DETECTION AND ECOLOGICAL RISK ASSESSMENT OF SELECTED PHARMACEUTICAL COMPOUNDS IN INDUSTRIAL EFFLUENT OF NATIONAL INDUSTRIAL ZONE, RCCI, RAWAT, PAKISTAN

SAEED AYAZ KHAN
01-262211-015

Department of Earth and Environmental Sciences

Bahria University, Islamabad, Pakistan

SEPTEMBER 2023

DETECTION AND ECOLOGICAL RISK ASSESSMENT OF SELECTED PHARMACEUTICAL COMPOUNDS IN INDUSTRIAL EFFLUENT OF NATIONAL INDUSTRIAL ZONE, RCCI, RAWAT, PAKISTAN

SAEED AYAZ KHAN

01-262211-015

A thesis submitted in fulfillment of the requirements for the award of the Master's in Science (Environmental Sciences)

Department of Earth and Environmental Sciences

Bahria University, Islamabad, Pakistan

SEPTEMBER 2023

THESIS COMPLETION CERTIFICATE

Name: Saeed Ayaz Khan

Registration No: 74838

Program of Study: MS Environmental Sciences

Thesis Title: DETECTION AND ECOLOGICAL RISK ASSESSMENT OF SELECTED PHARMACEUTICAL COMPOUNDS IN INDUSTRIAL EFFLUENT OF NATIONAL INDUSTRIAL ZONE, RCCI RAWAT, PAKISTAN.

It is to certify that the above scholar's thesis has been completed to my satisfaction, and to, the best of my belief, its standard is appropriate for submission of examination. I have also conducted a plagiarism test of this thesis using HEC prescribed software and found similarity index 10% that is within permissible limit set by the HEC for the MS degree thesis. I have also found the thesis in the format recognized by the BU for the MS thesis.

Supervisor's Signature: _____

Name: Dr. Asma Jamil

Dated:

AUTHOR'S DECLARATION

I, Saeed Ayaz Khan, hereby certify that all material in this dissertation titled "DETECTION AND ECOLOGICAL RISK ASSESSMENT OF SELECTED PHARMACEUTICAL COMPOUNDS IN INDUSTRIAL EFFLUENT OF NATIONAL INDUSTRIAL ZONE, RCCI RAWAT, PAKISTAN." is my work and has not been submitted previously by me for taking any degree from Bahria University Islamabad or elsewhere in the country/world.

At any time, if my statement is found to be incorrect the university has the right to withdraw/cancel my MS degree.

Scholar's Signature: _____

Name of Student: Saeed Ayaz Khan

Dated:

PLAGIARISM UNDERTAKING

I solemnly declare that the research work presented in the thesis titled "DETECTION AND ECOLOGICAL RISK ASSESSMENT OF SELECTED PHARMACEUTICAL COMPOUNDS IN INDUSTRIAL EFFLUENT OF NATIONAL INDUSTRIAL ZONE, RCCI RAWAT, PAKISTAN." is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has duly acknowledged, and I have written that complete thesis.

I understand the zero-tolerance policy of the HEC and Bahria University towards plagiarism. Therefore, I as author of the above titled thesis, declare that no portion of my thesis has been plagiarized, and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above-titled thesis even after awarding of MS degree, the university reserves the right to withdraw/revoke my MS degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of scholars are placed who submitted plagiarized thesis.

Scholar's Signature: _____

Name of Scholar: Saeed Ayaz Khan

Date:

DEDICATION

I dedicate this thesis to my beloved parents who have offered unconditional love and support and have always been there for me.

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to all those who have supported and contributed to the completion of this thesis. First and foremost, I am sincerely thankful to my supervisor Dr. Asma Jamil for their invaluable guidance, encouragement, and unwavering support throughout the research process. Their expertise and insightful feedback have been instrumental in shaping this work. I am thankful to Head of Department Earth and Environmental Sciences, Dr. Said Akbar Khan for providing all support.

I am also grateful to the research participants Syeda Areej Fatima, Muhammad Sareer and organizations that provided access to essential data and facilities, enabling the successful execution of this study. Their cooperation and willingness to share knowledge have been vital in advancing our understanding of the subject matter.

Furthermore, I extend my appreciation to my family and friends for their constant encouragement, understanding, and belief in my abilities. Their emotional support has been a source of strength and motivation during challenging times.

ABSTRACT

The National Industrial Zone, RCCI Rawat, Pakistan, houses multiple industries, contributing to the release of industrial effluent into adjacent aquatic environments. These compounds have demonstrated ecotoxicological impacts on aquatic organisms, and disruptions in the ecological balance of aquatic environments. Understanding the occurrence, distribution, and ecological risks associated with pharmaceutical compounds in the industrial effluent is crucial for effective environmental management and water resource protection. This research aims to address this knowledge gap by investigating the detection and ecological risk Assessment of selected pharmaceutical compounds (Ciprofloxacin, Levofloxacin, Cefixime, Cefoperazone and Ceftriaxone) in industrial effluent within the National Industrial Zone, RCCI Rawat, Pakistan. Selected PCs in effluent and in surface water (Ling Stream) were analyzed through Liquid Chromatography (LC) and UV-Detector. Cefixime fund at high concentration in effluent (173.53-380.9 µg/L followed by Cefoperazone (111.06-320.3 µg/L), Ciprofloxacin (67.72-212.55 µg/L), Levofloxacin (47.71-80.45 µg/L) were detected at medium concentration and Ceftriaxone (7.1-15.06 µg/L) at lowest level. The concentration of PCs (Ciprofloxacin, Levofloxacin, cefixime and ceftriaxone) in Surface water (Ling stream) were (2.47 – 7.22 µg/L), (1.92 – 3.73 µg/L), (0.00 – 9.94 µg/L) and (2.80 – 9.00 µg/L) respectively. Ecological risk Assessment were conducted against several freshwater species. Risk quotient in case of Ciprofloxacin (247 – 722) and Levofloxacin (192 – 373) Pharmaceutical compounds were highest against Green Algae greater than 1.0 that is and made high risk against the species. And Risk quotient of ciprofloxacin and levofloxacin for Fish and Daphnia were too low and less the 1.0. The RQ value was determined for Cefixime (156.5 – 248.5) against Bacteria (*P. putida*) pose high level risk against the species. The conducted ecological risk Assessment using the Risk Quotient (RQ, the potential impact on the aquatic environment of Ling stream, revealing significant variations in pharmaceutical compound concentrations and highlighting the limitations of current wastewater treatment processes in removing these compounds. In conclusion, this research addresses the need for improved treatment technologies and environmental management.

TABLE OF CONTENTS

Chapter	Title	Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

APIs	Active Pharmaceutical Ingredients
Approx	Approximately
EDTA	Ethylene Diamine Tetra Acetate
HPLC	High performance Liquid Chromatography
LC	Liquid chromatography
LC-MS	Liquid Chromatophraphy- Mass Spectrometry
MEC	Measured Environmental Concentration
Min.	Minimum
Max.	Maximum
POCIS	Polar Organic Chemical Integrative Samplers
PCs	Pharmaceutical Compounds
PPPs	Persistent Pharmaceutical Pollutants
PNEC	Predicted No- Effect Concentration
RQ or HQ	Risk Quotient or Hazard Quotient
RCCI	Rawalpindi Chamber of Commerce and Industry
UV	Ultra-Violet
VOCs	Volatile Organic Compounds

CHAPTER 1

INTRODUCTION

The emergence of Pharmaceutical compounds in the environment has garnered considerable attention during modern times (Papagiannaki et al., 2022). A variety of active pharmaceutical ingredients (APIs), metabolites, and transformation products constitute these compounds, which have been identified in multiple environmental compartments, such as surface water, groundwater, and sediments. The discharge of effluent from Pharmaceutical manufacturing facilities, healthcare facilities, and domestic wastewater is the predominant origin of Pharmaceutical compounds in the environment (Gadipelly et al., 2014).

The RCCI Rawat, Pakistan's National Industrial Zone, is a significant industrial region that encompasses a variety of industries. The aforementioned sectors, encompassing Pharmaceutical production facilities, chemical processing plants, and healthcare institutions, are responsible for producing industrial effluent that are released into adjacent aquatic environments (Ngwuluka et al., 2011). The release of untreated or insufficiently treated effluent that contain Pharmaceutical compounds into the environment can result in unfavorable ecological outcomes, which can impact the well-being of aquatic organisms and the overall health of the ecosystem (Edokpayi et al., 2017).

It has been observed that pharmaceutical compounds demonstrate ecotoxicological impacts on aquatic organisms, even at concentrations that are considered low. The impacts may

encompass alterations in conduct, stunted development, reproductive anomalies, and perturbations to the inherent equilibrium of aquatic environments. The environmental persistence, bioaccumulation, and transformative potential of Pharmaceutical compounds have elicited apprehension regarding their enduring effects and potential hazards to human health via the ingestion of contaminated water or aquatic organisms (Ferrari et al., 2003).

Understanding the occurrence, distribution, and ecological risks associated with pharmaceutical compounds in the industrial effluent of the National Industrial Zone, RCCI Rawat, Pakistan, is crucial for effective environmental management and the protection of water resources. This research addresses this knowledge gap by investigating the detection and ecological risk Assessment of selected pharmaceutical compounds in industrial effluent within this industrial zone. By gaining insights into the presence, concentrations, and potential ecological impacts of these compounds, it will be possible to develop strategies and measures to minimize their environmental impacts and ensure the sustainability of water resources in the region (Abdudeen et al., 2023).

The focus on the National Industrial Zone, RCCI Rawat, Pakistan, highlights the relevance and significance of studying pharmaceutical compounds' detection and ecological risk Assessment in this area. The subsequent sections of this thesis will delve into the problem statement, objectives, and significance of the research, followed by a comprehensive literature review, methodology, results, and discussions (Ahmad et al., 2020)

1.1 Background

The Pharmaceutical industry in Pakistan is experiencing a robust growth, exhibiting an annual expansion rate of 10%. In addition to its growth, this industry is found to be non-compliant with environmental regulations by discharging its effluent into the domestic wastewater system. The study aimed to investigate the presence and ecological risk assessment of 11 Pharmaceutical compounds from diverse therapeutic classes in pharmaceutical industry wastewater and receiving environmental matrices, including sludge, solid waste, and soil samples near pharmaceutical formulation units. This was undertaken due to the scarcity of information regarding the prevalence of Pharmaceutical compounds in Pakistani environmental matrices (Ashfaq et al., 2017). The concentrations of ciprofloxacin, enrofloxacin, and levofloxacin were observed in wastewater samples collected from two regions, namely Kahuta and Hattar. The results indicated that the concentrations of these antibiotics were slightly elevated in the Kahuta region (58, 32.9, and 36.7 µg/L, respectively) as compared to the Hattar region (42.1, 41.2, and 48.9 µg/L, respectively). The concentrations of Ciprofloxacin, Enrofloxacin, and Levofloxacin in samples of sludge were found to be significantly greater in the Hattar region (159, 153, and 164 µg kg-1, respectively) as compared to the Kahuta region (129, 58, and 91 µg kg-1, respectively). The utilization of Fluoroquinolones in the healthcare industry has resulted in the emergence of water pollution, thereby presenting an ecological apprehension to aquatic organisms (Riaz et al., 2017). Most of the indicators used to measure water quality in the nation surpassed the National Environmental Quality Standards established by the country. The presence of these contaminants has the potential to cause adverse effects on the composition of the soil, the quality of groundwater, and the ecosystems of freshwater habitats (Hussein et al., 2011). A study examined the quality of groundwater and surface

water in Rawalpindi with regards to potential contamination by hazardous substances, as well as the associated risk assessment for both drinking and agricultural purposes. This study involved the collection of 30 groundwater samples, 27 samples of surface water from River Soan, and 21 samples of wastewater. Each sample was analyzed for the presence of potentially hazardous elements and its physiochemical characteristics were measured. Based on the available data, it can be observed that the physiochemical parameters and potentially hazardous elements contaminations were found to be at their lowest levels in groundwater (Khan et al., 2019). Many Pharmaceutical industries produce effluent containing toxic metals, reagents, chemical catalysts, and organic compounds (Javed et al., 2021). The present investigation centered on the detection and evaluation of potential hazards posed by 52 pharmaceutical and personal care products belonging to diverse categories in water and sediment specimens obtained from the urban drains and canals of Lahore. Non-steroidal anti-inflammatory medications were identified as the predominant pharmaceutical compound in wastewater from urban drains and surface water from canals. Acetaminophen exhibited the highest concentration among the substances analyzed, with a median concentration of 13,880 ng/L (Ashfaq et al., 2019). The introduction of novel pollutants, including antibiotics, into the environment is a matter of apprehension for both environmental advocates and decision-makers.

