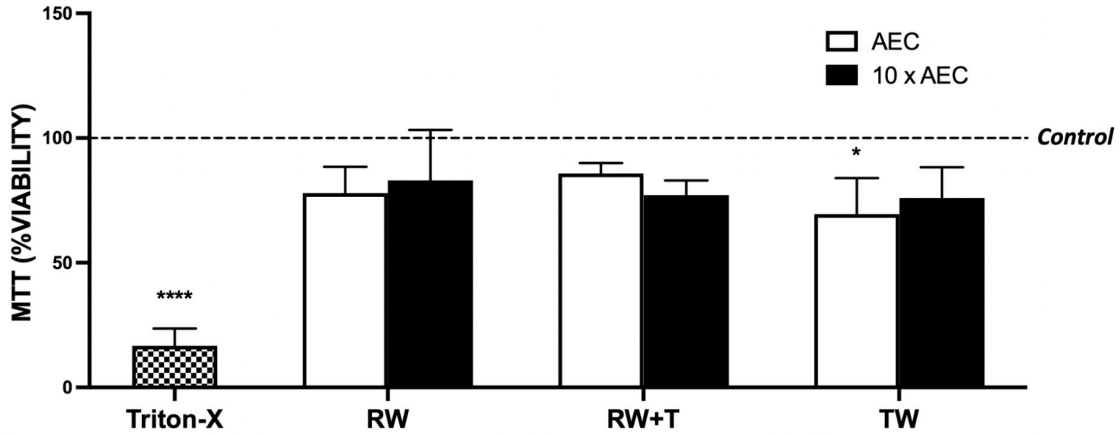


Figure 4. 41. Cytotoxicity of various water samples without spiking obtained from raw water and the tap water on HepG2 cells by MTT test. Triton-X (171 μM) was used as a positive control. AEC (Average Environmental Concentration which is 20 ng/L), RW (Raw Water), RW+T (Raw Water + Treatment) and TW (Tap Water). *p<0.05 compared to the medium control (dashed line in the graphic)

RW was not cytotoxic to HK-2 cells, while it is significantly cytotoxic after treatment. This observation was also in well accordance with the former observation with RW+T in HK-2 cells (Fig. 4.40.). Different than the observation of HepG2 cells, tap water was marginally cytotoxic at 10-fold concentrated samples instead of as it is (Fig. 4.42).

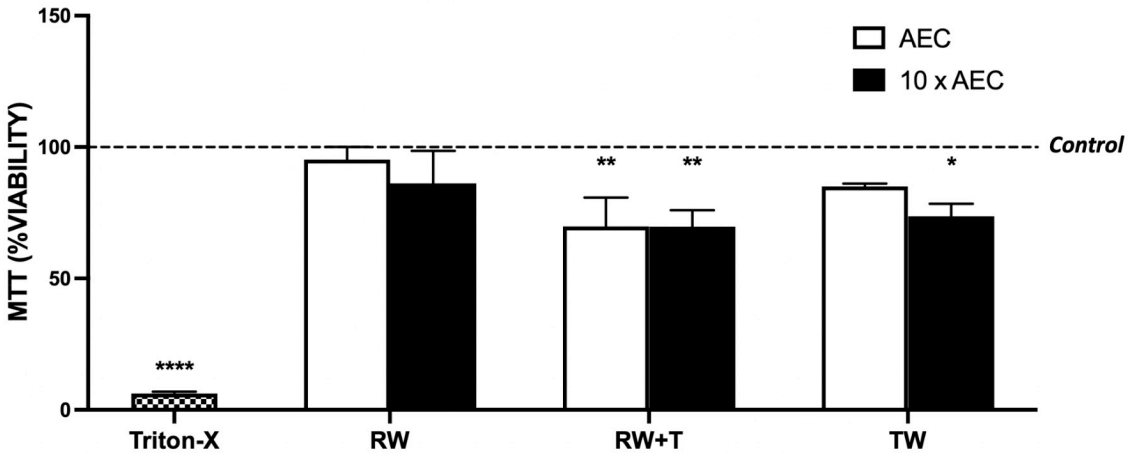


Figure 4. 42. Cytotoxicity of various water samples without spiking obtained from raw water and the tap water on HK-2 cells by MTT test. Triton-X (171 μM) was used as a positive control. AEC (Average Environmental Concentration), RW (Raw Water), RW+T (Raw Water + Treatment) and TW (Tap Water). *p<0.05, **p<0.01 compared to the medium control (dashed line in the graphic)

In aggregate, these results suggest that although treatment prevents primarily microbiological contamination of water sources, they may generate even more toxic degradation products from pharmaceuticals together with a wide range of other organic pollutants. Various phenomena are taking place in the mechanism such as concentration dependency of toxicity, nonmonotonic toxicity (low dose effect), and different (cyto)toxicities on liver and kidney cells.

5. CONCLUSIONS AND RECOMMENDATIONS

Based on experimental results, at least one or more pharmaceutical compounds (PCs) were determined in 77% of all samples, but it is also certain that the presence of pharmaceutical compounds (PCs) in Istanbul's drinking water sources in terms of mean and maximum values are not worse than other big cities around the world. In addition to this, on average 70% of PCs found in raw water are removed (in terms of degradation of the parent molecules) in drinking water treatment processes and 80% of the samples where PCs are detected, these molecules have been completely eliminated with a removal efficiency exceeding 99%. However, it should not be overlooked that certain PCs, such as valsartan and iopromide can not be completely eliminated during the treatment process. As a result, some compounds are still found in drinking water in a range of 5-50 ng/L.

Although drinking water treatment plants in Istanbul are observed to be working efficiently in terms of eliminating PCs, in avoiding one danger, we may potentially face another. As the experimental part of this study has also demonstrated, the pharmaceutical molecules eliminated through treatment are in fact, not mineralized but transformed into some organic degradation products. Furthermore, as a result of cytotoxicity studies, it was observed that the degradation products of carbamazepine (CBZ) in distilled water exhibit a significant cytoxicity of 38% on kidney cells (HK-2) after treatment. It was also observed that the cytotoxicity of the degradation products of CBZ on kidney cells after-treatment are significantly increased compared to before-treatment. Similarly, degradation products of sulfamethoxazole (SMX) after treatment has also shown a significant cytotoxicity of 24% and 25% on liver cells (HepG2) and HK-2 cells, respectively. Interestingly, when the same experiments were conducted with raw water, it was observed that the cytotoxic activity observed in pure water completely disappears for all concentrations of PCs, and even cell viability shows positive improvement. This situation can be explained by the instant reaction of electrophilic molecules that are formed in the treatment process from the spiked molecules with the nucleophilic molecules which may intrinsically be present in the raw water. However, this data is undoubtedly not sufficient to make a generalization. Factors such as concentrations of macro & micro pollutants in raw water, long term exposure to minute concentrations of PCs and their degradation products, the combined effects of mixtures of PCs and their degradation products, process conditions in the treatment plants, and seasonality, which can affect toxicity, should not be overlooked.

Turkey is one of the the countries where climate change will be most strongly felt. As a result, due to decreased rainfall and low water levels in water reservoirs, we can predict that the concentrations of pollutants in drinking water sources will increase even more in the coming years. Based on the the outcomes from this study and the anticipation that pharmaceutical residues and emerging organic pollutants in drinking water will become more serious problems in the next years, the following recommendations can be made:

1. Conducting toxicity/ecotoxicity assessments, especially for degradation products and cytotoxicity of other frequently detected PCs in raw water sources
2. Conducting further toxicity studies such as genotoxicity, especially in cases where cytotoxicity shows a positive signal
3. Conducting further toxicity studies for the degradation products of the PCs on the long term and combined toxicity
4. Adding the effect of seasonality to the study and monitoring changes in water abundance and scarcity times.
5. Determining the structure of unknown degradation products of the PCs (identification of the structures after each step; floculation, ozonation, chlorination, and the final treated water)
6. Determining the effect of sludge and sorption process in the removal of PCs
7. Screening studies in other regions of Turkey to perform a risk assessment for the country.
8. Identifying the best end-of-pipe technologies for the effective elimination of persistent PCs both in waste water treatment plants and drinking water treatment plants.
9. As a an alternative approach, employing the Effect-Driven Analysis approach proposed by (Escher & Fenner, 2011). It involves initially screening toxicity through an *in vitro/ex vivo* test battery and, in the case of detecting positive toxicity, proceeding with the elucidation of the responsible molecules' structures.

Lastly, although this study primarily focuses on the occurrence, fate, toxicity and removal of PCs, it is crucial not to overlook that the most important aspect for sustainable living is proactively reducing pollution at its source, including reducing unnecessary usage of pharmaceuticals, avoiding the disposal of expired medications into toilets, ensuring proper disposal of pharmaceutical waste from manufacturing facilities, protecting water basins from anthropogenic pollution, and increasing the number and the efficiency of biological wastewater treatment plants in Istanbul.

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APPENDIX A: LODs & LOQs OF THE ANALYTES

Table A. 1. LODs and LOQ of the Analytes

Compound	LOD ng / L	LOQ ng/L
Acesulfam K	2	10
Acetyl-Sulfadimidine (=Acetyl-Sulfamethazine)	1	2,5
Acetyl-Sulfamethoxazole	0,5	1
Ciprofloxacin	1	2,5
Clarithromycin	1	2,5
Danofloxacin	1	2,5
DEET	2	5
Difloxacin	1	2,5
Enrofloxacin	1	2,5
Erythromycin	0,5	1
Flumequine	0,5	1
Gabapentin	1	10
Ioxaglic acid	3	10
Irbesartan	2	5
Josamycin	1	2,5
Lamotrigine	1	2,5
Lincomycin	1	2,5
Marbofloxacin	1	5
Metformin-HCl	1	5
Nalidixic acid	1	2,5
Norfloxacin	1	2,5
Oxolinic acid	1	2,5
Paracetamol	3	10
Propyphenazone	1	5
Roxithromycin	1	2,5
Sarafloxacin	1	2,5
Spiramycin	1	2,5
Sulfadiazine	1	2,5
Sulfadimethoxine	1	2,5
Sulfadimidine (= Sulfamethazine)	1	2,5
Sulfadoxine	1	2,5
Sulfamerazine	1	2,5
Sulfamethoxazole	1	2,5
Sulfamethoxypyridazine	1	2,5
Sulfamonomethoxine	0,5	1
Sulfaquinoxalin	1	2,5

Sulfathiazole	1	2,5
Sulfisoxazol	1	2,5
Telmisartan	3	10
Tramadol-HCl	2	5
Trimethoprim	0,5	1
Tylosin	1	2,5
Valsartan	1	5
Valsartan acid	2	10

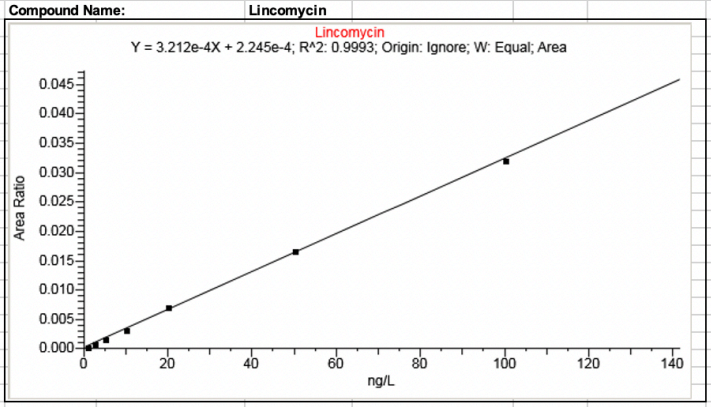
APPENDIX B: CALIBRATION REPORTS

Figure B. 1. Calibration Report of Lyncomycin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Lincomycin**