The present study aimed to investigate the presence and real-world situation analysis of selected antibiotics in wastewater channels of Rawalpindi/Islamabad, Pakistan, through detection and quantification methods. The results indicate that Ciprofloxacin exhibited the highest concentration (332.154 µg/mL) among the tested substances, with Ofloxacin, Ampicillin, Levofloxacin, and Sulfamethoxazole following in descending order. The

results of the study indicate the existence of antibiotics resulting from unselective utilization, potentially leading to the development of resistant strains and consequent dissemination of antibiotic resistance (Zafar et al., 2021). The results indicate that Faisalabad exhibited the highest concentration of antibiotics, with a mean value of 13.8 ng/L. Gujrat followed with a concentration of 7.8 ng/L, while Lahore, Quetta, Rawalpindi/Islamabad, and Peshawar exhibited mean concentrations of 4.04 ng/L, 3.9 ng/L, 2.29 ng/L, and 2.03 ng/L, respectively. The outcomes of the study indicate that Tigecycline and ciprofloxacin were the prevailing antibiotics detected in groundwater, with mean concentrations of 21.3 ng/L and 18.2 ng/L, correspondingly. The results of the spatial distribution analyses indicate that Faisalabad, which is widely recognized as the industrial hub of the country, exhibits the highest levels of groundwater pollution. The dominant antibiotic classes detected in the groundwater include quinolones (excluding flumequine), -Lactams, tetracyclines, sulfonamides, and amphenicols, which are indicative of elevated levels of human and veterinary medication usage within the city (Zainab et al., 2021).

1.2 Literature Review

The discharge of industrial effluent, encompassing the wastewater produced by manufacturing plants, pharmaceutical industries, and associated operations, has been acknowledged as a noteworthy contributor to the presence of pharmaceutical compounds in the environment. Pharmaceutical compounds are frequently present in effluent because of improper disposal practices, and inadequate treatment processes, leading to residual amounts. Consequently, these chemical compounds are introduced into aquatic environments via the discharge of wastewater, which may result in unfavorable ecological consequences (Gadipelly et al., 2014).

The potential impacts of pharmaceutical compounds on aquatic organisms and ecosystem health are a cause for concern, given their presence in industrial effluent. These chemical substances have been identified in diverse aquatic environments globally, encompassing inland waterways, freshwater reservoirs, and oceanic regions. Pharmaceutical compounds, upon being discharged into the environment, have the potential to endure, accumulate in living organisms, and cause prolonged impacts on both aquatic and terrestrial organisms that rely on water resources. The presence of Pharmaceutical compounds in the environment has the potential to cause ecological disturbances, impact biodiversity, and jeopardize the overall health and stability of ecosystems (Grenni et al., 2018).

Comprehending the origins, destiny, and ecological ramifications of pharmaceutical compounds present in industrial effluent is of paramount importance for the purpose of efficient environmental management and safeguarding. There is a current endeavor to establish reliable detection techniques and monitoring initiatives aimed at evaluating the magnitude of pharmaceutical pollution in wastewater effluent. Furthermore, ecotoxicological investigations and risk evaluations are being carried out to assess the possible hazards that may be caused by these substances to aquatic organisms and ecosystems (Chapman et al., 1998).

The present literature review aims to explore the current state of knowledge and research pertaining to pharmaceutical compounds present in industrial effluent. The present study aims to investigate the origins and routes of pharmaceutical contamination, the subsequent behavior and movement of these substances within the environment, and the consequential ecological ramifications that have been observed in aquatic ecosystems. This review seeks to identify research gaps, limitations, and potential areas for further investigation in the

field of pharmaceutical compound detection and ecological risk Assessment through a synthesis of the current literature.

The term "Pharmaceutical compounds" refers to a diverse group of chemical substances that are purposefully designed and formulated for medical or therapeutic applications (Nelson et al., 2017). These compounds serve as the active agents in pharmaceutical products and play a crucial role in diagnosing, preventing, treating, or alleviating symptoms of various diseases. Pharmaceutical compounds can be classified into different types based on their therapeutic properties, chemical structure, mode of action, and intended use (Abubakar et al., 2020).

Pharmaceutical contamination in industrial effluent arises from a variety of sources within the manufacturing and healthcare sectors. Pharmaceutical manufacturing facilities contribute to the contamination through the production, formulation, and packaging processes of medications. During these processes, the incomplete utilization or disposal of raw materials, intermediates, and final products can lead to the release of pharmaceutical compounds into wastewater. Spillages, leaks, or improper handling of pharmaceutical substances within manufacturing units can also contribute to contamination. The presence of pharmaceutical compounds in industrial effluent from healthcare facilities, such as hospitals and clinics, is primarily attributed to the disposal of unused or expired medications and the excretion of pharmaceutical residues by patients receiving treatment. These healthcare-related sources add to the complexity of Pharmaceutical contamination in industrial effluent and highlight the need for effective management strategies (Gadipelly et al., 2014).

The pathways through which pharmaceutical compounds enter industrial effluent can be diverse and multifaceted. One of the primary pathways is through direct wastewater discharges from pharmaceutical manufacturing plants or healthcare facilities. Wastewater streams generated during production processes, cleaning procedures, and patient care activities can carry pharmaceutical compounds into the effluent streams. Additionally, improper disposal practices, such as flushing unused medications down the drain or toilet, contribute to the direct entry of Pharmaceutical compounds into the wastewater system. (Pal et al., 2014).

The transport of Pharmaceutical compounds in the environment occurs through various pathways, including surface water, groundwater, and atmospheric deposition (Ritter, 2002). Surface runoff and drainage systems can transport pharmaceutical compounds from contaminated soils or agricultural areas into nearby rivers, lakes, or estuaries. Groundwater contamination can occur when pharmaceutical compounds infiltrate the soil and leach into the groundwater, potentially affecting drinking water sources. The fate and transport of Pharmaceutical compounds in the environment are complex phenomena influenced by chemical properties, environmental conditions, and hydrological processes (Tong et al., 2022).

Understanding the fate and transport of pharmaceutical compounds in the environment is essential for assessing their potential impacts on ecosystems and human health. These compounds can be distributed widely, potentially exposing aquatic organisms, wildlife, and even humans. The persistence and long-range transport of pharmaceutical compounds raises concerns about their ability to accumulate in organisms, potentially leading to bioaccumulation and biomagnification in food chains. Furthermore, the fate and transport

of pharmaceutical compounds can vary depending on the specific compound, environmental conditions, and other chemicals or contaminants. Therefore, investigating the fate and transport of Pharmaceutical compounds in different environmental compartments and ecosystems is crucial for assessing their ecological risks and designing effective strategies for their detection, monitoring, and mitigation in industrial effluent (Gavrilescu et al., 2015).

Pharmaceutical compounds can directly affect aquatic organisms, ranging from acute to chronic impacts. Many pharmaceuticals are designed to target specific receptors or biological pathways in humans or animals, but similar targets may exist in aquatic organisms. The direct effects of Pharmaceutical compounds can occur at various levels of biological organization, from individual organisms to entire populations (Frye et al., 2012).

Pharmaceutical compounds can also indirectly affect aquatic ecosystems by altering the ecological interactions and dynamics within these systems. For instance, the presence of Pharmaceutical in water bodies can affect the feeding behaviors of aquatic organisms, leading to changes in predation patterns and trophic cascades. Additionally, Pharmaceutical compounds can influence the composition and structure of microbial communities, disrupting critical ecological processes such as nutrient cycling or decomposition. The indirect effects of Pharmaceutical compounds can propagate through the food web, impacting higher trophic levels and potentially altering aquatic ecosystems' overall functioning and stability (Mor et al., 2022).

The widespread use of antibiotics, both in human medicine and agriculture, has led to the emergence of antibiotic-resistant bacteria. Pharmaceutical compounds containing antibiotics can enter aquatic ecosystems through wastewater discharges, contributing to the

selection and proliferation of antibiotic-resistant bacteria in the environment. Moreover, the loss of sensitive bacterial populations due to the selective pressure of antibiotics can disrupt essential ecological functions bacteria perform, such as nutrients.

Pharmaceutical compounds' ecological impacts on aquatic ecosystems are crucial for effective environmental management and conservation. It highlights the importance of implementing proper wastewater treatment methods, promoting responsible pharmaceutical disposal practices, and considering the potential ecological risks when designing and using pharmaceutical compounds. Additionally, studying the specific impacts of different Pharmaceutical compounds and their interactions in aquatic ecosystems is essential for developing mitigation strategies and regulations to minimize their ecological footprint (Khan et al., 2020).

The ecological impacts of pharmaceutical compounds on aquatic ecosystems can have implications for providing essential ecosystem services. For instance, the contamination of water bodies with pharmaceutical can affect the quality and availability of freshwater resources. This can affect human activities dependent on clean water, such as agriculture, industry, and drinking water supply. Additionally, the loss or impairment of biodiversity and ecological processes caused by pharmaceutical compounds can compromise the ability of aquatic ecosystems to provide other essential services, such as nutrient cycling, water purification, and flood regulation. Mitigating the ecotoxicological effects of Pharmaceutical compounds on ecosystem services is crucial for the sustainable management and conservation of aquatic ecosystems (Reid et al., 2019).

1.2.1 Environmental concerns in The National Industrial Zone, located in RCCI Rawat, Pakistan

The National Industrial Zone, located in RCCI Rawat, Pakistan, is a significant industrial hub that plays a vital role in economic development (Liu et al., 2021). However, this zone's rapid industrialization and expansion of industrial activities have raised several environmental concerns. One primary concern is the discharge of industrial effluent containing various pollutants, including pharmaceutical compounds, into the surrounding environment. The improper management and treatment of these effluent pose risks to nearby ecosystems, particularly aquatic ecosystems, as they can become contaminated with pharmaceutical residues and other harmful substances. Releasing Pharmaceutical compounds into the environment can harm water quality, soil health, and biodiversity. These compounds can accumulate in water bodies, affecting the resident flora and fauna and potentially disrupting the ecological balance. Moreover, the contamination of groundwater sources can impact the availability of clean drinking water, posing risks to human health (Fida et al., 2022). The National Industrial Zone in RCCI Rawat, therefore, requires careful attention to ensure the implementation of effective environmental management practices, including the proper treatment and disposal of industrial effluent, in order to mitigate the environmental concerns associated with Pharmaceutical compound contamination and safeguard the surrounding ecosystems and public health (Landrigan et al., 2020).

The National Industrial Zone in RCCI Rawat, Pakistan, is characterized by various industrial activities, including pharmaceutical manufacturing, chemical processing, and other industrial processes. These activities often involve using and producing hazardous substances, which can pose significant environmental risks if not properly managed.

Inadequate waste management practices, such as the improper handling and disposal of industrial waste and by-products, can result in soil and water pollution and air pollution through emissions. The release of pollutants into the environment can have far-reaching consequences, impacting not only the immediate surroundings but also downstream ecosystems and communities. Therefore, it is crucial to address the environmental concerns in the National Industrial Zone to prevent further degradation of the natural environment and to ensure sustainable industrial development (Misra et al., 2005).

The presence of pharmaceutical compounds in the industrial effluent of the National Industrial Zone raises specific environmental concerns. Even at low concentrations, pharmaceutical compounds can adversely affect aquatic organisms and ecosystems. The release of these compounds into water bodies can contaminate surface water and groundwater sources. As water resources are essential for agriculture, drinking water supply, and maintaining ecological integrity, their pollution can have significant implications for the local communities and the environment. Furthermore, the persistence of pharmaceutical compounds in the environment and their potential to bioaccumulate in organisms highlight the need for proactive measures to mitigate their ecological impacts. Implementing appropriate treatment technologies, improving waste management practices, and monitoring the effluent discharge from industrial facilities are crucial steps to minimize the environmental risks associated with pharmaceutical compounds in the National Industrial Zone.

In addition to the contamination of water bodies, the National Industrial Zone in RCCI Rawat is also susceptible to air pollution concerns. Industrial activities, such as combustion processes, chemical reactions, and exhaust emissions, can release various pollutants into

the atmosphere. These pollutants include volatile organic compounds (VOCs), particulate matter, and toxic gases, which can contribute to air pollution and adversely affect air quality and human health. The emission of air pollutants from the industrial zone can lead to the formation of smog, contribute to respiratory diseases, and negatively impact local ecosystems and vegetation. Therefore, implementing stringent air pollution control measures, promoting the use of cleaner technologies, and monitoring air quality are crucial for minimizing the environmental impact of industrial activities in the National Industrial Zone (Kampa et al., 2008).

To address the environmental concerns in the National Industrial Zone, collaborative efforts between the government, industries, and relevant stakeholders are essential. It is important to establish and enforce stringent environmental regulations and standards that govern industrial practices, waste management, and emissions control. The implementation of pollution prevention and control measures, such as the adoption of cleaner production technologies and the promotion of sustainable practices, can help minimize the environmental footprint of industrial activities. Raising awareness among industries and the local community about the importance of environmental conservation and sustainable development is crucial for fostering a culture of environmental responsibility and ensuring long-term environmental sustainability in the National Industrial Zone, RCCI Rawat, Pakistan (Tseng et al., 2013).

Industrial effluent can pose several potential environmental risks due to their composition and discharge into the environment. Water pollution can have detrimental effects on aquatic ecosystems, leading to the loss of biodiversity, the disruption of ecological processes, and

the impairment of water resources for various purposes such as drinking water supply, agriculture, and aquatic habitats (Munter et al., 2003).