Linear
Fail A

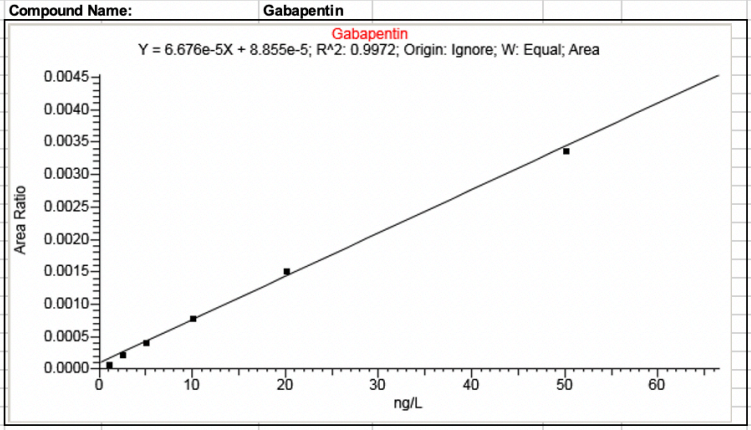
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	704669	50	2000922725	0	0,398	ng/L	N/A	N/A	A
2	2,5	1727389	50	2024722520	0,001	1,957	ng/L	N/A	N/A	A
3	5	3459726	50	2045695699	0,002	4,567	ng/L	N/A	N/A	
5	10	6837718	50	2069755028	0,003	9,587	ng/L	N/A	N/A	
7	20	14573388	50	2034089306	0,007	21,609	ng/L	N/A	N/A	
9	50	34486895	50	2067344675	0,017	51,242	ng/L	N/A	N/A	
11	100	70926811	50	2211983133	0,032	99,14	ng/L	N/A	N/A	

Figure B. 2. Calibration Report of Gabapentin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Gabapentin**



Linear
Fail A

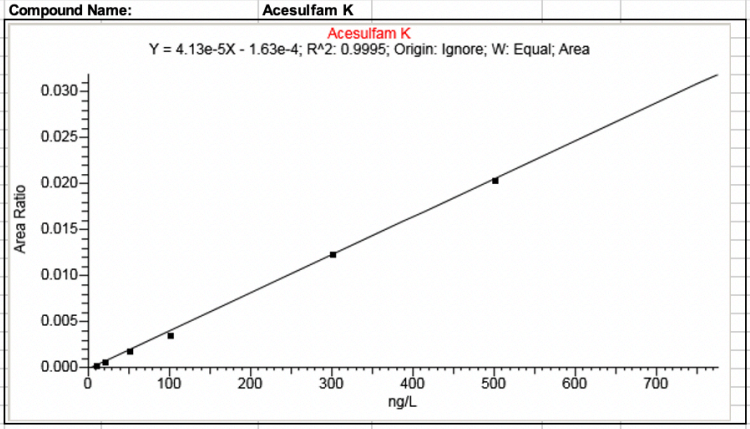
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	163561	50	2000922725	0	-0,102	ng/L	N/A	N/A	A
2	2,5	475587	50	2024722520	0	2,192	ng/L	N/A	N/A	
3	5	847915	50	2045695699	0	4,883	ng/L	N/A	N/A	
5	10	1654638	50	2069755028	0,001	10,649	ng/L	N/A	N/A	
7	20	3112924	50	2034089306	0,002	21,598	ng/L	N/A	N/A	
9	50	6984213	50	2067344675	0,003	49,28	ng/L	N/A	N/A	

Figure B. 3. Calibration Report of Acesulfam K

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Acesulfam K**



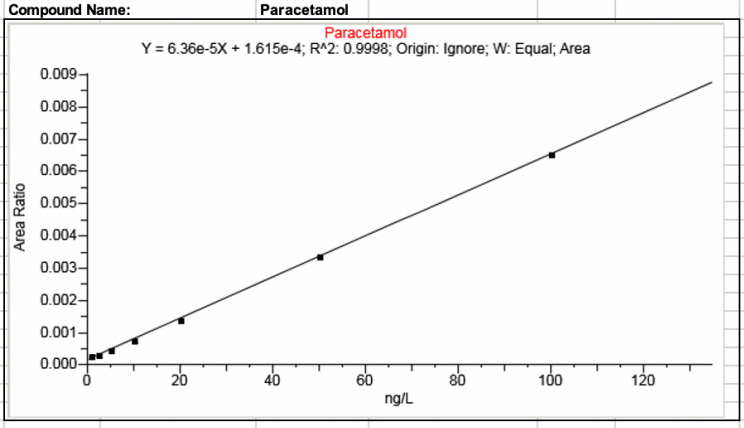
Linear
Pass

Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
5	10	608600	50	2069755028	0	11,067	ng/L	N/A	N/A
7	20	1590006	50	2034089306	0,001	22,875	ng/L	N/A	N/A
9	50	4042361	50	2067344675	0,002	51,295	ng/L	N/A	N/A
11	100	8035979	50	2211983133	0,004	91,918	ng/L	N/A	N/A
13	300	22101166	50	1785172323	0,012	303,738	ng/L	N/A	N/A
15	500	41119943	50	2010901818	0,02	499,108	ng/L	N/A	N/A

Figure B. 4. Calibration Report of Paracetamol

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Paracetamol**



Linear
Fail A

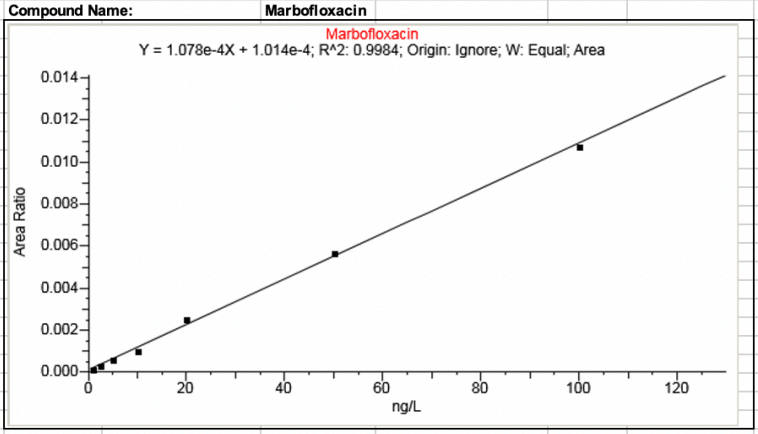
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	574774	50	2000922725	0	1,977	ng/L	N/A	N/A A
2	2,5	673883	50	2024722520	0	2,693	ng/L	N/A	N/A
3	5	924062	50	2045695699	0	4,563	ng/L	N/A	N/A
5	10	1600049	50	2069755028	0,001	9,616	ng/L	N/A	N/A
7	20	2827539	50	2034089306	0,001	19,317	ng/L	N/A	N/A
9	50	6947832	50	2067344675	0,003	50,304	ng/L	N/A	N/A
11	100	14429104	50	2211983133	0,007	100,03	ng/L	N/A	N/A

Figure B. 5. Calibration Report of Marbofloxacin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Marbofloxacin**



Linear
Fail A

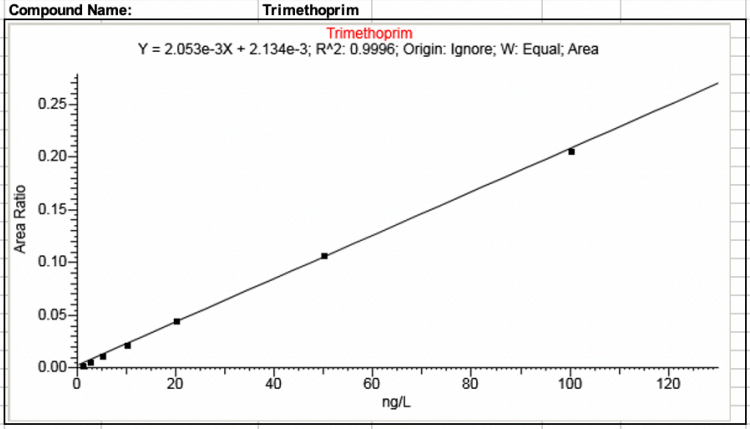
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	261368	50	2000922725	0	0,271	ng/L	N/A	N/A	A
2	2,5	662936	50	2024722520	0	2,097	ng/L	N/A	N/A	
3	5	1222726	50	2045695699	0,001	4,603	ng/L	N/A	N/A	
5	10	2133424	50	2069755028	0,001	8,619	ng/L	N/A	N/A	
7	20	5093351	50	2034089306	0,003	22,282	ng/L	N/A	N/A	
9	50	11760263	50	2067344675	0,006	51,816	ng/L	N/A	N/A	
11	100	23791768	50	2211983133	0,011	98,811	ng/L	N/A	N/A	

Figure B. 6. Calibration Report of Trimethropim

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Trimethoprim**



Linear
Fail A

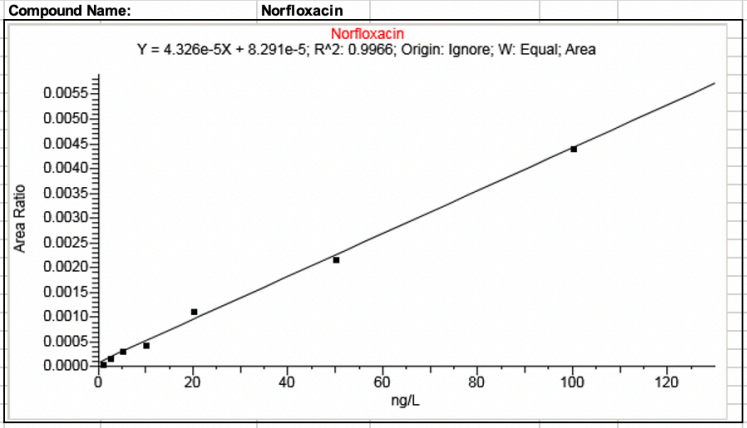
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	2859872	50	911580639	0,003	0,489	ng/L	N/A	N/A	A
2	2,5	5547970	50	903771339	0,006	1,951	ng/L	N/A	N/A	A
3	5	10452952	50	891937333	0,012	4,669	ng/L	N/A	N/A	
5	10	20282301	50	891138248	0,023	10,047	ng/L	N/A	N/A	
7	20	43718827	50	971919030	0,045	20,871	ng/L	N/A	N/A	
9	50	93056963	50	867081628	0,107	51,235	ng/L	N/A	N/A	
11	100	178926806	50	869101290	0,206	99,239	ng/L	N/A	N/A	

Figure B. 7. Calibration Report of Norfloxacin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Norfloxacin**



Linear
Fail A

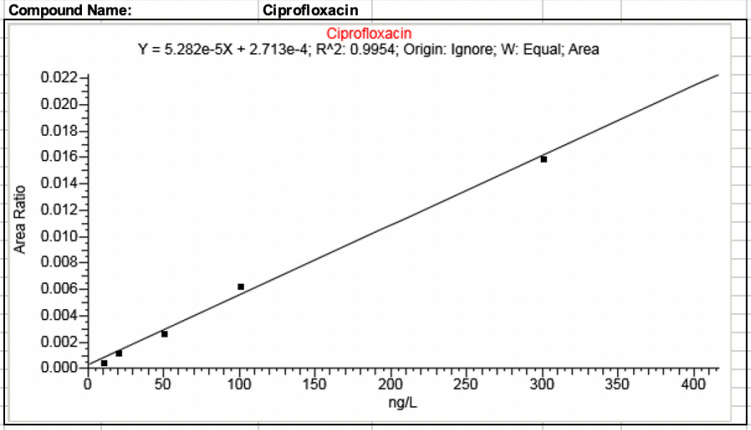
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	112017	50	2000922725	0	-0,623	ng/L	N/A	N/A	A
2	2,5	361622	50	2024722520	0	2,212	ng/L	N/A	N/A	
3	5	660930	50	2045695699	0	5,552	ng/L	N/A	N/A	
5	10	932937	50	2069755028	0	8,503	ng/L	N/A	N/A	
7	20	2315881	50	2034089306	0,001	24,402	ng/L	N/A	N/A	A
9	50	4498064	50	2067344675	0,002	48,378	ng/L	N/A	N/A	
11	100	9759782	50	2211983133	0,004	100,076	ng/L	N/A	N/A	

Figure B. 8. Calibration Report of Ciprofloxacin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Ciprofloxacin**



Linear
Fail A

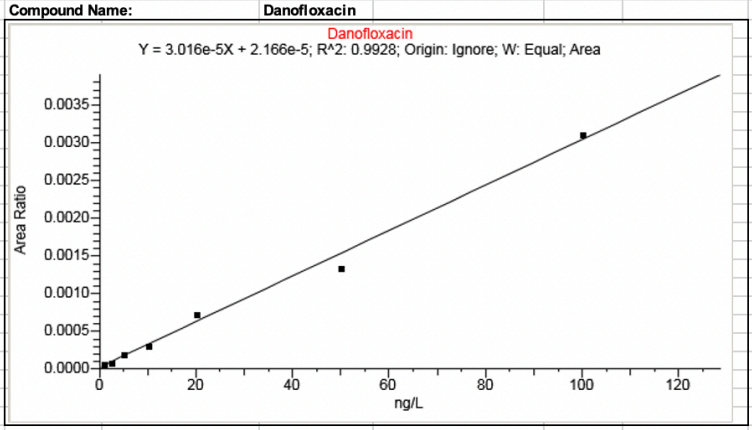
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
5	10	1048942	50	2069755028	0,001	4,458	ng/L	N/A	N/A	A
7	20	2509412	50	2034089306	0,001	18,22	ng/L	N/A	N/A	
9	50	5688872	50	2067344675	0,003	46,961	ng/L	N/A	N/A	
11	100	13957650	50	2211983133	0,006	114,328	ng/L	N/A	N/A	
13	300	28397753	50	1785172323	0,016	296,034	ng/L	N/A	N/A	

Figure B. 9. Calibration Report of Danofloxacin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Danofloxacin**



Linear
Fail A

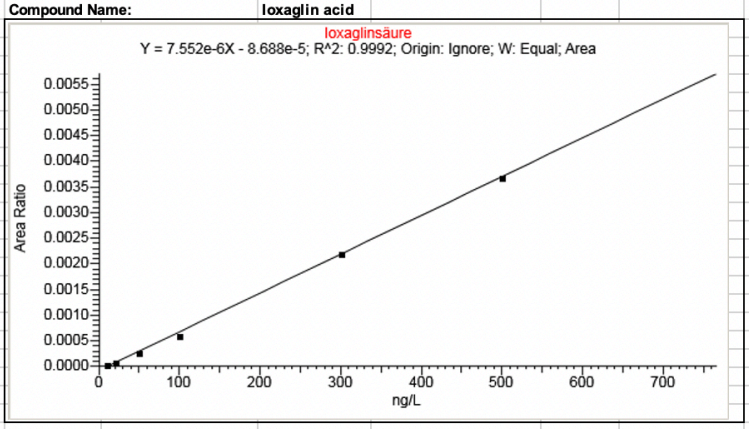
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	137341	50	2000922725	0	1,558	ng/L	N/A	N/A	A
2	2,5	166055	50	2024722520	0	2,001	ng/L	N/A	N/A	
3	5	408937	50	2045695699	0	5,91	ng/L	N/A	N/A	
5	10	637112	50	2069755028	0	9,489	ng/L	N/A	N/A	
7	20	1474074	50	2034089306	0,001	23,311	ng/L	N/A	N/A	
9	50	2773114	50	2067344675	0,001	43,761	ng/L	N/A	N/A	
11	100	6883582	50	2211983133	0,003	102,47	ng/L	N/A	N/A	

Figure B. 10. Calibration Report of Ioxaglic acid

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Ioxaglin acid**



Linear
Fail A

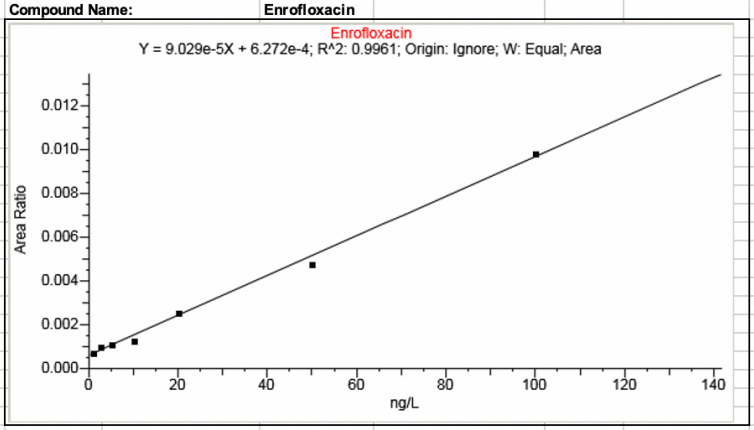
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
5	10	89378	50	2069755028	0	17,222	ng/L	N/A	N/A	A
7	20	150358	50	2034089306	0	21,291	ng/L	N/A	N/A	
9	50	566374	50	2067344675	0	47,779	ng/L	N/A	N/A	
11	100	1323750	50	2211983133	0,001	90,743	ng/L	N/A	N/A	
13	300	3926220	50	1785172323	0,002	302,718	ng/L	N/A	N/A	
15	500	7422553	50	2010901818	0,004	500,247	ng/L	N/A	N/A	

Figure B. 11. Calibration Report of Enrofloxacin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Enrofloxacin**



Linear
Fail A

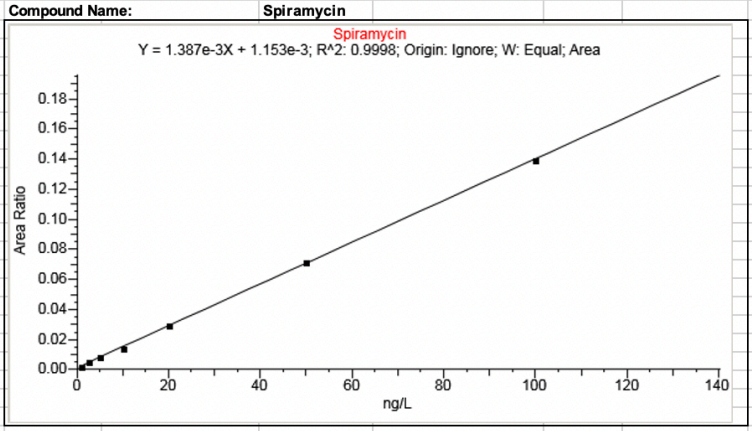
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	1524548	50	2000922725	0,001	1,492	ng/L	N/A	N/A	A
2	2,5	2031292	50	2024722520	0,001	4,164	ng/L	N/A	N/A	A
3	5	2324678	50	2045695699	0,001	5,639	ng/L	N/A	N/A	
5	10	2718153	50	2069755028	0,001	7,598	ng/L	N/A	N/A	A
7	20	5278367	50	2034089306	0,003	21,793	ng/L	N/A	N/A	
9	50	9886941	50	2067344675	0,005	46,021	ng/L	N/A	N/A	
11	100	21717540	50	2211983133	0,01	101,793	ng/L	N/A	N/A	

Figure B. 12. Calibration Report of Spiramycin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Spiramycin**



Linear
Fail A

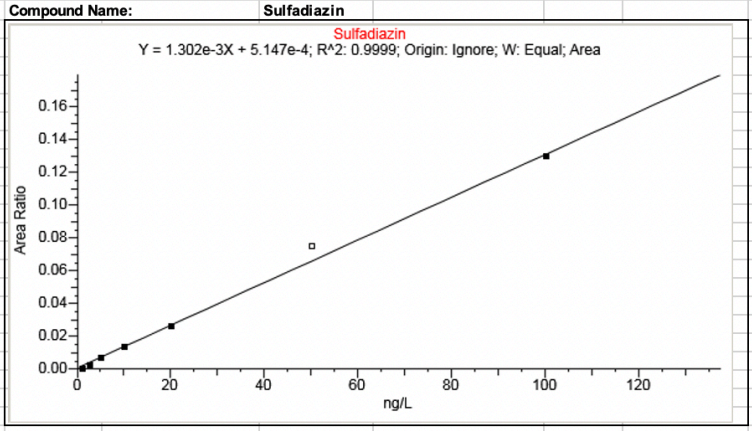
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	300687	50	174992276	0,002	0,408	ng/L	N/A	N/A	A
2	2,5	884330	50	176492628	0,005	2,782	ng/L	N/A	N/A	
3	5	1495455	50	180773097	0,008	5,134	ng/L	N/A	N/A	
5	10	2559087	50	179648520	0,014	9,441	ng/L	N/A	N/A	
7	20	5383124	50	183421832	0,029	20,332	ng/L	N/A	N/A	
9	50	13100958	50	182820717	0,072	50,843	ng/L	N/A	N/A	
11	100	28179330	50	202408031	0,139	99,56	ng/L	N/A	N/A	

Figure B. 13. Calibration Report of Sulfadiazine

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sulfadiazin**



Linear
Fail A

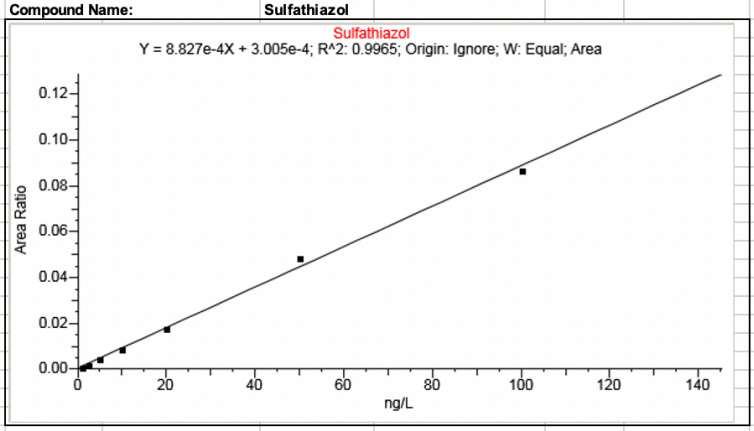
Level	Std Amount		Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1		190306	50	174992276	0,001	0,44	ng/L	N/A	N/A	A
2	2,5		575894	50	176492628	0,003	2,111	ng/L	N/A	N/A	
3	5		1349262	50	180773097	0,007	5,337	ng/L	N/A	N/A	
5	10		2531384	50	179648520	0,014	10,426	ng/L	N/A	N/A	
7	20		4939889	50	183421832	0,027	20,288	ng/L	N/A	N/A	
9	50	X		50	N/F	N/F	N/A	ng/L	N/A	N/A	
11	100		26432872	50	202408031	0,131	99,898	ng/L	N/A	N/A	