The pollutants present in the effluent can accumulate in the soil, potentially affecting soil fertility, microbial communities, and plant growth. Contaminated soil can also pose risks to human health if the pollutants enter the food chain through the consumption of contaminated crops or grazing animals (Murtaza et al., 2010). Some industrial effluent release volatile organic compounds (VOCs), particulate matter, or toxic gases into the atmosphere, contributing to air pollution. Emissions from industrial processes can lead to the formation of smog, contribute to the depletion of the ozone layer, and release greenhouse gases, thereby exacerbating climate change. Exposure to air pollutants from industrial effluent can have adverse effects on human health, including respiratory problems and increased risk of cardiovascular diseases (Barabad et al., 2018).

Inadequate management of industrial effluent can lead to the contamination of groundwater, which is a vital source of drinking water in many regions. If industrial effluent infiltrates the soil and reach the underlying aquifers, they can contaminate the groundwater, making it unsuitable for human consumption and posing health risks to local communities. Contaminated groundwater can be challenging and costly to remediate, potentially impacting the availability of clean water resources for an extended period (Idrees et al., 2018).

Addressing these environmental risks requires the implementation of effective waste management practices, including proper treatment and disposal of industrial effluent. Strict regulations, monitoring programs, and enforcement of environmental standards are essential to ensure that industrial activities do not pose significant harm to the environment.

Promoting sustainable industrial practices, resource efficiency, and the use of cleaner technologies can also help minimize the potential environmental risks associated with industrial effluent (Singh et al., 2016).

1.2.2 Studies on Detection of Pharmaceutical Compounds in Industrial Effluent

Several studies have been conducted to detect and quantify pharmaceutical compounds in industrial effluent. These studies aim to understand the presence, concentration, and potential risks associated with the release of pharmaceutical compounds into the environment. Here are a few examples of studies conducted on the detection of pharmaceutical compounds in industrial effluent:

Focused on assessing the presence of pharmaceutical residues in industrial wastewater and evaluating the effectiveness of advanced treatment technologies in their removal. The researchers analyzed samples from different industries and employed techniques such as solid-phase extraction and high-performance liquid chromatography to detect and quantify pharmaceutical compounds. The study highlighted the need for implementing advanced treatment methods, such as activated carbon adsorption, membrane filtration, and advanced oxidation processes, to effectively remove Pharmaceutical residues from industrial effluent (Ankush et al., 2018).

Studied specifically on the detection and analysis of antibiotic residues in industrial wastewater and their potential environmental impacts. The researchers collected samples from industrial effluent and nearby surface water bodies and analyzed them using techniques such as liquid chromatography-tandem mass spectrometry. The study found the presence of various antibiotics in industrial effluent, highlighting the need for improved

wastewater treatment processes to prevent the release of these compounds into the environment (Wang et al., 2021).

Evaluated passive sampling techniques, such as polar organic chemical integrative samplers, to monitor the presence of selected pharmaceutical compounds in industrial wastewater. The researchers deployed POCIS samplers in different locations within industrial facilities and analyzed the accumulated compounds using LC-MS. The study demonstrated the applicability of passive sampling techniques in monitoring Pharmaceutical compounds in industrial effluent and provided insights into their spatial and temporal variations (Grabicová et al., 2020).

Explored on the occurrence, fate, and removal strategies of emerging contaminants, including pharmaceutical compounds, in industrial effluent. The researchers conducted a comprehensive analysis of effluent samples from different industrial sectors using advanced analytical techniques. The study highlighted the need for improved monitoring and treatment methods to effectively detect and remove emerging contaminants from industrial wastewater (Chaturvedi et al., 2021).

The study revealed the widespread occurrence of antibiotics in industrial effluent, highlighting the importance of advanced treatment processes to mitigate their environmental impacts (Wang et al., 2021).

These studies highlight the significance of detecting and monitoring pharmaceutical compounds in industrial effluent to understand their environmental fate, assess potential risks, and develop effective wastewater treatment strategies. By identifying the presence and concentration of pharmaceutical compounds, these studies contribute to the overall

understanding of the ecological risks associated with the discharge of industrial effluent and help inform regulatory measures and pollution prevention strategies.

1.2.3 Ecological risk assessment of pharmaceutical compounds

The release of pharmaceutical compounds into aquatic ecosystems can have detrimental effects on aquatic organisms. Studies have shown that certain compounds, such as antibiotics and hormones, can disrupt the endocrine system of fish and other aquatic organisms, leading to reproductive impairments and altered behavior. Additionally, Pharmaceutical compounds with cytotoxic properties can induce cellular damage and oxidative stress in aquatic organisms, affecting their overall health and survival. Chronic exposure to sublethal concentrations of these compounds can also lead to long-term ecological impacts, such as reduced growth, impaired development, and changes in community structure. Understanding the specific ecotoxicological effects of Pharmaceutical compounds is crucial for assessing the potential risks they pose to aquatic organisms and ecosystems (Ebele et al., 2017).

Ecological risk assessment frameworks and approaches play a fundamental role in evaluating the potential ecological impacts of pharmaceutical compounds in aquatic ecosystems. These frameworks provide a systematic and structured approach to identify and evaluate the risks associated with the presence of pharmaceutical compounds. They involve a series of steps, starting with problem formulation, which includes defining the scope of the assessment, identifying the potential receptors of concern, and setting the assessment goals. Exposure assessment is another critical step that focuses on quantifying the concentrations of pharmaceutical compounds in the environment and determining the extent and duration of exposure for aquatic organisms. Effects assessment involves

evaluating the toxicity of these compounds to different species and their potential impacts on population dynamics and ecosystem functioning. The integration of exposure and effects data enables the characterization of the risks posed by Pharmaceutical compounds in aquatic systems (Laurenson et al., 2014).

Selection of appropriate assessment endpoints and consideration of species sensitivity are crucial aspects of ecotoxicological risk assessment for pharmaceutical compounds. Assessment endpoints are specific ecological or biological responses that are measured to evaluate the effects of these compounds on aquatic organisms. Examples of commonly used endpoints include reproductive success, growth rates, and survival. The selection of appropriate endpoints depends on the specific objectives of the risk assessment and the characteristics of the organisms being studied. Moreover, the sensitivity of different species to pharmaceutical compounds can vary significantly. Some species may be more susceptible to the toxic effects of certain compounds, while others may exhibit higher tolerance levels. Considering species sensitivity allows for a more accurate assessment of the potential ecological risks posed by Pharmaceutical compounds in aquatic ecosystems and facilitates the development of targeted management strategies to protect vulnerable species and maintain ecosystem integrity (Caldwell et al., 2014).

Ecological risk assessment frameworks provide a structured and systematic approach for evaluating the potential risks associated with pharmaceutical compounds in aquatic systems. These frameworks typically involve several key steps. Problem formulation is the initial step, which involves clearly defining the objectives of the risk assessment, identifying the potential receptors of concern, and setting the assessment boundaries. This step ensures that the assessment is focused and tailored to the specific context. Exposure

assessment is another critical component, where the concentrations and duration of exposure to pharmaceutical compounds in the environment are quantified. This step considers factors such as emission sources, transport pathways, and environmental fate to estimate the potential exposure levels of aquatic organisms. Effects assessment involves evaluating the potential adverse effects of the compounds on different ecological receptors, considering both acute and chronic toxicity. Risk characterization integrates the exposure and effects data to estimate the likelihood and magnitude of adverse effects, leading to an overall assessment of ecological risk (Ahrens et al., 2014).

Several ecological risk assessment approaches are employed to evaluate the risks associated with pharmaceutical compounds in aquatic ecosystems. These approaches may include probabilistic modeling, species sensitivity distributions, and ecosystem-based Assessment. Probabilistic modeling considers the uncertainties associated with exposure and effects data, providing a more realistic estimation of risks. Species sensitivity distributions involve analyzing the sensitivity of different species to the compounds to determine safe levels and potential impacts on sensitive species. Ecosystem-based Assessment take a broader perspective, considering the potential effects on entire ecosystems and their functioning, including ecological interactions and community dynamics. These approaches allow for a comprehensive understanding of the ecological risks posed by Pharmaceutical compounds and aid in decision-making and risk management strategies (Katsanevakis et al., 2011).

The selection of appropriate assessment endpoints and the consideration of species sensitivity are vital in ecological risk Assessment of Pharmaceutical compounds. Assessment endpoints are specific measurable variables or attributes used to assess the

effects of these compounds on ecological receptors. They can include population parameters, community metrics, or ecosystem functions. The selection of appropriate endpoints depends on the specific goals of the assessment and the ecological relevance of the endpoints to the ecosystem under study. Furthermore, considering species sensitivity is crucial in understanding the potential impacts of pharmaceutical compounds on different organisms within the ecosystem. Species may vary in their sensitivity to specific compounds, and accounting for these differences allows for a more accurate assessment of ecological risks. It enables the identification of vulnerable species and the development of targeted management strategies to protect biodiversity and ecosystem health (East et al., 2021).

The selection of assessment endpoints plays a crucial role in ecological risk Assessment of Pharmaceutical compounds. Assessment endpoints represent specific ecological or biological responses that are measured to evaluate the effects of these compounds on the environment. These endpoints can include individual-level responses (e.g., mortality, growth inhibition) as well as higher-level ecological parameters (e.g., population dynamics, community structure). The choice of assessment endpoints should consider the ecological relevance, sensitivity, and feasibility of measurement. It is important to select endpoints that capture the potential adverse effects of Pharmaceutical compounds on the target ecosystem and provide meaningful insights into the overall ecological risk (Walker et al., 2013).

Species sensitivity is a key consideration in ecological risk Assessment of Pharmaceutical compounds. Different species may exhibit varying sensitivities to these compounds due to differences in physiology, life history traits, and ecological interactions. Some species may

be more susceptible to the toxic effects of pharmaceutical compounds, while others may be more resilient or have specific adaptations that provide them with a higher tolerance. Understanding species sensitivity helps in identifying vulnerable species that may be at greater risk and require specific protection measures. It also aids in determining safe levels of exposure and establishing appropriate assessment criteria for ecological risk Assessment (Kroeker et al., 2013).

Exposure assessment is a crucial step in ecological risk Assessment of Pharmaceutical compounds in aquatic systems. It involves quantifying the concentrations and duration of exposure to these compounds in the environment, considering their sources, pathways, and fate. Various methods and techniques are employed to assess exposure to pharmaceutical compounds, including water sampling and analysis, sediment sampling and analysis, and bioaccumulation studies. Water sampling allows for the measurement of pharmaceutical compound concentrations in water bodies, while sediment sampling helps evaluate the accumulation and persistence of these compounds in sediments. Bioaccumulation studies involve measuring the concentrations of pharmaceutical compounds in aquatic organisms to assess their potential uptake and accumulation through the food chain. These exposure assessment methods provide valuable data for understanding the levels and patterns of exposure to Pharmaceutical compounds in aquatic systems (Previšić et al., 2021).

In addition to direct exposure assessment, modeling approaches are often employed to estimate exposure to pharmaceutical compounds in aquatic systems. These models integrate data on emission sources, environmental fate processes (such as degradation and transformation), and transport mechanisms to simulate the concentrations of pharmaceutical compounds in different compartments of the aquatic ecosystem. Modeling

allows for the assessment of exposure under different scenarios and provides a more comprehensive understanding of the spatial and temporal variations in exposure. It also helps identify hotspots of exposure and assists in prioritizing areas for further investigation or management actions (Boxall et al., 2014).

Risk characterization is a critical component of ecological risk Assessment of Pharmaceutical compounds. It involves integrating the exposure and effects data to estimate the likelihood and magnitude of adverse effects on the target ecosystem. Risk characterization combines quantitative and qualitative information to assess the potential risks associated with pharmaceutical compounds. This includes considering the uncertainties and variability in exposure and effects data, as well as the specific context and conditions of the assessment. The results of risk characterization provide a comprehensive assessment of the ecological risks posed by Pharmaceutical compounds, allowing for informed decision-making and risk management strategies (Turner et al., 2014).

The interpretation of results in ecological risk Assessment involves evaluating the significance of the findings and their implications for environmental management. It involves considering the magnitude and likelihood of adverse effects, as well as the potential ecological consequences. The interpretation of results helps in understanding the overall ecological risk posed by pharmaceutical compounds, identifying key areas of concern, and prioritizing management actions. Additionally, the interpretation of results contributes to the ongoing refinement and improvement of risk assessment methodologies, allowing for better-informed Assessment in the future (Boxall et al., 2012).

Several studies have been conducted to assess the ecological risks associated with pharmaceutical compounds in wastewater. These studies have focused on investigating the presence, fate, and effects of pharmaceutical compounds in different aquatic environments, such as rivers, lakes, and coastal areas, that receive wastewater discharges. They have employed various approaches, including laboratory toxicity tests, field surveys, and modeling, to assess the potential ecological risks. These studies have provided valuable insights into the concentrations of Pharmaceutical compounds, their effects on aquatic organisms, and the potential for ecological impacts in receiving water bodies (Rosi-Marshall et al., 2012).