Figure B. 14. Calibration Report of Sulfathiazole

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sulfathiazol**



Linear
Fail A

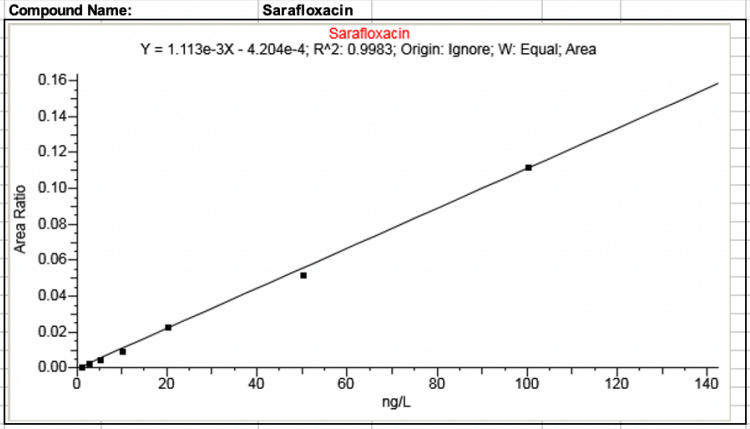
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	97513	50	174992276	0,001	0,291	ng/L	N/A	N/A	A
2	2,5	288403	50	176492628	0,002	1,511	ng/L	N/A	N/A	A
3	5	815061	50	180773097	0,005	4,767	ng/L	N/A	N/A	
5	10	1580932	50	179648520	0,009	9,629	ng/L	N/A	N/A	
7	20	3273797	50	183421832	0,018	19,88	ng/L	N/A	N/A	
9	50	8871967	50	182820717	0,049	54,636	ng/L	N/A	N/A	
11	100	17532183	50	202408031	0,087	97,787	ng/L	N/A	N/A	

Figure B. 15. Calibration Report of Sarafloxacin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sarafloxacin**



Linear
Fail A

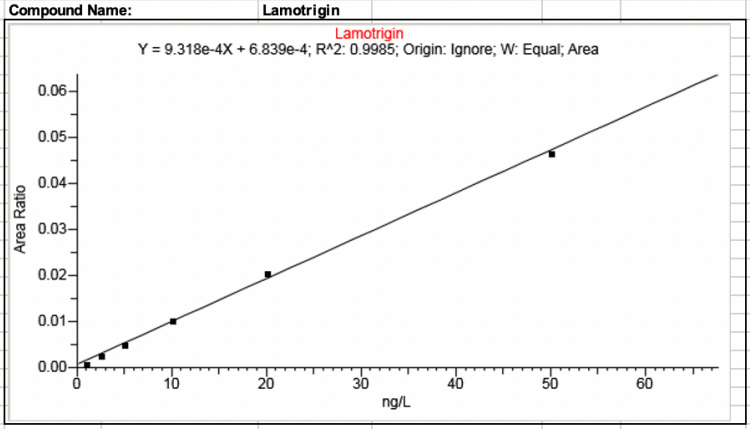
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	211752	50	174992276	0,001	1,465	ng/L	N/A	N/A	A
2	2,5	562254	50	176492628	0,003	3,24	ng/L	N/A	N/A	A
3	5	959238	50	180773097	0,005	5,145	ng/L	N/A	N/A	
5	10	1766172	50	179648520	0,01	9,211	ng/L	N/A	N/A	
7	20	4230987	50	183421832	0,023	21,102	ng/L	N/A	N/A	
9	50	9490539	50	182820717	0,052	47,018	ng/L	N/A	N/A	
11	100	22740661	50	202408031	0,112	101,319	ng/L	N/A	N/A	

Figure B. 16. Calibration Report of Lamotrigine

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Lamotrigin**



Linear
Fail A

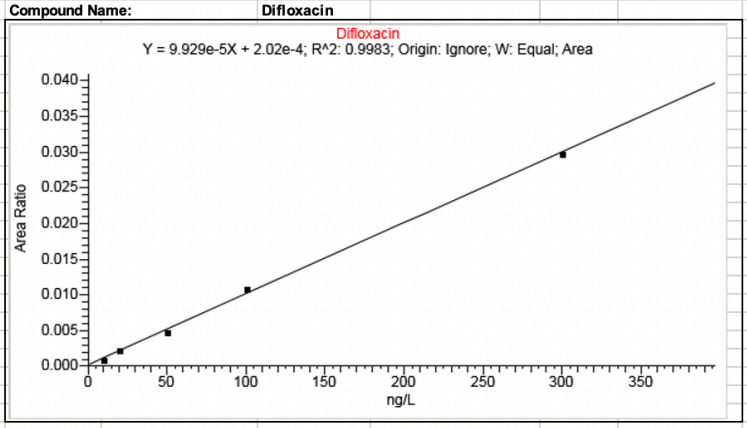
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	2041095	50	2000922725	0,001	0,361	ng/L	N/A	N/A	A
2	2,5	5688954	50	2024722520	0,003	2,281	ng/L	N/A	N/A	
3	5	10497452	50	2045695699	0,005	4,773	ng/L	N/A	N/A	
5	10	21333907	50	2069755028	0,01	10,327	ng/L	N/A	N/A	
7	20	41753536	50	2034089306	0,021	21,294	ng/L	N/A	N/A	
9	50	96702036	50	2067344675	0,047	49,463	ng/L	N/A	N/A	

Figure B. 17. Calibration Report of Difloxacin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Difloxacin**



Linear
Fail A

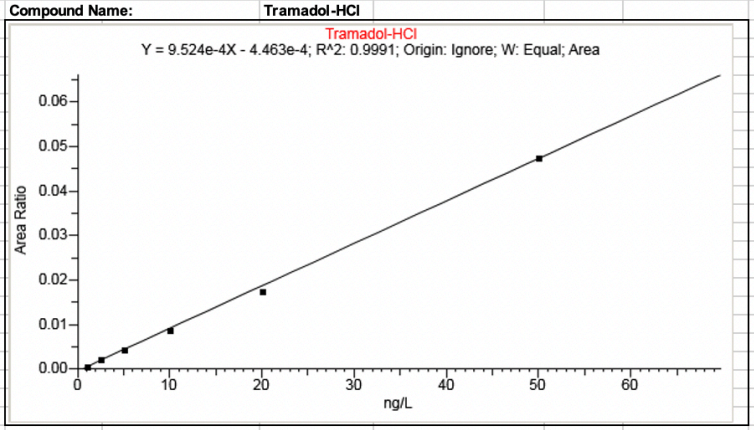
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
5	10	1901762	50	2069755028	0,001	7,22	ng/L	N/A	N/A	A
7	20	4650808	50	2034089306	0,002	20,994	ng/L	N/A	N/A	
9	50	9780146	50	2067344675	0,005	45,613	ng/L	N/A	N/A	
11	100	24193376	50	2211983133	0,011	108,124	ng/L	N/A	N/A	
13	300	53189012	50	1785172323	0,03	298,05	ng/L	N/A	N/A	

Figure B. 18. Calibration Report of Tramadol HCl

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Tramadol-HCl**



Linear
Fail A

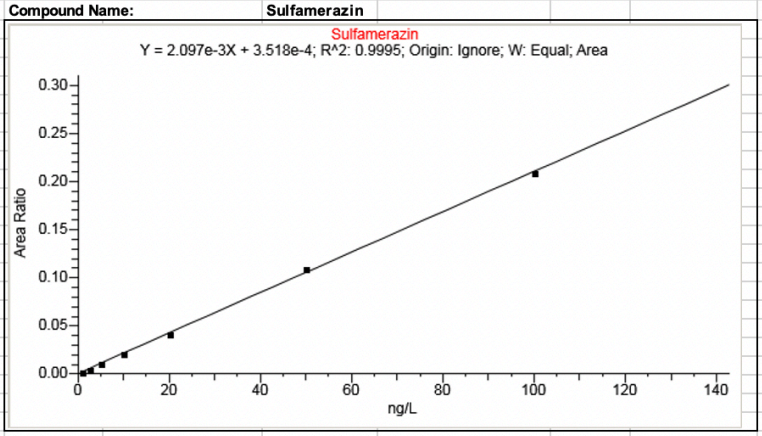
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	1628382	50	2000922725	0,001	1,323	ng/L	N/A	N/A	A
2	2,5	4535172	50	2024722520	0,002	2,82	ng/L	N/A	N/A	
3	5	9187151	50	2045695699	0,004	5,184	ng/L	N/A	N/A	
5	10	18369414	50	2069755028	0,009	9,787	ng/L	N/A	N/A	
7	20	35851221	50	2034089306	0,018	18,974	ng/L	N/A	N/A	
9	50	98340359	50	2067344675	0,048	50,412	ng/L	N/A	N/A	

Figure B. 19. Calibration Report of Sulfamerazine

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sulfamerazin**



Linear
Pass

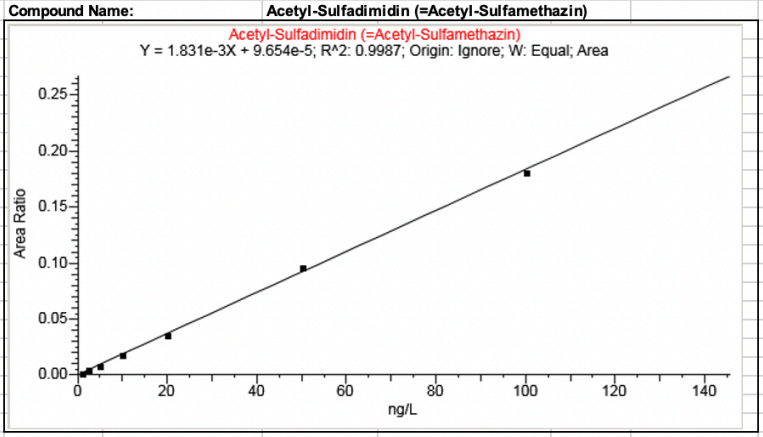
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	396914	50	174992276	0,002	0,914	ng/L	N/A	N/A
2	2,5	851033	50	176492628	0,005	2,132	ng/L	N/A	N/A
3	5	1908191	50	180773097	0,011	4,866	ng/L	N/A	N/A
5	10	3786314	50	179648520	0,021	9,882	ng/L	N/A	N/A
7	20	7666592	50	183421832	0,042	19,763	ng/L	N/A	N/A
9	50	19898982	50	182820717	0,109	51,734	ng/L	N/A	N/A
11	100	42182284	50	202408031	0,208	99,209	ng/L	N/A	N/A

Figure B. 20. Calibration Report of Acetyl Sulfadimidine

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Acetyl-Sulfadimidin (=Acetyl-Sulfamethazin)**



Linear
Pass

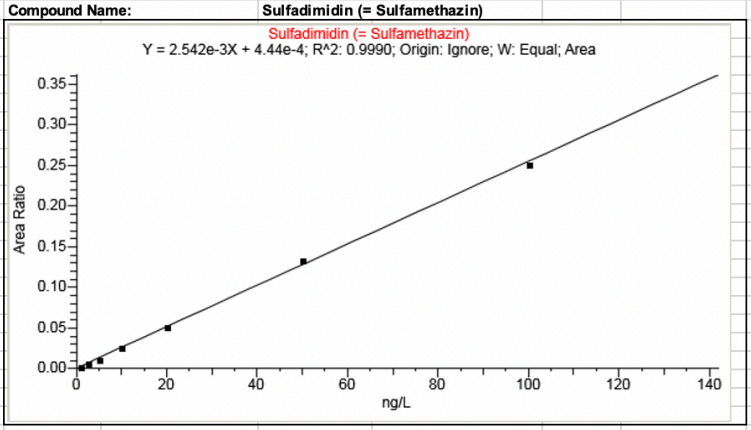
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	277543	50	174992276	0,002	0,814	ng/L	N/A	N/A
2	2,5	795574	50	176492628	0,005	2,41	ng/L	N/A	N/A
3	5	1527181	50	180773097	0,008	4,562	ng/L	N/A	N/A
5	10	3190314	50	179648520	0,018	9,649	ng/L	N/A	N/A
7	20	6560637	50	183421832	0,036	19,487	ng/L	N/A	N/A
9	50	17697550	50	182820717	0,097	52,83	ng/L	N/A	N/A
11	100	36607365	50	202408031	0,181	98,749	ng/L	N/A	N/A

Figure B. 21. Calibration Report of Sulfadimidine

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sulfadimidin (= Sulfamethazin)**



Linear
Fail A

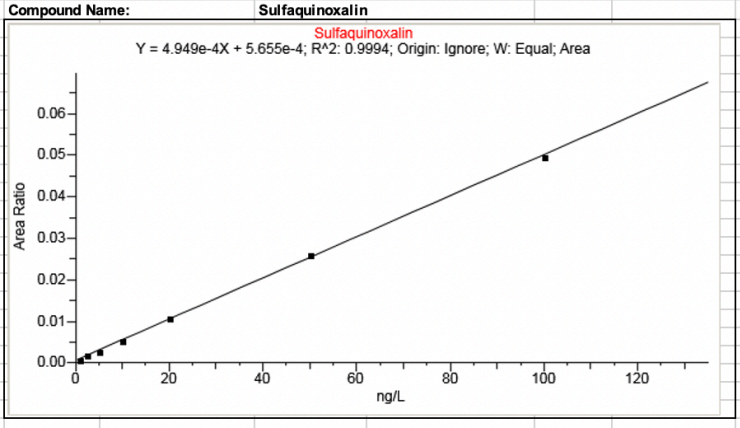
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	413056	50	174992276	0,002	0,754	ng/L	N/A	N/A	A
2	2,5	1065244	50	176492628	0,006	2,2	ng/L	N/A	N/A	
3	5	2117337	50	180773097	0,012	4,433	ng/L	N/A	N/A	
5	10	4564457	50	179648520	0,025	9,82	ng/L	N/A	N/A	
7	20	9398917	50	183421832	0,051	19,982	ng/L	N/A	N/A	
9	50	24482891	50	182820717	0,134	52,504	ng/L	N/A	N/A	
11	100	50932003	50	202408031	0,252	98,808	ng/L	N/A	N/A	

Figure B. 22. Calibration Report of Sulfaquinoxaline

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sulfaquinoxalin**



Linear
Fail A

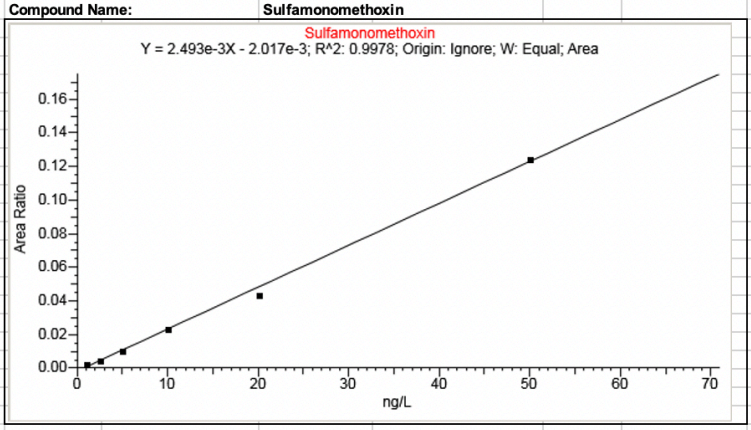
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	130319	50	174992276	0,001	0,362	ng/L	N/A	N/A	A
2	2,5	321032	50	176492628	0,002	2,533	ng/L	N/A	N/A	
3	5	500653	50	180773097	0,003	4,453	ng/L	N/A	N/A	
5	10	949575	50	179648520	0,005	9,537	ng/L	N/A	N/A	
7	20	2002526	50	183421832	0,011	20,916	ng/L	N/A	N/A	
9	50	4773083	50	182820717	0,026	51,607	ng/L	N/A	N/A	
11	100	10041639	50	202408031	0,05	99,093	ng/L	N/A	N/A	

Figure B. 23. Calibration Report of Sulfamonomethoxine

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sulfamonomethoxin**



Linear
Fail A

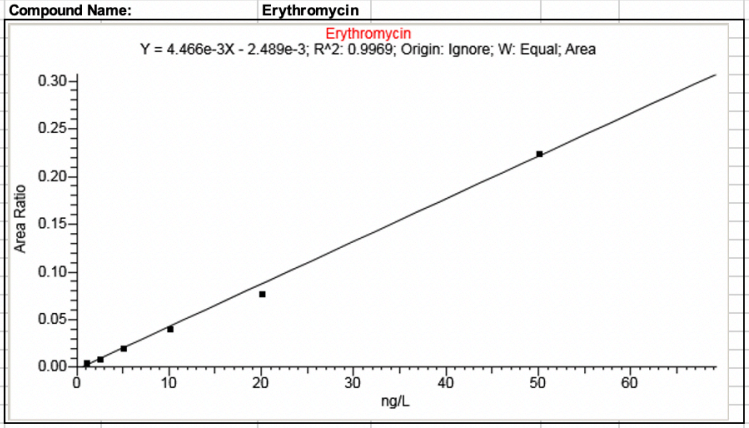
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	388233	50	174992276	0,002	1,699	ng/L	N/A	N/A	A
2	2,5	807365	50	176492628	0,005	2,644	ng/L	N/A	N/A	
3	5	1908935	50	180773097	0,011	5,045	ng/L	N/A	N/A	
5	10	4200582	50	179648520	0,023	10,188	ng/L	N/A	N/A	
7	20	8004666	50	183421832	0,044	18,313	ng/L	N/A	N/A	
9	50	22699755	50	182820717	0,124	50,611	ng/L	N/A	N/A	

Figure B. 24. Calibration Report of Erythromycin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Erythromycin**



Linear
Fail A

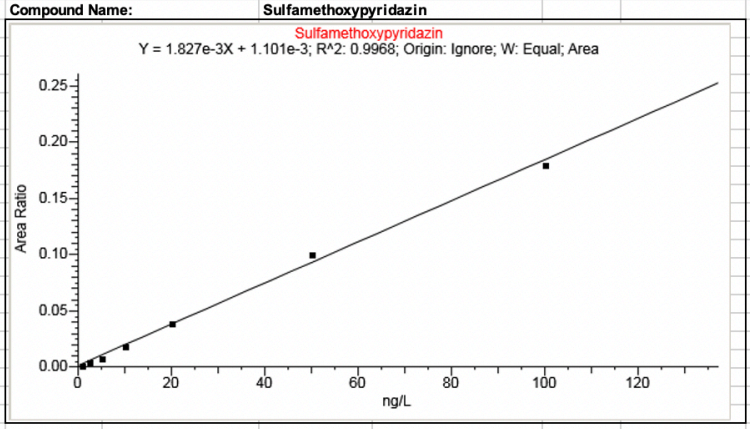
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	973314	50	174992276	0,006	1,803	ng/L	N/A	N/A	A
2	2,5	1760365	50	176492628	0,01	2,791	ng/L	N/A	N/A	
3	5	3793578	50	180773097	0,021	5,256	ng/L	N/A	N/A	
5	10	7486797	50	179648520	0,042	9,889	ng/L	N/A	N/A	
7	20	14281207	50	183421832	0,078	17,992	ng/L	N/A	N/A	
9	50	40995663	50	182820717	0,224	50,769	ng/L	N/A	N/A	

Figure B. 25. Calibration Report of Sulfamethoxypyridazine

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sulfamethoxypyridazin**



Linear
Fail A

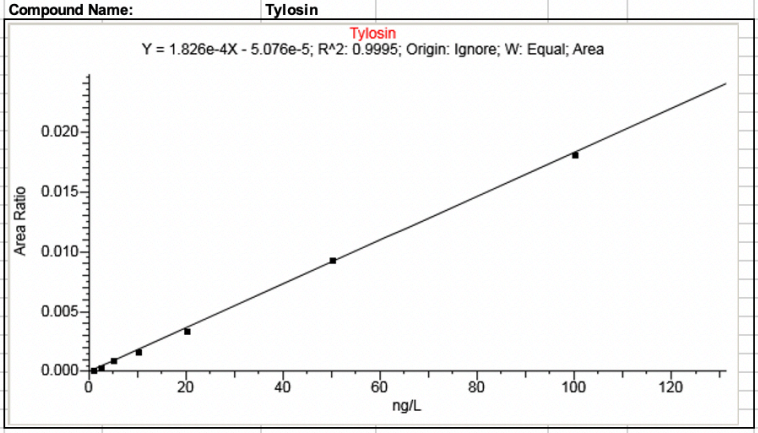
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	282478	50	174992276	0,002	0,281	ng/L	N/A	N/A	A
2	2,5	821320	50	176492628	0,005	1,944	ng/L	N/A	N/A	A
3	5	1463800	50	180773097	0,008	3,828	ng/L	N/A	N/A	A
5	10	3317728	50	179648520	0,018	9,503	ng/L	N/A	N/A	
7	20	7199129	50	183421832	0,039	20,875	ng/L	N/A	N/A	
9	50	18318728	50	182820717	0,1	54,228	ng/L	N/A	N/A	
11	100	36413032	50	202408031	0.18	97.84	ng/L	N/A	N/A	

Figure B. 26. Calibration Report of Tylosin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Tylosin**



Linear
Pass

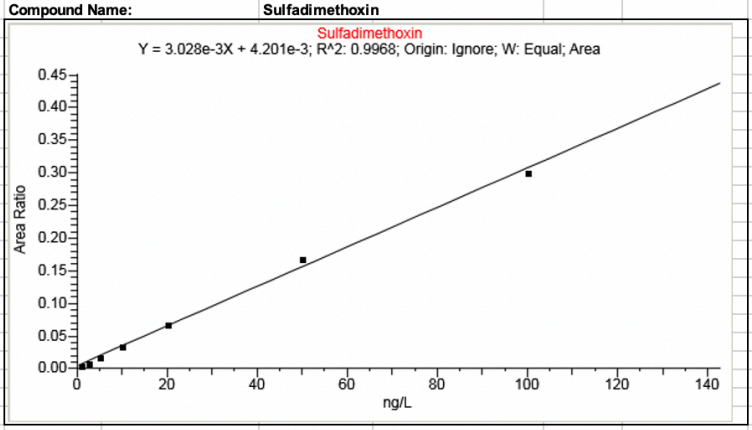
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	265268	50	2000922725	0	1,004	ng/L	N/A	N/A
2	2,5	793831	50	2024722520	0	2,425	ng/L	N/A	N/A
3	5	1891183	50	2045695699	0,001	5,34	ng/L	N/A	N/A
5	10	3569117	50	2069755028	0,002	9,72	ng/L	N/A	N/A
7	20	6956817	50	2034089306	0,003	19,004	ng/L	N/A	N/A
9	50	19374530	50	2067344675	0,009	51,591	ng/L	N/A	N/A
11	100	40050796	50	2211983133	0,018	99,416	ng/L	N/A	N/A