Findings from previous risk Assessment have highlighted the need for improved wastewater treatment technologies, monitoring programs, and regulatory measures to mitigate the ecological risks associated with pharmaceutical compounds. These Assessment have identified specific compounds of concern, such as antibiotics, hormones, and painkillers, and their potential impacts on aquatic ecosystems. They have also emphasized the importance of considering mixture effects and long-term exposure scenarios in risk Assessment. The recommendations from previous risk Assessment include implementing advanced wastewater treatment processes, promoting source control measures, and fostering collaboration between stakeholders to address the challenges posed by Pharmaceutical compounds in aquatic environments (Drechsel et al., 2022).

1.2.4 Significance of the literature review for the current research

The literature review conducted for this research on the detection and ecological risk Assessment of Pharmaceutical compounds in industrial effluent is significant for several reasons. Firstly, it provides a comprehensive understanding of the current state of

knowledge in the field, encompassing the sources, pathways, and fate of pharmaceutical compounds, as well as their ecological impacts. The review also highlights the gaps and limitations in existing research, which can guide the current study towards addressing these gaps and contributing to the field.

The literature review serves as a foundation for the current research by providing a theoretical framework and conceptual understanding of the topic. It informs the research methodology, including the selection of appropriate detection techniques, assessment endpoints, and exposure assessment methods. The review also helps in identifying potential areas for improvement and future research directions, contributing to the advancement of knowledge in the field of pharmaceutical compound detection and ecological risk Assessment.

In conclusion, the literature review on the detection and ecological risk Assessment of Pharmaceutical compounds in industrial effluent provides a comprehensive overview of the current understanding, challenges, and research gaps in the field. It serves as a valuable resource for the current research, enabling a focused and informed investigation into the detection and ecological risks associated with pharmaceutical compounds in the National Industrial Zone, RCCI Rawat, Pakistan. The findings from the literature review inform the research methodology and contribute to the overall significance and relevance of the current study.

1.3 Problem statement

The Pharmaceutical manufacturing facilities situated in Pakistan have been identified as sources of concern due to their release of substantial quantities of pharmaceutical compounds into the environment through their wastewater effluent. This issue has garnered significant attention from environmental experts, regulatory authorities, and the general populace, owing to the potential ramifications for aquatic ecosystems, human well-being, and overall ecological equilibrium. The effluent discharged by these facilities encompass a diverse array of active pharmaceutical ingredients, solvents, excipients, and chemical byproducts integral to medication production. This complex composition allows these compounds to infiltrate the environment, potentially leading to contamination of water bodies, including rivers, lakes, and groundwater reservoirs. This multifaceted challenge arises from factors such as inadequate treatment systems, lax regulatory frameworks, the intricate chemical nature of pharmaceutical, and insufficient monitoring mechanisms. This issue's consequences encompass ecological disruption, possible human health implications, the acceleration of antibiotic resistance, and the prolonged persistence of these compounds in the environment. Effectively addressing this issue necessitates collaborative efforts involving pharmaceutical manufacturers, regulatory bodies, environmental agencies, and public awareness initiatives, encompassing enhanced wastewater treatment, regulatory reforms, rigorous monitoring practices, and proactive education to foster responsible practices within both the pharmaceutical industry and the wider community.

1.4 Aim and objectives

I. Detection and quantification of selected pharmaceutical compounds in industrial effluent and surface water of ling stream.

II. Ecological risk assessment to assess the impact of pharmaceutical compounds on the aquatic environment.

1.5 Significance of the study

The present study holds considerable importance for various reasons. The study adds to the existing knowledge on the detection and ecological risk Assessment of Pharmaceutical compounds in industrial effluent, with a specific focus on the National Industrial Zone in RCCI Rawat, Pakistan. The results of the study will offer significant knowledge regarding the existence, levels, and probable ecological consequences of pharmaceutical substances. This will aid in making informed decisions and implementing environmental management strategies based on empirical evidence.

The present study aims to fill the gaps and overcome the challenges identified in the existing literature, including but not limited to the inadequacy of detection techniques, assessment endpoints, and exposure assessment methods. The research endeavors to enhance the comprehension and techniques pertaining to the detection of pharmaceutical compounds and ecological risk Assessment in industrial effluent by directing attention towards these gaps.

Finally, the results of this study will hold pragmatic ramifications for environmental regulators, wastewater treatment plants, and businesses functioning within the National Industrial Zone. The results of this study have the potential to provide valuable insights for the creation of specific mitigation approaches, enhanced wastewater treatment

methodologies, and regulatory frameworks aimed at reducing the ecological hazards linked to pharmaceutical substances present in industrial effluent.

CHAPTER 2

RESEARCH METHODOLOGY

2.1 Study Area

The research area was used of Pakistan's National Industrial Zone, RCCI Rawat (Latitude: 33.51819° N: Longitude: 73.24468° E) and the sampling area (Fig.2.1). People who rely on agriculture, cattle, aquaculture, and poultry as a source of income use treated or raw wastewater, specifically, to irrigate agricultural land. The research location is in an ecologically dry area and is covered in prickly vegetation. Medium rainfall, high summer, and low winter temperatures, and a low to medium diversity of plant species are all characteristics of the region (Zafar et al., 2021).

2.2 Pharmaceutical compounds of interest

The five ciprofloxacin, levofloxacin, cefixime, Cefoperazone sodium, and ceftriaxone sodium are the primary pharmaceutical chemicals that make up the designated classes of antibiotics. The main criteria used to choose antibiotics of interest were their persistence in surface water, frequency of manufacture in the area, availability of analytical tools in the lab, and frequency of use.

2.3 Samples collection and handling

Effluent samples were collected from five-point source pharmaceutical manufacturing units (A, B, C, D, and E) in National Industrial Zone, RCCI Rawat, Pakistan (Table 2.1) and Fig. 2.1).

Five sampling points of surface water Ling stream (K, L, M, N, and O) approximately a part 300-meter distance near National Industrial Zone (Table 2.2 and Fig. 2.1).

These facilities produce a wide range of pharmaceutical compounds in large volumes. For the collection of effluent, five pharmaceutical manufacturing units and Surface water

sampling points were chosen. The sampling campaign took place at times (morning and afternoon) of the day. After that, two samples from each sampling point (morning and afternoon) properly blended to create a composite sample for each site. To confirm the accuracy of the studies and account for any sampling uncertainty, these composite samples were evaluated independently. In amber glass vials, one liter of effluent and surface water samples were collected. Each collected wastewater and surface water sample treated with around 250 mg of disodium EDTA immediately after collection to complex the metal ions and then stored in the refrigerator at 4 °C to limit bacterial growth (Ashfaq et al., 2017). Within 48 hours of receiving the samples, they were extracted.

Table 2.1: Effluent Samples from different Point Source of Pharmaceutical Units

Sr. No	Sampling Points	Sample ID	Approx. Location
1	Sampling Point A	Sample A	33.516°N: 73.250°E
2	Sampling Point B	Sample B	33.519°N: 73.253°E
3	Sampling Point C	Sample C	33.516°N: 73.238°E
4	Sampling Point D	Sample D	33.521°N: 73.245°E
5	Sampling Point E	Sample E	33.515°N: 73.231°E

Table 2.2: Samples from Surface water of Ling Stream

Sr. No	Sampling Points	Sample ID	Approx. Location
1	Sampling Point K	Sample K	33.524°N: 73.244°E
2	Sampling Point L	Sample L	33.526°N: 73.240°E
3	Sampling Point M	Sample M	33.527°N: 73.237°E
4	Sampling Point N	Sample N	33.527°N: 73.235°E
5	Sampling Point O	Sample O	33.529°N: 73.235°E

Figure 2.1: Study Area and Sampling points.

2.4 Extraction and Chromatographic Conditions

Liquid-liquid extractions were used to extract the desired medicinal ingredient from wastewater and surface water samples. Among the initially chosen di-ethyl ether, n-hexane, and chloroform, chloroform was chosen as the final extractant. Filtration of liquid samples begins with 0.45 µm membrane filters. Each filtered sample was extracted three times, each time with 50 mL chloroform. The extracts were mixed and then evaporated. After dissolving the residue in 2 mL of methanol, it will be refrigerated for analysis. HPLC LC-20A system (Shimadzu, Japan) with UV detector is utilized to analyze the targeted substances with 20µL loop is used to inject the samples. All the targeted pharmaceutical compounds (Ceftriaxone, Ciprofloxacin, Levofloxacin, Cefoperazone and Cefixime) were detected using independents confirmed HPLC techniques (Ashfaq et al., 2017)

2.4.1 Reference Standards, Chemicals and Reagents used for analysis

Reference standards of all the selected pharmaceutical compounds (Ceftriaxone Sodium USP Reference standard lot. No. R16150, Levofloxacin Manufactured by Saakh Pharma (Pvt) ltd Karachi Pakistan, Ciprofloxacin HCl Manufactured by Citi Pharma ltd Kasur Pakistan, Cefoperazone Sodium manufactured by Shandong Luoxin Pharmaceutical group Hengxin Pharmaceutical Co., Ltd. And Cefixime USP Reference Standard lot. No. R095X0).Other chemicals(Chloroform Duksan pure chemicals Korea, Acetonitrile Duksan pure chemicals Korea, Monobasic potassium phosphate Honey well Germany , dibasic sodium phosphate dodecahydrate Duksan pure chemicals Korea, citric acid Chem-lab Belgium, sodium hydroxide Chem-lab Belgium, phosphoric acid Duksan pure chemicals Korea, disodium EDTA Duksan pure chemicals Korea, tetradecyl ammonium bromide Sigma-Aldrich Switzerland, tetraethylammonium bromide Sigma-Aldrich Switzerland, 0.4 N tetra butyl ammonium hydroxide Chem-lab Belgium, triethylamine Duksan pure chemicals Korea and methanol Chem-lab Belgium) used are either HPLC grade or Analytical grade without any further purification.

2.4.2 Analytical method for Ceftriaxone Sodium

Solution A- 9 g/L of Monobasic Potassium Phosphate was dissolved in distilled water. Solution B- 24 g/L of Dibasic Sodium Phosphate Dodecahydrate was dissolved in distilled water. Solution C- 20 g/L of citric acid was dissolved in water and adjusted with 10 Normal Sodium hydroxide to a pH of 5.0 prior to dilution. Buffer- Combined 389 mL of Solution A and 611 mL of Solution B. Adjusted with 10 Normal sodium hydroxide or phosphoric acid to a pH of 7.0.

Mobile phase- 2.0 g each of tetradecylammonium bromide and tetraethylammoniums bromide was dissolved in a mixture of 440 mL of water, 55 mL of Buffer, 5.0 mL of Solution C, and 500 mL of Acetonitrile.

Standard solution- 20,000 µg/L standard in Mobile phase

Sample solution in Mobile phase

Chromatographic Conditions:

Mode: LC

Detector: 254nm

Column: 4.6-mm x 250-mm; 5-µm packing L1 (C18)

Flow Rate: 1.5ml/min

Injection volume: 20 µL

Separately inject 20µl standard and sample solution.

 Results

$C_u = (r_u/r_s) \times (C_s)$

ru = peak response from the Sample solution

rs =peak response the Standard solution

Cs = concentration in the Standard solution

Cu = concentration in the Sample solution (Akl et al., 2011)

2.4.3 Analytical method for Cefixime and Cefoperazone

Solution A: Tetra butyl ammonium hydroxide solution A. Diluted 25 ml of 0.4 N tetra butyl ammonium hydroxide solutions with water to obtain 1000 ml of solution and adjusted with 1.5 molar phosphoric acid to a pH of 6.5.

Mobile Phase: Mobile phase preparation:

Prepare a filtered and degassed mixture of Tetra butyl ammonium hydroxide solutions pH 6.5 and Acetonitrile (3:1). Filter it through 0.45μm filter.

Solution C*:* Phosphate Buffer pH 7.0 Solution C

Dissolved 7.1g anhydrous dibasic sodium phosphate in water to make 500 ml of solution, adjust volume of this solution with sufficient volume of monobasic potassium phosphate solution to a pH of 7.0.

Monobasic potassium Phosphate solution B

Dissolved 6.8 g of monobasic potassium phosphate in water to make 500 ml of solution.

Standard Preparation:
20,000 μg/L of Cefixime and Cefoperazone each standard in Solution C.

Sample Preparation*:* Dilute samples with solution C

Chromatographic Parameters
Detector: UV

Wavelength: 254 nm

Column: Packing L1 (C18), 4μm (120.5 mm x 4.6 mm)

Flow Rate: 2 ml/min,

Injection Volume: 10 μL.

Column temperature maintained at: 40ºC

Procedure
Separately inject 20μl standard and sample solution.

Results

$$C_u = (r_u/r_s) \times (C_s)$$

ru = peak response from the Sample solution

rs = peak response from the Standard solution

Cs = concentration of the Standard solution

Cu = concentration of the Sample solution (Tripathi et al., 2015)

2.4.4 Analytical method for Levofloxacin and Ciprofloxacin

Solution A: 0.025 M phosphoric acid. Adjusted with triethylamine to a pH of 2.0 ± 0.1.

Solution B: Acetonitrile and Solution A (13:87)

3.3.4.3 Solution C: 0.025 M phosphoric acid. Adjusted with triethylamine to a pH of 3.0 ± 0.1.

Mobile phase: Acetonitrile and Solution C (13:87)

Standard solution: 30,000 µg/L of Ciprofloxacin Hydrochloride and Levofloxacin each standard in Solution B

Sample solution: Diluted the sample with Solution B.

Chromatographic system

Mode: LC

Detector: UV 278 nm.

Column: 4.6-mm x 25-cm; 5-micron m packing L1 (C18)

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Analysis

Samples: Standard solution and Sample solution

Results

$$C_u = (r_u/r_s) \times (C_s)$$

r_u = peak response from the Sample solution

r_s = peak response from the Standard solution

Cs = concentration of in the Standard solution

Cu = concentration of sample solution (Kassab et al., 2005)

2.5 Ecological Risk Assessment

Pharmaceutical compounds can directly affect aquatic organisms, ranging from acute to chronic impacts. Many pharmaceuticals are designed to target specific receptors or biological pathways in humans or animals, but similar targets may exist in aquatic organisms. Exposure to Pharmaceutical compounds can disrupt normal physiological processes in aquatic organisms, affecting their behavior, growth, reproduction, and survival. For example, certain pharmaceutical, such as antibiotics or antiparasitic drugs, may target bacterial or fungal populations in the aquatic environment, leading to alterations in microbial community structures. Other Pharmaceutical compounds, such as endocrine disruptors, can interfere with the hormonal systems of aquatic organisms, leading to reproductive abnormalities or developmental disorders. The direct effects of Pharmaceutical compounds can occur at various levels of biological organization, from individual organisms to entire populations (Frye et al., 2012).

Pharmaceutical compounds can also indirectly affect aquatic ecosystems by altering the ecological interactions and dynamics within these systems. For instance, the presence of pharmaceutical in water bodies can affect the feeding behaviors of aquatic organisms, leading to changes in predation patterns and trophic cascades. Additionally, Pharmaceutical compounds can influence the composition and structure of microbial communities, disrupting critical ecological processes such as nutrient cycling or decomposition. The indirect effects of Pharmaceutical compounds can propagate through the food web, impacting higher trophic levels and potentially altering aquatic ecosystems' overall functioning and stability (Mor et al., 2022).

Risk assessment measurements are used to assess the impact of pharmaceutical pollution on the aquatic environment. The European Agency for the Evaluation of Medicinal Products' criteria will be utilized to determine the ecological risk assessment. This risk

evaluation estimates the PC's hazardous dose to a specific species in the receiving aquatic habitat.

The (RQ) or (HQ) is the ratio of the PCs' predicted environmental concentration (PEC) or measured environmental concentration (MEC) to their predicted no-effect concentration (PNEC).

$$RQ = \frac{\text{MEC}}{\text{PNEC}}$$

MEC is the maximum observed ambient concentration in µg/L, and PNEC is the Predicated no effect concentration (µg/L), where RQ stands for risk quotient or hazard quotient.

RQ greater than one implies a high risk to the aquatic community, while RQ less than one indicates a medium or no risk. This study will be used to calculate MEC values, while PNEC values will be taken from the literature (Ashfaq et al., 2017)

CHAPTER 3

RESULTS AND DISCUSSION

Pakistan's Pharmaceutical industry is expanding significantly because of increased local population and export potential. The Pharmaceutical sector, however, does not adhere to any national or international environmental norms. Without any treatment, the effluent from the pharmaceutical industry is released into the domestic wastewater system. We have concentrated on evaluating the presence of five frequently manufactured medications in the effluent from the various pharmaceutical formulation factories. (Table 3.1) Most of the targeted PCs could easily be found. The concentration of certain PCs did, however, varied depending on the sampling site (Fig. 3.1).

Table 3.1: Mean concentration of PCs in Effluent from different Point Source of Pharmaceutical Units

Mean concentration of PCs in µg/L					
Pharmaceutical Compounds	**Sampling Point A**	**Sampling Point B**	**Sampling Point C**	**Sampling Point D**	**Sampling Point E**
Ciprofloxacin	212.55	90.68	116.49	137.98	67.72
Levofloxacin	66.40	49.84	80.45	63.34	47.71
Cefixime	380.90	173.53	289.54	200.98	191.92
Cefoperazone	229.98	170.13	111.06	320.30	211.06
Ceftriaxone	7.10	15.06	8.60	7.79	10.33
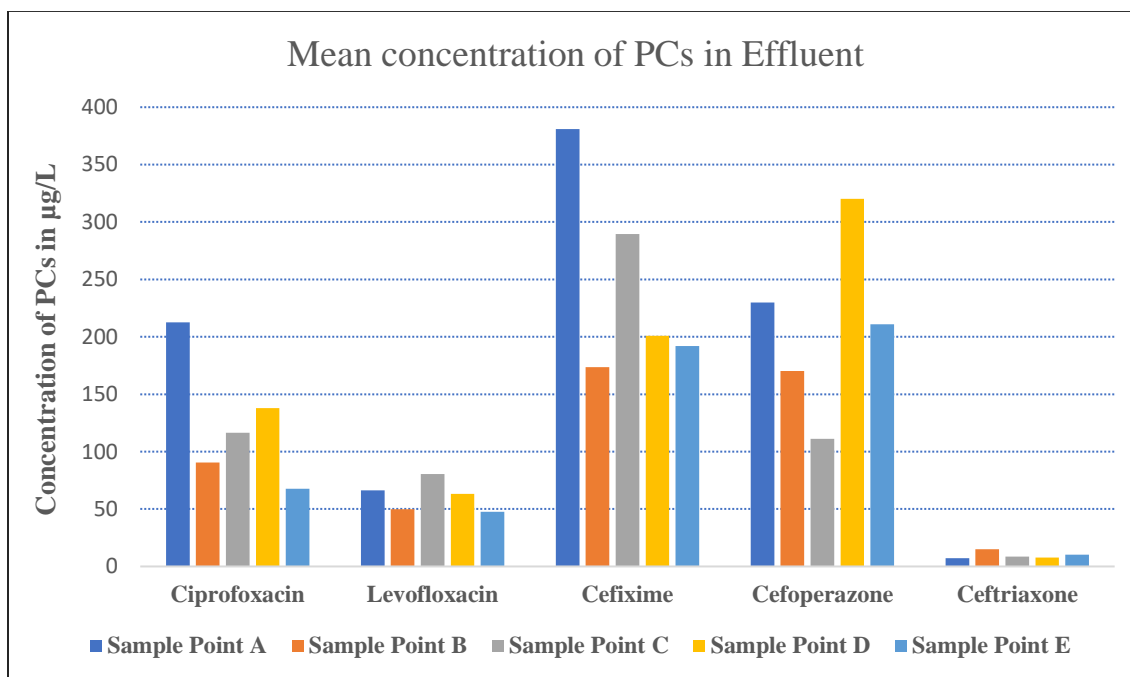
Figure 3.1: Mean concentration of PCs in Effluent from different Point Source of Pharmaceutical Units.

The evaluation of the concentrations of individual pharmaceutical compounds in the effluent offers useful information about the predominant compounds present in the discharge. Cefixime was found at high concentration in effluent (173.53-380.9 µg/L) followed by Cefoperazone (111.06-320.3 µg/L), Ciprofloxacin (67.72-212.55 µg/L), Levofloxacin (47.71-80.45 µg/L) were detected at medium concentration and Ceftriaxone (7.1-15.06 µg/L) at lowest level. Cefixime 380.9 µg/L in sample point 'A' and Cefoperazone 320.3 µg/L in sample point 'D' are found in the highest concentrations among the compounds under examination, according to the data presented, with Ciprofloxacin, Levofloxacin, and Ceftriaxone following closely behind. The increased concentrations of Cefixime and Cefoperazone suggest a higher utilization or greater manufacturing of these substances inside the pharmaceutical facilities being examined. Persistent Pharmaceutical pollutants (PPPs) have been recognized as potential endocrine disruptors, imitating growth hormones when present at concentrations ranging from nanograms per liter to micrograms per liter. Their presence in drinking water poses a significant risk to both human health and

aquatic species (Massima Mouele et al., 2021). This discovery holds significance for future research endeavors focused on comprehending the origins and routes of these medicinal chemicals inside the environment. The study's findings verify the detection of 156 pharmaceuticals in environmental media, including surface water, groundwater, and drinking water, within Germany (Loos et al., 2013). Of specific interest is the potential exposure of non-target organisms to pharmaceutical, given their inherent biological activity, even at low levels of exposure, which can lead to physiological alterations. Studies conducted over the past two decades have shed light on the impact of Pharmaceutical exposure on organisms, with ethinyloestradiol being identified as a contributor to the feminization of male fish in rivers dominated by effluent discharges (Loos et al., 2013).

Concentration of five selected pharmaceutical compounds in surface water (Ling stream) sampling points (K, L, M, N, and O) near National Industrial Zone (RCCI), Rawat. Each Composite sample of sampling point K, L, M, N and O is analyzed for pharmaceutical compounds in surface water through High performance liquid chromatography with UV-Visible detector. (Table 3.2 and Fig. 3.2) showed the detected pharmaceutical compounds in surface water of ling stream.

Table 3.2: Mean concentration of PCs in surface water of Ling stream

Mean concentration of PCs in µg/L					
Pharmaceutical Compounds	Sampling Point K	Sampling Point L	Sampling Point M	Sampling Point N	Sampling Point O
Ciprofloxacin	5.37	3.52	2.47	7.22	4.72
Levofloxacin	2.27	2.36	1.92	3.73	2.93
Cefixime	6.95	0.00	9.94	0.00	6.26
Cefoperazone	0.00	0.00	0.00	0.00	0.00
Ceftriaxone	7.68	3.71	9.00	5.98	2.80
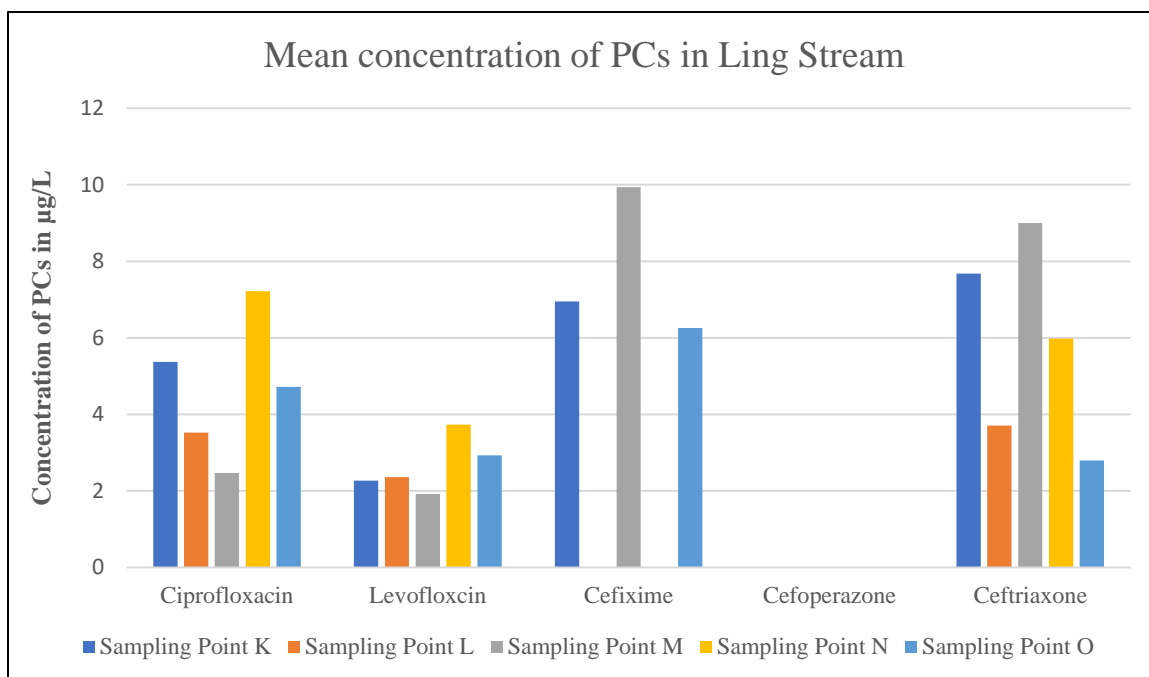
Figure 3.2: Mean concentration of PCs in surface water of Ling stream

The concentration of PCs (Ciprofloxacin, Levofloxacin, cefixime and ceftriaxone) in Surface water (Ling stream) were (2.47 – 7.22 µg/L), (1.92 – 3.73 µg/L), (0.00 – 9.94 µg/L) and (2.80 – 9.00 µg/L) respectively. The Ling stream exhibits spatial heterogeneity in the concentrations of medicinal compounds across different sampling stations. The large fluctuation seen for Cefixime is of particular concern, as certain data points indicate the absence of compound detection. The observed spatial variance can be ascribed to a confluence of elements, encompassing diverse point source discharges along the stream, the dilution effects stemming from tributaries, and the influence of environmental conditions on degradation processes. The findings derived from the examination of quantities of medicinal compounds in the Ling stream reveal notable regional variability across various sampling points. A matter of notable concern pertains to the significant variability seen in the case of Cefixime, wherein certain data points exhibit an absence of detection for the aforementioned molecule (Mirzaei et al., 2019). The observed geographic variation gives rise to significant inquiries regarding the elements that exert influence on the distribution and ultimate destiny of medicinal compounds within the stream (Omuferen et al., 2022).