Figure B. 27. Calibration Report of Sulfadimethoxine

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: Sulfadimethoxin



Linear
Fail A

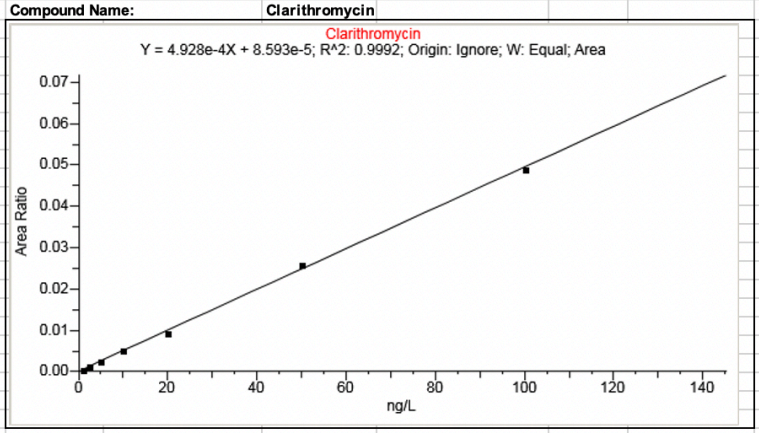
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	679718	50	174992276	0,004	-0,105	ng/L	N/A	N/A A
2	2,5	1489509	50	176492628	0,008	1,4	ng/L	N/A	N/A A
3	5	3143300	50	180773097	0,017	4,356	ng/L	N/A	N/A
5	10	6055840	50	179648520	0,034	9,747	ng/L	N/A	N/A
7	20	12573460	50	183421832	0,069	21,254	ng/L	N/A	N/A
9	50	30659857	50	182820717	0,168	54,006	ng/L	N/A	N/A
11	100	60807461	50	202408031	0,3	97,842	ng/L	N/A	N/A

Figure B. 28. Calibration Report of Clarithromycin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: Clarithromycin



Linear
Pass

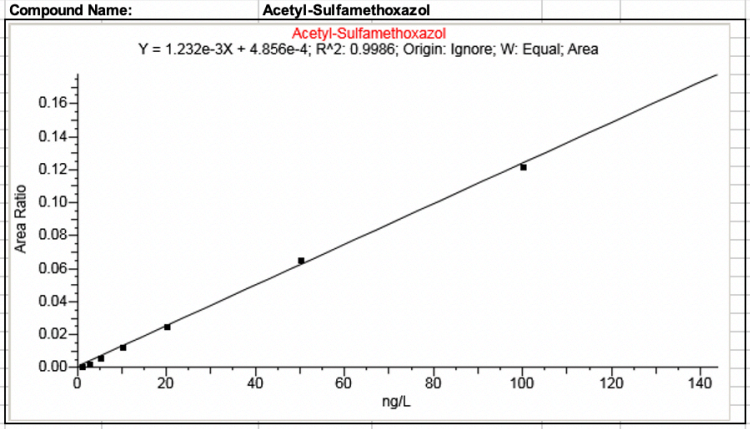
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	1004327	50	2000922725	0,001	0,844	ng/L	N/A	N/A
2	2,5	2439601	50	2024722520	0,001	2,27	ng/L	N/A	N/A
3	5	5226581	50	2045695699	0,003	5,01	ng/L	N/A	N/A
5	10	10489742	50	2069755028	0,005	10,109	ng/L	N/A	N/A
7	20	19220843	50	2034089306	0,009	18,999	ng/L	N/A	N/A
9	50	53305939	50	2067344675	0,026	52,144	ng/L	N/A	N/A
11	100	108251775	50	2211983133	0,049	99,124	ng/L	N/A	N/A

Figure B. 29. Calibration Report of Acetyl Sulfamethoxazole

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Acetyl-Sulfamethoxazol**



Linear
Fail A

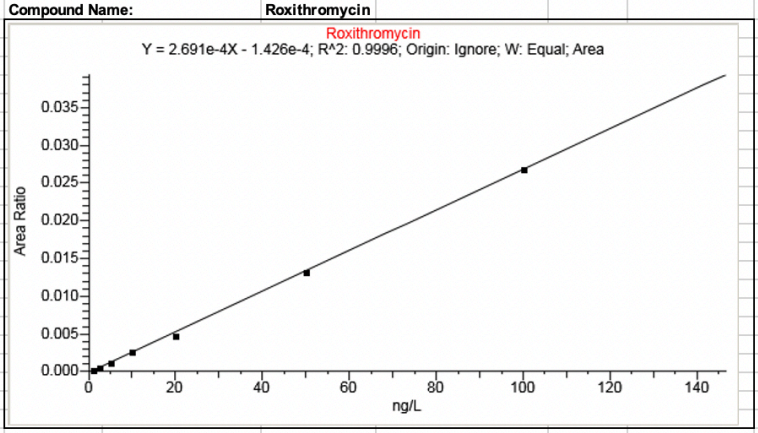
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	189559	50	174992276	0,001	0,485	ng/L	N/A	N/A	A
2	2,5	487963	50	176492628	0,003	1,85	ng/L	N/A	N/A	A
3	5	1129471	50	180773097	0,006	4,678	ng/L	N/A	N/A	
5	10	2256907	50	179648520	0,013	9,804	ng/L	N/A	N/A	
7	20	4669446	50	183421832	0,025	20,271	ng/L	N/A	N/A	
9	50	11984480	50	182820717	0,066	52,819	ng/L	N/A	N/A	
11	100	24682185	50	202408031	0,122	98,593	ng/L	N/A	N/A	

Figure B. 30. Calibration Report of Roxithromycin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Roxithromycin**



Linear
Fail A

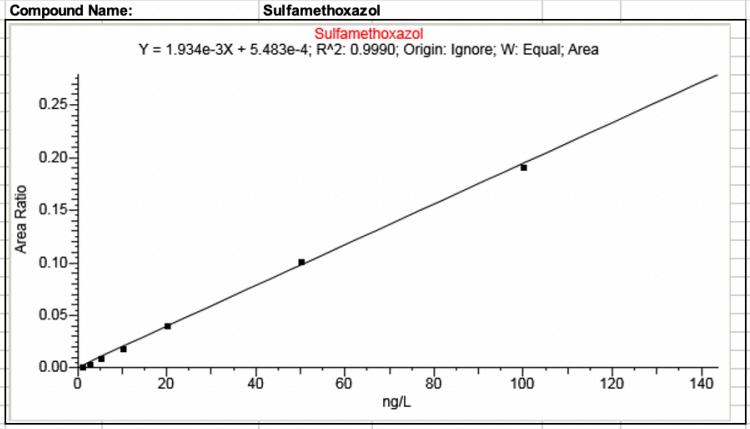
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	596355	50	2000922725	0	1,637	ng/L	N/A	N/A	A
2	2,5	1211016	50	2024722520	0,001	2,753	ng/L	N/A	N/A	
3	5	2623260	50	2045695699	0,001	5,296	ng/L	N/A	N/A	
5	10	5424616	50	2069755028	0,003	10,27	ng/L	N/A	N/A	
7	20	9846507	50	2034089306	0,005	18,52	ng/L	N/A	N/A	
9	50	27275947	50	2067344675	0,013	49,564	ng/L	N/A	N/A	
11	100	59476135	50	2211983133	0,027	100,459	ng/L	N/A	N/A	

Figure B. 31. Calibration Report of Sulfamethoxazole

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sulfamethoxazol**



Linear
Fail A

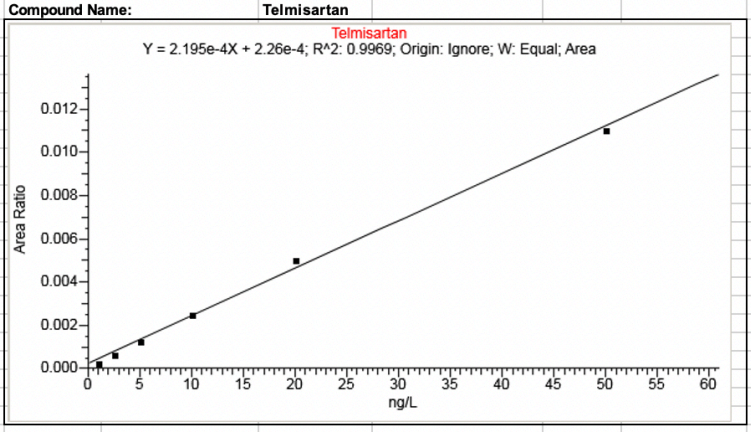
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	347046	50	174992276	0,002	0,742	ng/L	N/A	N/A	A
2	2,5	748384	50	176492628	0,004	1,909	ng/L	N/A	N/A	A
3	5	1711735	50	180773097	0,009	4,611	ng/L	N/A	N/A	
5	10	3393408	50	179648520	0,019	9,481	ng/L	N/A	N/A	
7	20	7419879	50	183421832	0,04	20,628	ng/L	N/A	N/A	
9	50	18608052	50	182820717	0,102	52,332	ng/L	N/A	N/A	
11	100	38795076	50	202408031	0,192	98,797	ng/L	N/A	N/A	

Figure B. 32. Calibration Report of Telmisartan

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Telmisartan**



Linear
Fail A

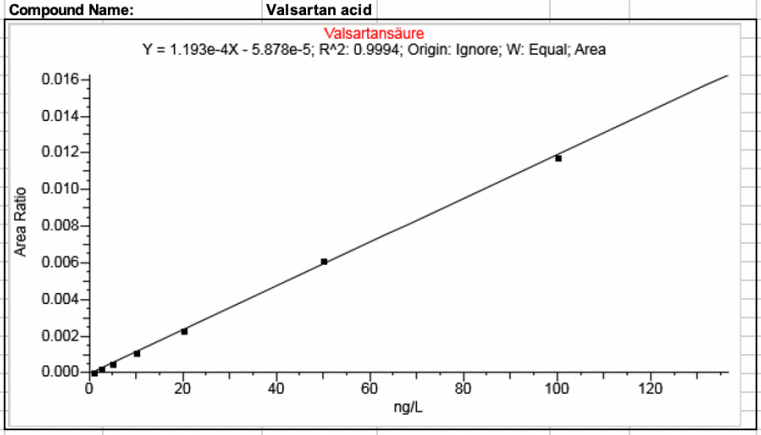
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	543537	50	2000922725	0	0,208	ng/L	N/A	N/A	A
2	2,5	1324727	50	2024722520	0,001	1,951	ng/L	N/A	N/A	A
3	5	2639558	50	2045695699	0,001	4,848	ng/L	N/A	N/A	
5	10	5179445	50	2069755028	0,003	10,37	ng/L	N/A	N/A	
7	20	10238490	50	2034089306	0,005	21,899	ng/L	N/A	N/A	
9	50	22807810	50	2067344675	0,011	49,225	ng/L	N/A	N/A	

Figure B. 33. Calibration Report of Valsartan acid

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Valsartan acid**



Linear
Pass

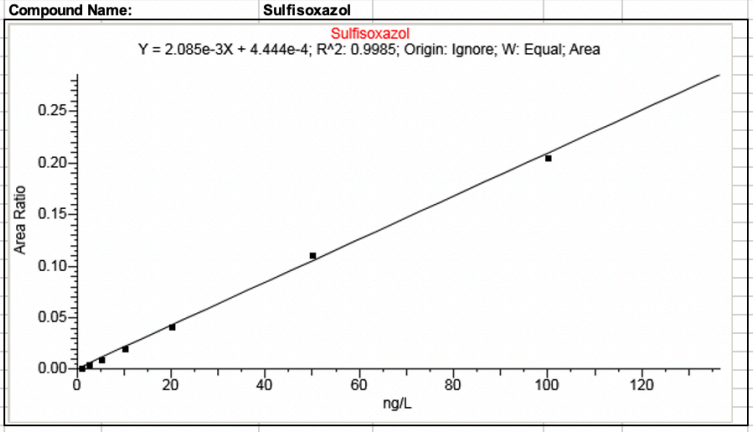
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	114501	50	2000922725	0	0,972	ng/L	N/A	N/A
2	2,5	438784	50	2024722520	0	2,31	ng/L	N/A	N/A
3	5	1021533	50	2045695699	0	4,679	ng/L	N/A	N/A
5	10	2280403	50	2069755028	0,001	9,73	ng/L	N/A	N/A
7	20	4679851	50	2034089306	0,002	19,781	ng/L	N/A	N/A
9	50	12669964	50	2067344675	0,006	51,873	ng/L	N/A	N/A
11	100	26031876	50	2211983133	0,012	99,156	ng/L	N/A	N/A

Figure B. 34. Calibration Report of Sulfisoxazole

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sulfisoxazol**



Linear
Fail A

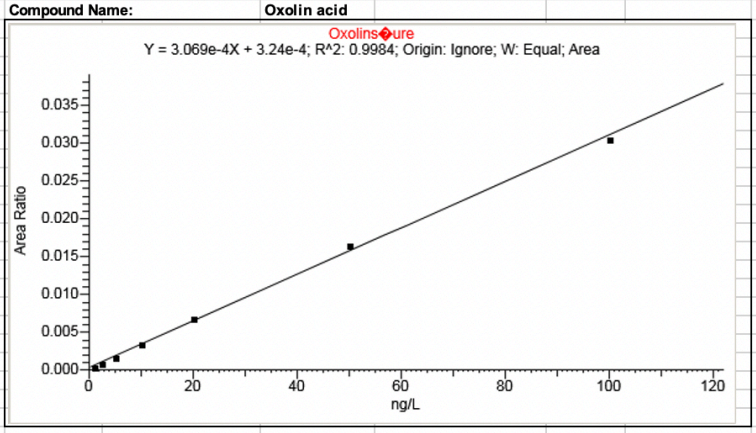
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	283772	50	174992276	0,002	0,565	ng/L	N/A	N/A A
2	2,5	843945	50	176492628	0,005	2,08	ng/L	N/A	N/A
3	5	1814741	50	180773097	0,01	4,601	ng/L	N/A	N/A
5	10	3675847	50	179648520	0,02	9,598	ng/L	N/A	N/A
7	20	7765913	50	183421832	0,042	20,089	ng/L	N/A	N/A
9	50	20296293	50	182820717	0,111	53,021	ng/L	N/A	N/A
11	100	41687798	50	202408031	0,206	98,547	ng/L	N/A	N/A

Figure B. 35. Calibration Report of Oxolinic acid

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Oxolin acid**



Linear
Fail A

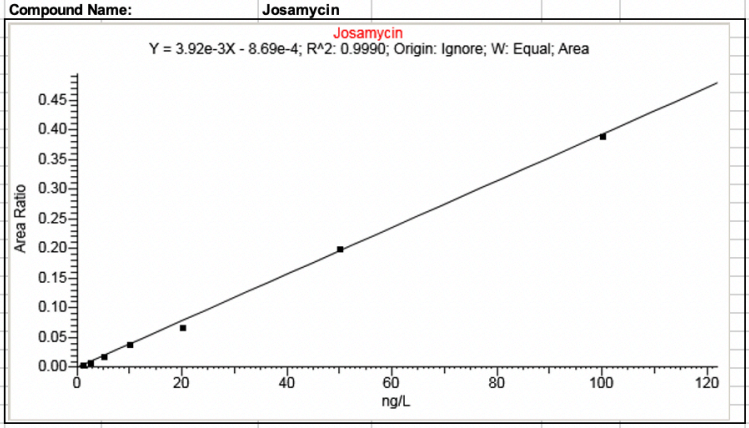
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	680992	50	2000922725	0	0,053	ng/L	N/A	N/A	A
2	2,5	1645296	50	2024722520	0,001	1,592	ng/L	N/A	N/A	A
3	5	3499633	50	2045695699	0,002	4,519	ng/L	N/A	N/A	
5	10	6998261	50	2069755028	0,003	9,963	ng/L	N/A	N/A	
7	20	13935314	50	2034089306	0,007	21,27	ng/L	N/A	N/A	
9	50	34035211	50	2067344675	0,016	52,594	ng/L	N/A	N/A	
11	100	67582877	50	2211983133	0,031	98,509	ng/L	N/A	N/A	

Figure B. 36. Calibration Report of Josamycin

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Josamycin**



Linear
Fail A

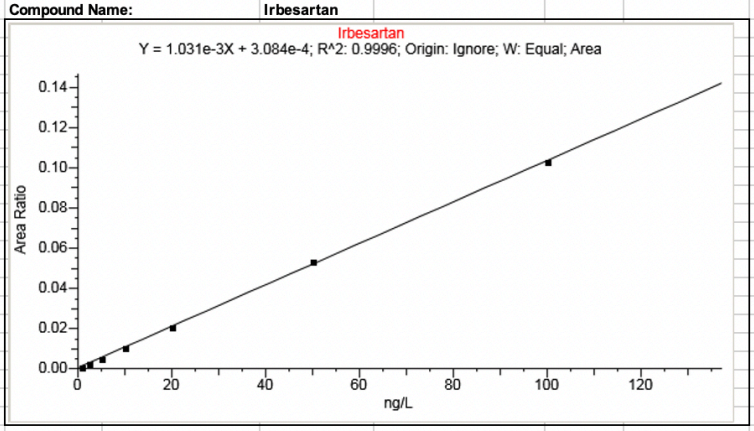
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	861091	50	174992276	0,005	1,477	ng/L	N/A	N/A	A
2	2,5	1537692	50	176492628	0,009	2,444	ng/L	N/A	N/A	
3	5	3374120	50	180773097	0,019	4,983	ng/L	N/A	N/A	
5	10	7307342	50	179648520	0,041	10,598	ng/L	N/A	N/A	
7	20	12669334	50	183421832	0,069	17,841	ng/L	N/A	N/A	
9	50	36805459	50	182820717	0,201	51,576	ng/L	N/A	N/A	
11	100	78840598	50	202408031	0,39	99,582	ng/L	N/A	N/A	

Figure B. 37. Calibration Report of Irbesartan

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Irbesartan**



Linear
Fail A

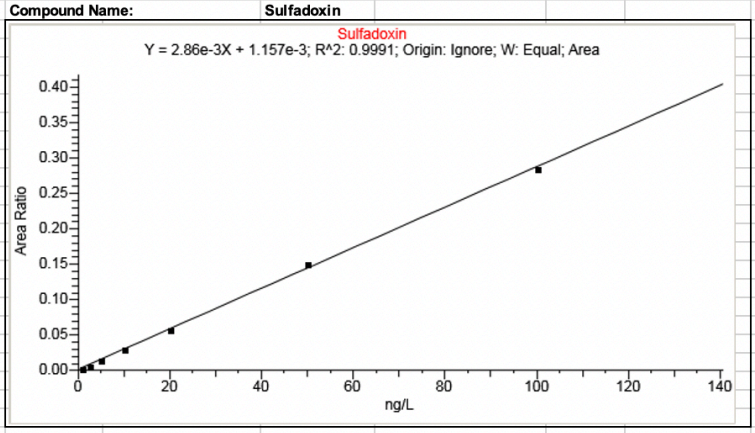
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	2042352	50	2000922725	0,001	0,691	ng/L	N/A	N/A	A
2	2,5	5092051	50	2024722520	0,003	2,141	ng/L	N/A	N/A	
3	5	10901260	50	2045695699	0,005	4,871	ng/L	N/A	N/A	
5	10	21905742	50	2069755028	0,011	9,969	ng/L	N/A	N/A	
7	20	42710001	50	2034089306	0,021	20,071	ng/L	N/A	N/A	
9	50	110385601	50	2067344675	0,053	51,501	ng/L	N/A	N/A	
11	100	226994255	50	2211983133	0,103	99,257	ng/L	N/A	N/A	

Figure B. 38. Calibration Report of Sulfadoxine

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Sulfadoxin**



Linear
Fail A

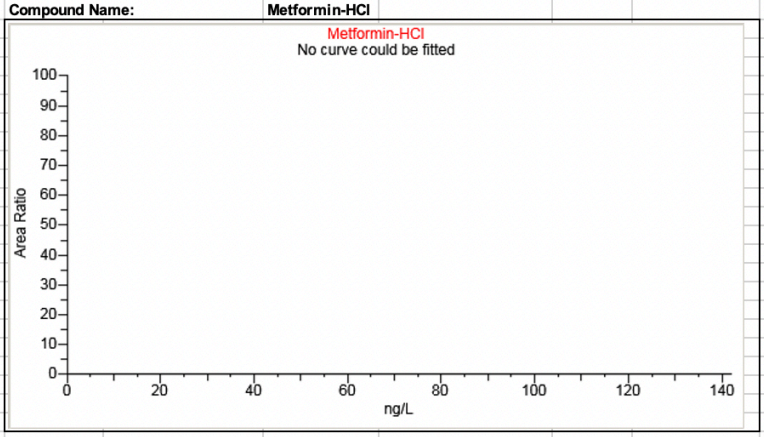
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	524857	50	174992276	0,003	0,644	ng/L	N/A	N/A	A
2	2,5	1205089	50	176492628	0,007	1,983	ng/L	N/A	N/A	A
3	5	2619132	50	180773097	0,014	4,661	ng/L	N/A	N/A	
5	10	5444869	50	179648520	0,03	10,193	ng/L	N/A	N/A	
7	20	10607029	50	183421832	0,058	19,815	ng/L	N/A	N/A	
9	50	27559441	50	182820717	0,151	52,304	ng/L	N/A	N/A	
11	100	57485260	50	202408031	0,284	98,899	ng/L	N/A	N/A	

Figure B. 39. Calibration Report of Metformin HCl

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Metformin-HCl**



Linear
Pass

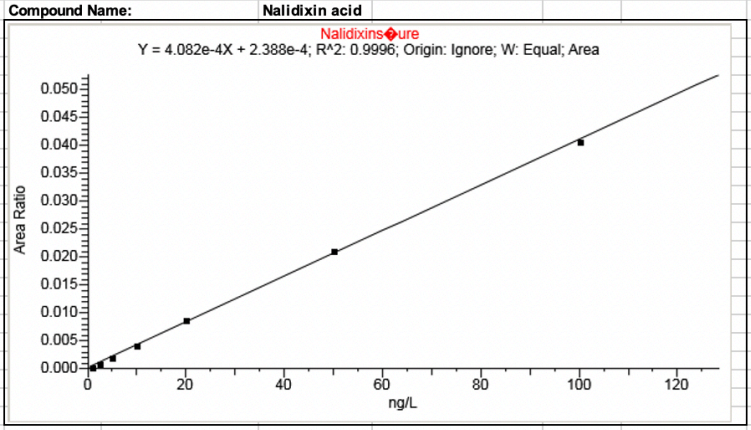
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	N/F	50	N/F	N/F	N/F	ng/L	N/A	N/A
2	2,5	N/F	50	N/F	N/F	N/F	ng/L	N/A	N/A
3	5	N/F	50	N/F	N/F	N/F	ng/L	N/A	N/A
5	10	N/F	50	N/F	N/F	N/F	ng/L	N/A	N/A
7	20	N/F	50	N/F	N/F	N/F	ng/L	N/A	N/A
9	50	N/F	50	N/F	N/F	N/F	ng/L	N/A	N/A
11	100	N/F	50	N/F	N/F	N/F	ng/L	N/A	N/A