The investigation reveals a notable difference in the levels of pharmaceutical compounds present in the effluent discharged from point source pharmaceutical units and the Ling stream in the vicinity of RCCI. The levels of pharmaceutical chemicals in the effluent, namely at sample points A, B, C, D, and E, exhibit significantly greater amounts compared to those found in the Ling stream, specifically at sampling points K, L, M, N, and O. The observed difference indicates that the pharmaceutical facilities are the predominant sources of pollution in the Ling stream. The findings were consistent that numerous pollutants, including pesticides, heavy metals, polycyclic aromatic hydrocarbons, microplastic particles, and pharmaceutical, are introduced into water bodies because of human activities. These pollutants pose significant threats to the well-being of plants, animals, and humans due to their acute toxicity and the potential risk of accumulating in the environment, leading to chronic effects (Parolini, 2020). To obtain a thorough comprehension of the geographical variability of medicinal compounds in the Ling stream, it is imperative to consider the combined effects of several components, such as point source discharges, tributary influences, and ambient circumstances. Moreover, further investigation is necessary to ascertain the precise origins of Pharmaceutical contamination and to clarify the underlying factors contributing to the observed variations (Huang et al., 2020).

3.1 The Ecological Risk Assessment of Pharmaceutical compounds in Ling Stream

The discharge from pharmaceutical facilities consists of a diverse combination of pharmaceutical chemicals that are introduced into the surrounding ecosystem because of their production procedures and due to inadequate elimination during the treatment of wastewater. Upon entering the Ling stream, these substances can potentially have significant impacts on aquatic life and the surrounding environment. Freshwater ecosystems, including aquatic environments, are subjected to greater contamination compared to other habitats. This heightened exposure results from the extensive use of water in various industrial processes, as well as the discharge of effluent originating from industrial activities and urban developments (Demirak et al., 2006).

To estimate the ecological risk connected to PCs in the various environmental matrices, ecological risk assessment has become essential. In this risk evaluation, the potentially detrimental dose of a PC to a certain specie existing in the receiving aquatic habitat is approximately calculated (Ashfaq et al., 2017). Ecological Risk Assessment of pharmaceutical compounds in Ling stream (Table 3.3).

Table 3.3: The Ecological Risk Assessment of Pharmaceutical Compounds in Ling
Stream

Pharmaceutical compounds	*PNEC µg/L	Species	*MEC min µg/L	MEC max µg/L	*RQ min	RQ max	References
Ciprofloxacin	7285	Fish	2.47	7.22	0.0003	0.0009	(Iatrou et al., 2014)
	3415	Daphnia	2.47	7.22	0.0007	0.0021	
	0.01	Green algae	2.47	7.22	247	722	
Levofloxacin	20240	Fish	1.92	3.73	0.00009	0.00018	(Iatrou et al., 2014)
	8950	Daphnia	1.92	3.73	0.0002	0.0004	
	0.01	Green algae	1.92	3.73	192	373	
Cefixime	0.04	Bacteria (*P. putida*)	6.26	9.94	156.5	248.5	(Mirzaei et al., 2019)
Ceftriaxone	--	----	2.8	9.0	--	--	----

*MEC is the maximum environmental concentration in µg/L,

*PNEC is the Predicated no effect concentration (µg/L),

*RQ stands for risk quotient or hazard quotient.

Only three pharmaceutical compounds in surface water were calculated for ecological risk
in the Table 3.3. Due to the unavailability of PNEC values, the Ceftriaxone sodium PC was
not evaluated. Cefoperazone was not detected in surface water (ling stream). Using the
measured concentration of certain PCs in the surface water, ecological risk Assessment was
conducted against several freshwater species. Risk quotient in case of Ciprofloxacin (247

– 722) and Levofloxacin (192 – 373) pharmaceutical compounds were highest against Green Algae greater than 1 that is and made high risk against the species. Risk quotient of ciprofloxacin and levofloxacin for Fish and Daphnia were too low and less the 1. The RQ value was determined for Cefixime (156.5 – 248.5) against Bacteria *(P. putida*) pose high level risk against the species.

The growing environmental contamination caused by pharmaceutical can be attributed not only to their rising consumption but also to the limitations of conventional wastewater treatments. These treatments often fail to completely remove many Pharmaceutical compounds, contributing to their presence in the environment (Kolpin et al., 2002). Subsequently, ecotoxicologists primarily directed their attention towards sewage effluent and receiving rivers while investigating pharmaceutical, potentially overlooking other potential sources of Pharmaceutical residues. Additionally, the Pharmaceutical industry argued against significant discharge of active Pharmaceutical ingredients (APIs) from manufacturing, citing economic reasons, as drugs are highly valuable and unlikely to be released on a large scale (Williams, 2005).

The presence of elevated levels of pharmaceutical chemicals in the effluent discharged from pharmaceutical facilities underscores the necessity for the development and implementation of more effective technologies for treating wastewater. The efficacy of conventional treatment techniques in removing complex and diverse substances may be limited, resulting in their subsequent release into the environment.

The incorporation of sophisticated treatment methodologies, such as activated carbon filtration, ozonation, or alternative advanced oxidation procedures, has the potential to yield substantial enhancements in the elimination of pharmaceutical compounds during the wastewater treatment process. Conducting thorough research and implementing efficacious treatment options are crucial in mitigating the adverse environmental consequences associated with Pharmaceutical contamination (Rekhate et al., 2020). This study highlights the significance of consistent monitoring and adherence to rules pertaining to the release of pharmaceutical chemicals from industrial facilities. The implementation of wastewater treatment regulations and the establishment of rigorous thresholds for Pharmaceutical

chemical concentrations are essential measures in mitigating their discharge into the natural environment (Litter et al., 2010).

Furthermore, the implementation of regular monitoring programs can effectively assess the efficacy of treatment procedures and guarantee the adherence of pharmaceutical facilities to environmental requirements. The establishment of collaborative initiatives between regulatory agencies and companies plays a crucial role in ensuring the preservation of public health and the safeguarding of the environment from the adverse effects of Pharmaceutical pollution (Coha et al., 2021).

To formulate complete mitigation methods, it is imperative to do additional study to have a deeper understanding of the fate and transit mechanisms of pharmaceutical chemicals within the Ling stream. The investigation of the persistence and transformation mechanisms of these chemicals under different environmental settings will provide valuable insights into their enduring environmental consequences. It is imperative to do research on the possible bioaccumulation of Pharmaceutical substances in aquatic creatures in order to evaluate their transmission within the food chain and their potential impacts on higher trophic levels (Kahlon et al., 2018). Furthermore, the establishment of monitoring initiatives specifically targeting pharmaceutical chemicals in surface water and sediments can yield continuous data to enhance environmental governance.

In summary, this study provides significant findings regarding the levels of pharmaceutical chemicals found in wastewater discharged from pharmaceutical facilities and the Ling stream in the vicinity of RCCI. The results emphasize the necessity of implementing advanced wastewater treatment technology, implementing rigorous monitoring practices, and enforcing stringent regulatory measures to effectively address the environmental consequences and safeguard public health from the adverse effects of pharmaceutical pollution. Subsequent investigations can expand upon this existing body of knowledge and facilitate the development of efficacious approaches for the sustainable management of water resources in the context of pharmaceutical pollutants.

CONCLUSIONS

1 The study successfully detected and quantified several pharmaceutical compounds (Ciprofloxacin, Levofloxacin, Cefixime, Cefoperazone, and Ceftriaxone) in industrial effluent and surface water (Ling stream) samples from different points.

2 Variations in concentration levels among these compounds reflect differences in manufacturing processes, usage, and treatment practices within the pharmaceutical units.

3 Cefixime was found at high concentration in effluent (173.53-380.9 µg/L) followed by Cefoperazone (111.06-320.3 µg/L), Ciprofloxacin (67.72-212.55 µg/L), Levofloxacin (47.71-80.45 µg/L) were detected at medium concentration and Ceftriaxone (7.1-15.06 µg/L) at lowest level. The concentration of PCs (Ciprofloxacin, Levofloxacin, cefixime and ceftriaxone) in Surface water (Ling stream) were (2.47 – 7.22 µg/L), (1.92 – 3.73 µg/L), (0.00 – 9.94 µg/L) and (2.80 – 9.00 µg/L) respectively.

4 An ecological risk assessment was conducted to evaluate the potential impact of pharmaceutical compounds on the aquatic environment, with a focus on the Ling stream. Risk quotient of Ciprofloxacin (247 – 722) and Levofloxacin (192 – 373) pharmaceutical compounds were highest against Green Algae greater than 1 indicating that it can pose high risk against the species. Risk quotient of ciprofloxacin and levofloxacin for Fish and Daphnia were too low and less the 1. The RQ value was determined for Cefixime (156.5 – 248.5) against Bacteria (*P. putida*) pose high level risk against the species.

5 Understanding the sources and distribution mechanisms of pharmaceutical is crucial for implementing targeted management strategies, involving advanced monitoring techniques and collaborative efforts to mitigate their environmental impact.

RECOMMENDATIONS

- To formulate complete mitigation methods, it is imperative to do additional study to have a deeper understanding of the fate and transit mechanisms of pharmaceutical chemicals within the Ling stream.

- The establishment of monitoring initiatives specifically targeting pharmaceutical chemicals in surface water and sediments can yield continuous data to enhance environmental governance.

- The presence of elevated levels of pharmaceutical chemicals in the effluent discharged from pharmaceutical facilities underscores the necessity for the development and implementation of more effective technologies for treating wastewater.

- Pharmaceutical units should develop more efficient manufacturing procedure and equipment for manufacturing lifesaving drugs for wellbeing.

REFERENCES

Abdudeen, A., Selim, M. Y., Sekar, M., and Elgendi, M. (2023). Jatropha's Rapid Developments and Future Opportunities as a Renewable Source of Biofuel—A Review. *Energies, 16*(2), 828.

Abubakar, A. R., and Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of pharmacy and bioallied sciences, 12*(1), 1.

Ahmad, I., and Taidong, Z. (2020). Special economic zones in Pakistan: Promises and perils. *Pakistan Institute of Development Economics (PIDE), Islamabad*.

Ahrens, L., and Bundschuh, M. (2014). Fate and effects of poly-and perfluoroalkyl substances in the aquatic environment: A review. *Environmental toxicology and chemistry, 33*(9), 1921-1929.

Akl, M. A., Ahmed, M. A., and Ramadan, A. (2011). Validation of an HPLC-UV method for the determination of ceftriaxone sodium residues on stainless steel surface of Pharmaceutical manufacturing equipments. *Journal of Pharmaceutical biomedical analysis, 55*(2), 247-252.

Ankush, Mandal, M. K., Sharma, M., Khushboo, Pandey, S., and Dubey, K. K. (2018). Membrane technologies for the treatment of Pharmaceutical industry wastewater. In *Water and Wastewater Treatment Technologies* (pp. 103-116): Springer.

Ashfaq, M., Li, Y., Rehman, M. S. U., Zubair, M., Mustafa, G., Nazar, M. F., Yu, C.-P., and Sun, Q. (2019). Occurrence, spatial variation and risk assessment of pharmaceutical and personal care products in urban wastewater, canal surface water, and their

sediments: A case study of Lahore, Pakistan. *Science of the Total Environment, 688*, 653-663.

Ashfaq, M., Nawaz Khan, K., Saif Ur Rehman, M., Mustafa, G., Faizan Nazar, M., Sun, Q., Iqbal, J., Mulla, S. I., and Yu, C. P. (2017). Ecological risk assessment of in the receiving environment of Pharmaceutical wastewater in Pakistan. *Ecotoxicology and environmental safety, 136*, 31-39. doi:10.1016/j.ecoenv.2016.10.029

Barabad, M. L. M., Jung, W., Versoza, M. E., Lee, Y.-i., Choi, K., and Park, D. (2018). Characteristics of particulate matter and volatile organic compound emissions from the combustion of waste vinyl. *International journal of environmental research and public health, 15*(7), 1390.

Boxall, A. B., Rudd, M. A., Brooks, B. W., Caldwell, D. J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J. P., and Verslycke, T. (2012). Pharmaceutical and personal care products in the environment: what are the big questions? *Environmental health perspectives, 120*(9), 1221-1229.

Boxall, A. B. A., Keller, V., Straub, J., Monteiro, S., Fussell, R., and Williams, R. (2014). Exploiting monitoring data in environmental exposure modelling and risk assessment of pharmaceutical. *Environment international, 73*, 176-185.

Caldwell, D. J., Mastrocco, F., Margiotta-Casaluci, L., and Brooks, B. W. (2014). An integrated approach for prioritizing pharmaceutical found in the environment for risk assessment, monitoring and advanced research. *Chemosphere, 115*, 4-12.

Chapman, P. M., Fairbrother, A., and Brown, D. (1998). A critical evaluation of safety (uncertainty) factors for ecological risk assessment. *Environmental Toxicology and Chemistry: An International Journal, 17*(1), 99-108.

Chaturvedi, P., Shukla, P., Giri, B. S., Chowdhary, P., Chandra, R., Gupta, P., and Pandey, A. (2021). Prevalence and hazardous impact of Pharmaceutical and personal care products and antibiotics in environment: A review on emerging contaminants. *Environmental Research, 194*, 110664.

Coha, M., Farinelli, G., Tiraferri, A., Minella, M., and Vione, D. (2021). Advanced oxidation processes in the removal of organic substances from produced water: Potential, configurations, and research needs. *Chemical Engineering Journal, 414*, 128668.

Demirak, A., Yilmaz, F., Tuna, A. L., and Ozdemir, N. (2006). Heavy metals in water, sediment and tissues of Leuciscus cephalus from a stream in southwestern Turkey. *Chemosphere, 63*(9), 1451-1458.

Drechsel, P., Qadir, M., and Galibourg, D. (2022). The WHO guidelines for safe wastewater use in agriculture: a review of implementation challenges and possible solutions in the global south. *Water, 14*(6), 864.

East, A., Anderson, R. H., and Salice, C. J. (2021). Per-and polyfluoroalkyl substances (PFAS) in surface water near US Air Force bases: Prioritizing individual chemicals and mixtures for toxicity testing and risk assessment. *Environmental toxicology and chemistry, 40*(3), 859-870.

Ebele, A. J., Abdallah, M. A.-E., and Harrad, S. (2017). pharmaceutical and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging contaminants, 3*(1), 1-16.

Edokpayi, J. N., Odiyo, J. O., and Durowoju, O. S. (2017). Impact of wastewater on surface water quality in developing countries: a case study of South Africa. *Water quality, 10*, 66561.

Ferrari, B. t., Paxéus, N., Giudice, R. L., Pollio, A., and Garric, J. (2003). Ecotoxicological impact of pharmaceutical found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. *Ecotoxicology and environmental safety, 55*(3), 359-370.

Fida, M., Li, P., Wang, Y., Alam, S. K., and Nsabimana, A. (2022). Water contamination and human health risks in Pakistan: a review. *Exposure and Health*, 1-21.

Frye, C., Bo, E., Calamandrei, G., Calza, L., Dessì-Fulgheri, F., Fernández, M., Fusani, L., Kah, O., Kajta, M., and Le Page, Y. (2012). Endocrine disrupters: a review of some sources, effects, and mechanisms of actions on behaviour and neuroendocrine systems. *Journal of neuroendocrinology, 24*(1), 144-159.

Gadipelly, C., Pérez-González, A., Yadav, G. D., Ortiz, I., Ibáñez, R., Rathod, V. K., and Marathe, K. V. (2014). Pharmaceutical industry wastewater: review of the technologies for water treatment and reuse. *Industrial and Engineering Chemistry Research, 53*(29), 11571-11592.

Gavrilescu, M., Demnerová, K., Aamand, J., Agathos, S., and Fava, F. (2015). Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *New biotechnology, 32*(1), 147-156.

Grabicová, K., Grabic, R., Fedorova, G., Kolářová, J., Turek, J., Brooks, B. W., and Randák, T. (2020). Psychoactive Pharmaceutical in aquatic systems: A comparative

assessment of environmental monitoring approaches for water and fish. *Environmental pollution, 261*, 114150.

Grenni, P., Ancona, V., and Caracciolo, A. B. (2018). Ecological effects of antibiotics on natural ecosystems: A review. *Microchemical Journal, 136*, 25-39.

Huang, B., Yuan, Z., Li, D., Zheng, M., Nie, X., and Liao, Y. (2020). Effects of soil particle size on the adsorption, distribution, and migration behaviors of heavy metal (loid) s in soil: A review. *Environmental Science: Processes and Impacts, 22*(8), 1596-1615.

Hussein, M. A., Obuid-Allah, A. H., Mahmoud, A. A., and Fangary, H. M. (2011). Population dynamics of freshwater snails (Mollusca: Gastropoda) at Qena Governorate, upper Egypt. *Egyptian Academic Journal of Biological Sciences, B. Zoology, 3*(1), 11-22.

I Litter, M., and Quici, N. (2010). Photochemical advanced oxidation processes for water and wastewater treatment. *Recent Patents on Engineering, 4*(3), 217-241.

Iatrou, E. I., Stasinakis, A. S., and Thomaidis, N. S. (2014). Consumption-based approach for predicting environmental risk in Greece due to the presence of antimicrobials in domestic wastewater. *Environ Sci Pollut Res Int, 21*(22), 12941-12950. doi:10.1007/s11356-014-3243-7

Idrees, N., Tabassum, B., Abd_Allah, E. F., Hashem, A., Sarah, R., and Hashim, M. (2018). Groundwater contamination with cadmium concentrations in some West UP Regions, India. *Saudi journal of biological sciences, 25*(7), 1365-1368.

Javed, M., Qamar, M. A., Shahid, S., Alsaab, H. O., and Asif, S. (2021). Highly efficient visible light active Cu–ZnO/SgC 3 N 4 nanocomposites for efficient photocatalytic degradation of organic pollutants. *RSC advances, 11*(59), 37254-37267.

Kahlon, S. K., Sharma, G., Julka, J., Kumar, A., Sharma, S., and Stadler, F. J. (2018). Impact of heavy metals and nanoparticles on aquatic biota. *Environmental chemistry letters, 16*, 919-946.

Kampa, M., and Castanas, E. (2008). Human health effects of air pollution. *Environmental pollution, 151*(2), 362-367.

Kassab, N. M., Singh, A. K., Kedor-Hackmam, E. R. M., and Santoro, M. I. R. M. J. R. B. d. C. F. (2005). Quantitative determination of ciprofloxacin and norfloxacin in Pharmaceutical preparations by high performance liquid chromatography. *Revista Brasileira de Ciências Farmacêuticas, 41*, 507-513.

Katsanevakis, S., Stelzenmüller, V., South, A., Sørensen, T. K., Jones, P. J., Kerr, S., Badalamenti, F., Anagnostou, C., Breen, P., and Chust, G. (2011). Ecosystem-based marine spatial management: review of concepts, policies, tools, and critical issues. *Ocean and coastal management, 54*(11), 807-820.

Khan, H. K., Rehman, M. Y. A., and Malik, R. N. (2020). Fate and toxicity of Pharmaceutical in water environment: An insight on their occurrence in South Asia. *Journal of environmental management, 271*, 111030.

Khan, S. A., Abeer, N., Hussain, S. Z., Muhammad, S., Jawad, F., and Khan, T. (2019). Potentially toxic elements contamination in water and evaluation for risk assessment in the Rawalpindi, Pakistan. *Desalin Water Treat, 159*, 327-337.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B., and Buxton, H. T. (2002). pharmaceutical, hormones, and other organic wastewater contaminants in US streams, 1999− 2000: A national reconnaissance. *Environmental Science and Technology, 36*(6), 1202-1211.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., and Gattuso, J. P. (2013). Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global change biology, 19*(6), 1884-1896.

Landrigan, P. J., Stegeman, J. J., Fleming, L. E., Allemand, D., Anderson, D. M., Backer, L. C., Brucker-Davis, F., Chevalier, N., Corra, L., and Czerucka, D. (2020). Human health and ocean pollution. *Annals of global health, 86*(1).

Laurenson, J. P., Bloom, R. A., Page, S., and Sadrieh, N. (2014). Ethinyl estradiol and other human Pharmaceutical estrogens in the aquatic environment: a review of recent risk assessment data. *The AAPS journal, 16*, 299-310.

Liu, Y., and Jiang, Y. (2021). Urban growth sustainability of Islamabad, Pakistan, over the last 3 decades: a perspective based on object-based backdating change detection. *GeoJournal, 86*, 2035-2055.

Loos, R., Carvalho, R., António, D. C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., and Blaha, L. (2013). EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluent. *Water Research, 47*(17), 6475-6487.

Massima Mouele, E. S., Tijani, J. O., Badmus, K. O., Pereao, O., Babajide, O., Zhang, C., Shao, T., Sosnin, E., Tarasenko, V., and Fatoba, O. O. (2021). Removal of

Pharmaceutical residues from water and wastewater using dielectric barrier discharge methods—A review. *International journal of environmental research and public health, 18*(4), 1683.

Mirzaei, R., Mesdaghinia, A., Hoseini, S. S., and Yunesian, M. (2019). Antibiotics in urban wastewater and rivers of Tehran, Iran: Consumption, mass load, occurrence, and ecological risk. *Chemosphere, 221*, 55-66.

Misra, V., and Pandey, S. (2005). Hazardous waste, impact on health and environment for development of better waste management strategies in future in India. *Environment international, 31*(3), 417-431.

Mor, J. R., Muñoz, I., Sabater, S., Zamora, L., and Ruhi, A. (2022). Energy limitation or sensitive predators? Trophic and non-trophic impacts of wastewater pollution on stream food webs. *Ecology, 103*(2), e03587.

Munter, R. (2003). Industrial wastewater characteristics. *The Baltic University Programme (BUP), Sweden, 185*.

Murtaza, G., Ghafoor, A., Qadir, M., Owens, G., Aziz, M., and Zia, M. (2010). Disposal and use of sewage on agricultural lands in Pakistan: A review. *Pedosphere, 20*(1), 23-34.

Nelson, K. M., Dahlin, J. L., Bisson, J., Graham, J., Pauli, G. F., and Walters, M. A. (2017). The essential medicinal chemistry of curcumin: miniperspective. *Journal of medicinal chemistry, 60*(5), 1620-1637.

Ngwuluka, N. C., Ochekpe, N. A., and Odumosu, P. O. (2011). An assessment of Pharmaceutical waste management in some Nigerian Pharmaceutical industries. *African Journal of Biotechnology, 10*(54), 11259-11264.

Omuferen, L. O., Maseko, B., and Olowoyo, J. (2022). Occurrence of antibiotics in wastewater from hospital and convectional wastewater treatment plants and their impact on the effluent receiving rivers: current knowledge between 2010 and 2019. *Environmental Monitoring and Assessment, 194*(4), 306.

Pal, A., He, Y., Jekel, M., Reinhard, M., and Gin, K. Y.-H. (2014). Emerging contaminants of public health significance as water quality indicator compounds in the urban water cycle. *Environment international, 71*, 46-62.

Papagiannaki, D., Belay, M. H., Gonçalves, N. P., Robotti, E., Bianco-Prevot, A., Binetti, R., and Calza, P. (2022). From monitoring to treatment, how to improve water quality: The pharmaceutical case. *Chemical Engineering Journal Advances*, 100245.

Parolini, M. (2020). Toxicity of the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) acetylsalicylic acid, paracetamol, diclofenac, ibuprofen and naproxen towards freshwater invertebrates: A review. *Science of the Total Environment, 740*, 140043.

Previšić, A., Vilenica, M., Vučković, N., Petrović, M., and Rožman, M. (2021). Aquatic insects transfer pharmaceutical and endocrine disruptors from aquatic to terrestrial ecosystems. *Environmental Science and Technology, 55*(6), 3736-3746.

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T., Kidd, K. A., MacCormack, T. J., Olden, J. D., and Ormerod, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews, 94*(3), 849-873.

Rekhate, C. V., and Srivastava, J. (2020). Recent advances in ozone-based advanced oxidation processes for treatment of wastewater-A review. *Chemical Engineering Journal Advances, 3*, 100031.

Riaz, L., Mahmood, T., Kamal, A., Shafqat, M., and Rashid, A. (2017). Industrial release of fluoroquinolones (FQs) in the waste water bodies with their associated ecological risk in Pakistan. *Environmental Toxicology and Pharmacology, 52*, 14-20.

Ritter, K. S., Paul Sibley, Ken Hall, Patricia Keen, Gevan Mattu, Beth Linton, Len. (2002). Sources, pathways, and relative risks of contaminants in surface water and groundwater: a perspective prepared for the Walkerton inquiry. *Journal of Toxicology and Environmental Health Part A, 65*(1), 1-142.

Rosi-Marshall, E. J., and Royer, T. V. (2012). Pharmaceutical compounds and ecosystem function: an emerging research challenge for aquatic ecologists. *Ecosystems, 15*, 867-880.

Singh, P., and Sharma, V. (2016). Integrated plastic waste management: environmental and improved health approaches. *Procedia Environmental Sciences, 35*, 692-700.

Tong, X., Mohapatra, S., Zhang, J., Tran, N. H., You, L., He, Y., and Gin, K. Y.-H. (2022). Source, fate, transport and modelling of selected emerging contaminants in the aquatic environment: Current status and future perspectives. *Water Research*, 118418.

Tripathi, I., Gupta, P., Gupta, M., and Kamal, A. (2015). Charectarization and Validation of Cefixime Trihydrate Tablets with Ftir and Rp-Hplc Techeniques. *Indian journal of applied research, 5*(4), 691.