Figure B. 40. Calibration Report of Nalidixic acid

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Nalidixin acid**



Linear
Fail A

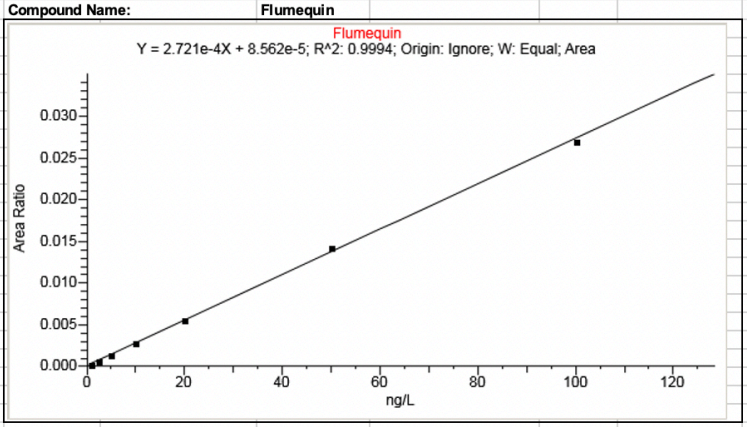
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	839142	50	2000922725	0	0,442	ng/L	N/A	N/A A
2	2,5	2197376	50	2024722520	0,001	2,074	ng/L	N/A	N/A
3	5	4371834	50	2045695699	0,002	4,651	ng/L	N/A	N/A
5	10	8879761	50	2069755028	0,004	9,925	ng/L	N/A	N/A
7	20	17936634	50	2034089306	0,009	21,018	ng/L	N/A	N/A
9	50	43619854	50	2067344675	0,021	51,105	ng/L	N/A	N/A
11	100	90173936	50	2211983133	0,041	99,285	ng/L	N/A	N/A

Figure B. 41. Calibration Report of Flumequine

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Flumequin**



Linear
Fail A

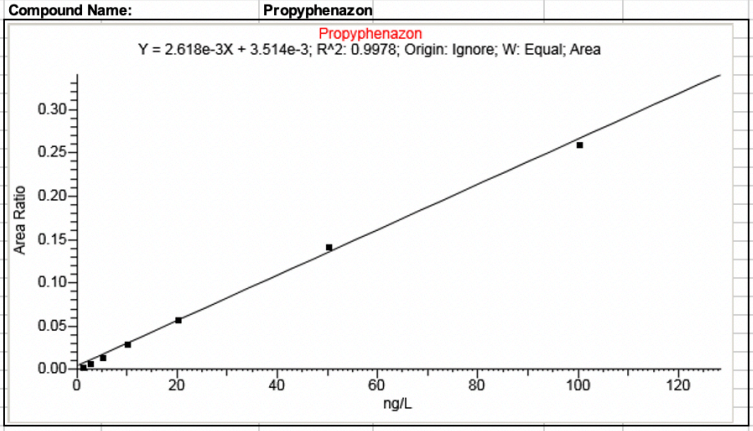
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	500461	50	2000922725	0	0,604	ng/L	N/A	N/A	A
2	2,5	1324474	50	2024722520	0,001	2,089	ng/L	N/A	N/A	
3	5	2855654	50	2045695699	0,001	4,815	ng/L	N/A	N/A	
5	10	5761408	50	2069755028	0,003	9,914	ng/L	N/A	N/A	
7	20	11297074	50	2034089306	0,006	20,094	ng/L	N/A	N/A	
9	50	29398456	50	2067344675	0,014	51,94	ng/L	N/A	N/A	
11	100	59809216	50	2211983133	0,027	99,043	ng/L	N/A	N/A	

Figure B. 42. Calibration Report of Propyphenazone

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Propyphenazon**



Linear
Fail A

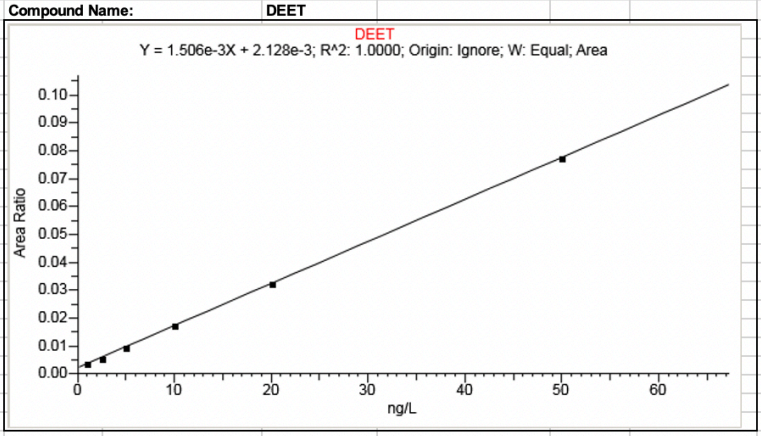
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD	
1	1	5999837	50	2000922725	0,003	-0,197	ng/L	N/A	N/A	A
2	2,5	15246552	50	2024722520	0,008	1,534	ng/L	N/A	N/A	A
3	5	30615383	50	2045695699	0,015	4,375	ng/L	N/A	N/A	
5	10	62718285	50	2069755028	0,03	10,233	ng/L	N/A	N/A	
7	20	119503382	50	2034089306	0,059	21,1	ng/L	N/A	N/A	
9	50	295509294	50	2067344675	0,143	53,261	ng/L	N/A	N/A	
11	100	576371461	50	2211983133	0,261	98,194	ng/L	N/A	N/A	

Figure B. 43. Calibration Report of DEET

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **DEET**



Linear
Pass

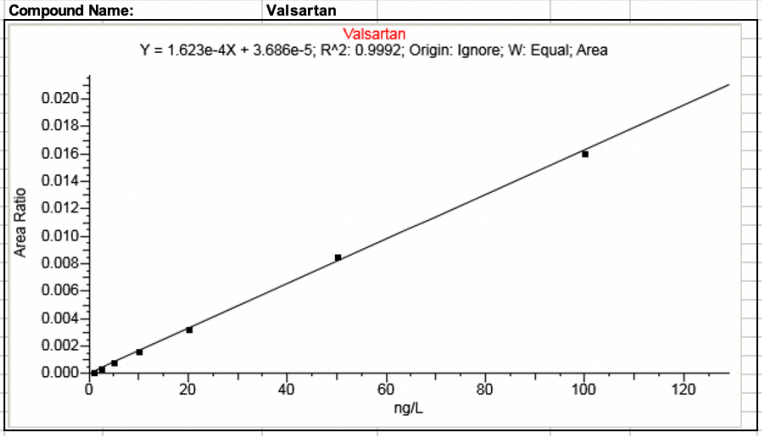
Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	7373651	50	2000922725	0,004	1,034	ng/L	N/A	N/A
2	2,5	11417138	50	2024722520	0,006	2,332	ng/L	N/A	N/A
3	5	19704102	50	2045695699	0,01	4,984	ng/L	N/A	N/A
5	10	35859349	50	2069755028	0,017	10,093	ng/L	N/A	N/A
7	20	65926099	50	2034089306	0,032	20,111	ng/L	N/A	N/A
9	50	159880335	50	2067344675	0,077	49,946	ng/L	N/A	N/A

Figure B. 44. Calibration Report of Valsartan

Compound Calibration Report

Lab Name:	AGES	**Method:**	Antibiotika_Serie1_Antibiotics_2020_final
Instrument:	Q-Exactive		Antibiotics_2020_final
User:	cchydro_Saner	**Cali File:**	Antibiotika_Serie1.calx
Batch:	Antibiotika_Serie1		

Compound Name: **Valsartan**



Linear
Fail A

Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
1	1	279986	50	2000922725	0	0,635	ng/L	N/A	N/A
2	2,5	742542	50	2024722520	0	2,033	ng/L	N/A	N/A
3	5	1671672	50	2045695699	0,001	4,808	ng/L	N/A	N/A
5	10	3375884	50	2069755028	0,002	9,823	ng/L	N/A	N/A
7	20	6689882	50	2034089306	0,003	20,038	ng/L	N/A	N/A
9	50	17608288	50	2067344675	0,009	52,255	ng/L	N/A	N/A
11	100	35587406	50	2211983133	0.016	98.907	ng/L	N/A	N/A

APPENDIX C: EXACT MASSES OF THE STANDARDS

Table C. 1. Exact masses (Dalton) of the standards used in the calibrations

			Molecular Formula	Exact Mass
Quinolone	1	Ciprofloxacin	C17H18FN3O3	332.14050
	2	Danofloxacin	C19H20FN3O3	358.15615
	3	Enrofloxacin	C19H22FN3O3	360.17180
	4	Marbofloxacin	C17H19FN4O4	363.14631
	5	Norfloxacin	C16H18FN3O3	320.14050
	6	Difloxacin	C21H19F2N3O3	400.14672
	7	Flumequine	C14H12FNO3	262.08740
	8	Nalidixic Acid	C12H12N2O3	233.09207
	9	Sarafloxacin	C20H17F2N3O3	386.13107
	10	Oxolinic Acid	C13H11NO5	262.07100
Macrolide	1	Clarithromycin	C38H69NO13	748.48417
	2	Erythromycin	C37H67NO13	734.46852
	3	Josamycin	C42H69NO15	828.47400
	4	Roxithromycin	C41H76N2O15	837.53185
	5	Erythromycin-anhydro	C37H65NO12	716.45795
	6	Spiramycin	C43H74N2O14	843.52128
	7	Tylosin	C46H77NO17	916.52643
Lincosamide	1	Lincomycin	C18H34N2O6S	407.22103
Diaminopyrimidine	1	Trimethoprim	C14H18N4O3	291.14517
Sulfonamide	1	Sulfadiazine	C10H10N4O2S	251.05972
	2	Sulfadimidine	C12H14N4O2S	279.09102
	3	Sulfadoxine	C12H14N4O4S	311.08085
	4	Sulfamethoxazole	C10H11N3O3S	254.05939
	5	Sulfathiazole	C9H9N3O2S2	256.02089
	6	Acetyl-sulfamethazine	C14H16N4O3S	321.10159
	7	Acetyl-sulfamethoxazole	C12H13N3O4S	296.06995
	8	Sulfadimethoxine	C12H14N4O4S	311.08085
	9	Sulfamerazine	C11H12N4O2S	265.07537
	10	Sulfamethoxypyridazine	C11H12N4O3S	281.07029
	11	Sulfamonomethoxine	C11H12N4O3S	281.07029
	12	Sulfaquinoxaline	C14H12N4O2S	301.07537
	13	Sulfisoxazole	C11H13N3O3S	268.07504
ISTDs	1	Atrazine D5		221.13243
	4	Ciprofloxacin D8		339.13322

	5	Erythromycin C13 D3		738.49070
	6	Gabapentin D10		182.19652
	7	Sulfamethoxazole D4		258.08450
	8	Trimethoprim D3		294.16400