Tseng, M.-L., Tan, R. R., and Siriban-Manalang, A. B. (2013). Sustainable consumption and production for Asia: sustainability through green design and practice. *Journal of Cleaner Production, 40*, 1-5.

Turner, M., Duncan, J., Shah, U., Metsvaht, T., Varendi, H., Nellis, G., Lutsar, I., Yakkundi, S., McElnay, J., and Pandya, H. (2014). Risk assessment of neonatal excipient exposure: lessons from food safety and other areas. *Advanced drug delivery reviews, 73*, 89-101.

Walker, S. D., and McEldowney, S. (2013). Molecular docking: A potential tool to aid ecotoxicity testing in environmental risk assessment of pharmaceutical. *Chemosphere, 93*(10), 2568-2577.

Wang, K., Zhuang, T., Su, Z., Chi, M., and Wang, H. (2021). Antibiotic residues in wastewaters from sewage treatment plants and Pharmaceutical industries: Occurrence, removal and environmental impacts. *Science of the Total Environment, 788*, 147811.

Williams, R. T. (2005). *Human pharmaceutical: assessing the impacts on aquatic ecosystems*: Allen Press/ACG Publishing.

Zafar, R., Bashir, S., Nabi, D., and Arshad, M. (2021). Occurrence and quantification of prevalent antibiotics in wastewater samples from Rawalpindi and Islamabad, Pakistan. *Science of the Total Environment, 764*, 142596.

Zainab, S. M., Junaid, M., Rehman, M. Y. A., Lv, M., Yue, L., Xu, N., and Malik, R. N. (2021). First insight into the occurrence, spatial distribution, sources, and risks assessment of antibiotics in groundwater from major urban-rural settings of Pakistan. *Science of the Total Environment, 791*, 148298.

APPENDIX A

HPLC Analysis Chromatograms of Selected Pharmaceutical compounds

5/31/2023 3:34:50 PM Page 1 / 1

SHIMADZU
LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: Cefoperazone		
Sample ID	: Standard		
Data Filename	: Standard.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 4:18:59 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 4:36:30 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
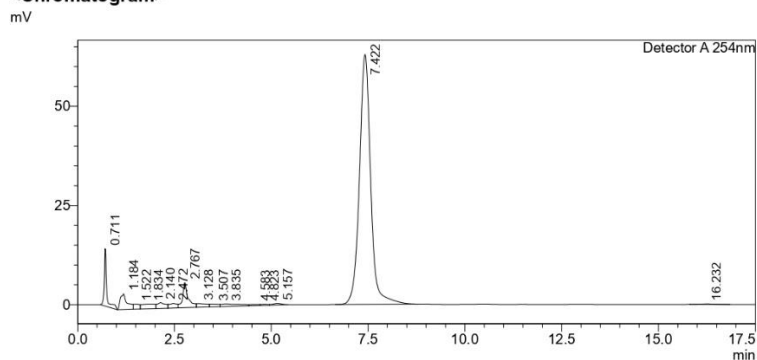
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.711	61457	14442	0.000			
2	1.184	49495	3933	0.000			
3	1.522	13250	1242	0.000		V	
4	1.834	27323	1172	0.000		V	
5	2.140	22208	1460	0.000		V	
6	2.472	16123	1157	0.000		V	
7	2.767	63463	6345	0.000		V	
8	3.128	16434	919	0.000		V	
9	3.507	10932	696	0.000		V	
10	3.835	21648	652	0.000		V	
11	4.583	4921	287	0.000		V	
12	4.823	3478	269	0.000		V	
13	5.157	6058	390	0.000		V	
14	7.422	1274516	63015	0.000			
15	16.232	2926	95	0.000			
Total		1594233	96074				

R _D - 1-12-1 - Standard.lcd

SHIMADZU
LabSolutions   Analysis Report

<Sample Information>

Sample Name	: Cefixime		
Sample ID	: Standard		
Data Filename	: Standard		
Method Filename	: Cefixime.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 10:50:28 AM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 11:10:50 AM	Processed by	: Ayaz Khan

<Chromatogram>

mV

<Peak Table>
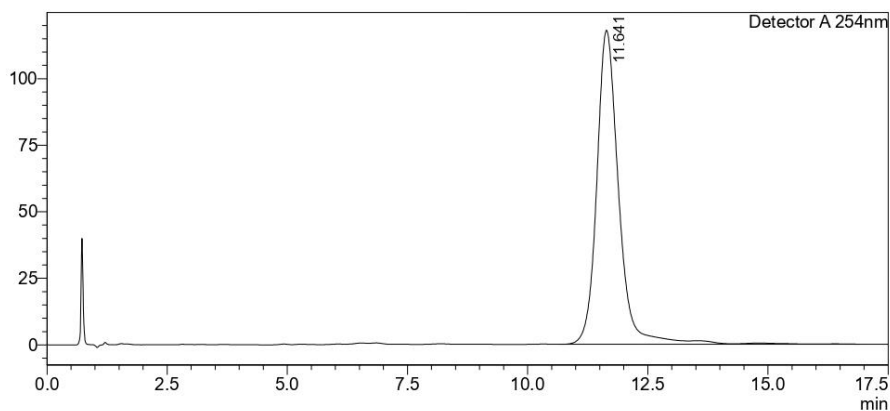
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	11.641	3741707	117968	100.000		SV	
Total		3741707	117968				

Tailing Factor
1.159

SHIMADZU LabSolutions Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample A		
Data Filename	: Sample 1.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 1:00:53 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 1:18:23 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

<Peak Table>
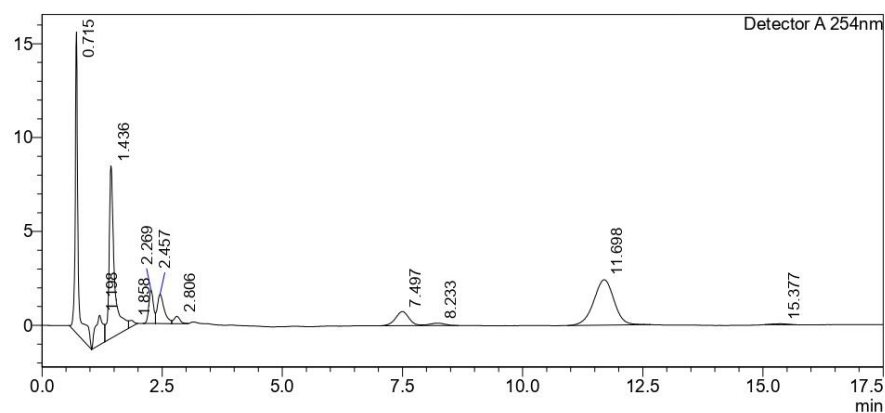
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.715	65998	16027	0.000			
2	1.198	17674	1576	0.000			
3	1.436	71089	9192	0.000		V	
4	1.858	2501	347	0.000		V	
5	2.269	11874	1769	0.000			
6	2.457	15546	1559	0.000		V	
7	2.806	3407	391	0.000		V	
8	7.497	14656	755	0.000			
9	8.233	2972	131	0.000		V	
10	11.698	71268	2419	0.000		S	
11	15.377	1239	51	0.000			
Total		278223	34217				

SHIMADZU LabSolutions   Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample B		
Data Filename	: Sample 2.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 1:32:41 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 1:50:11 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

<Peak Table>
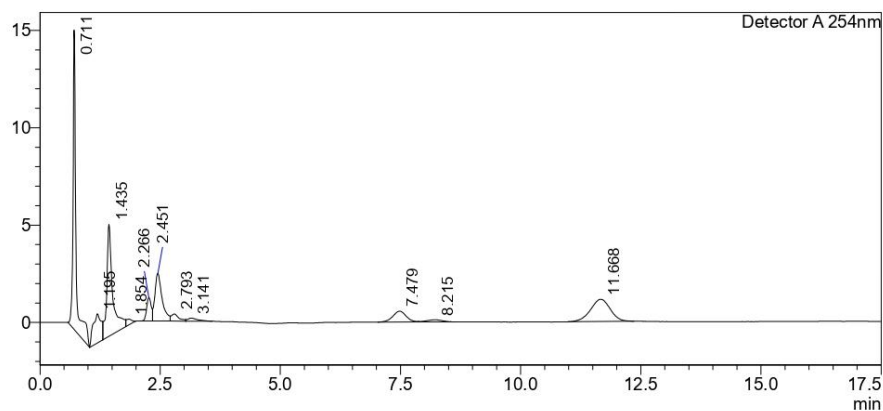
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.711	63652	15369	0.000			
2	1.195	17668	1494	0.000			
3	1.435	48774	5742	0.000		V	
4	1.854	2387	284	0.000		V	
5	2.266	8097	1206	0.000			
6	2.451	25397	2449	0.000		V	
7	2.793	3980	368	0.000		V	
8	3.141	2272	158	0.000		V	
9	7.479	10842	559	0.000			
10	8.215	2515	111	0.000		V	
11	11.668	32466	1138	0.000			
Total		218051	28878				

SHIMADZU LabSolutions # Analysis Report

\<Sample Information\>

Sample Name	: Sample		
Sample ID	: Sample C		
Data Filename	: Sample 3.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 1:51:10 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 2:08:40 PM	Processed by	: Ayaz Khan

\<Chromatogram\>

mV

Detector A 254nm

\<Peak Table\>
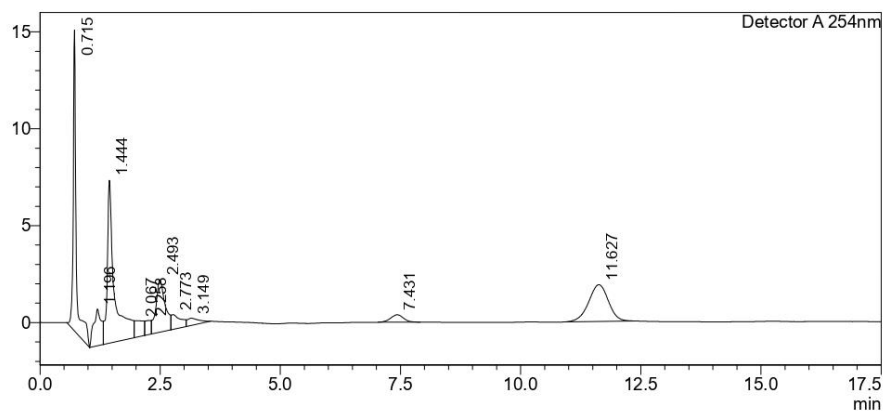
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.715	64855	15508	0.000			
2	1.196	21137	1884	0.000			
3	1.444	87592	8401	0.000		V	
4	2.067	10614	820	0.000		V	
5	2.258	6167	728	0.000		V	
6	2.493	36441	2714	0.000		V	
7	2.773	10196	757	0.000		V	
8	3.149	6120	370	0.000		V	
9	7.431	7078	382	0.000			
10	11.627	54170	1898	0.000			
Total		304369	33463				

SHIMADZU
LabSolutions # Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample D		
Data Filename	: Sample 4.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 2:09:50 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 2:27:20 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 254nm

<Peak Table>
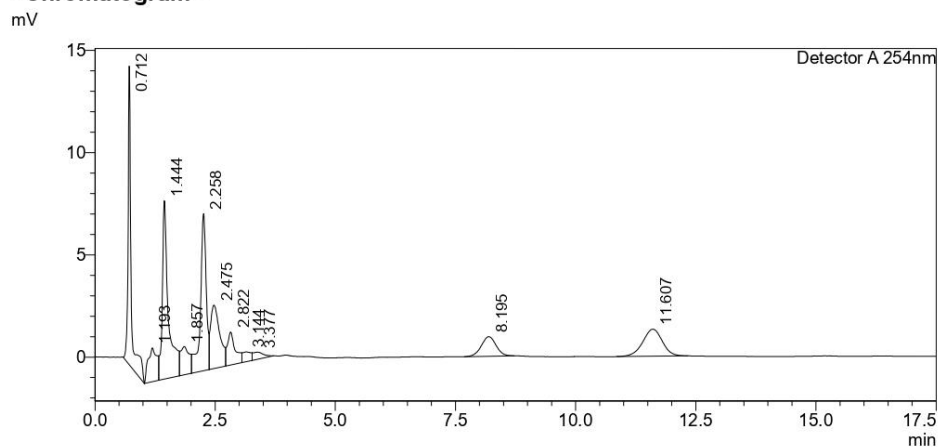
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.712	62488	14602	0.000			
2	1.193	21040	1656	0.000			
3	1.444	76796	8719	0.000		V	
4	1.857	17489	1390	0.000		V	
5	2.258	65211	7672	0.000		V	
6	2.475	41311	3101	0.000		V	
7	2.822	18550	1607	0.000		V	
8	3.144	5530	474	0.000		V	
9	3.377	5302	346	0.000		V	
10	8.195	20417	956	0.000			
11	11.607	37602	1322	0.000			
Total		371735	41845				

SHIMADZU LabSolutions   # Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample E		
Data Filename	: Sample 5.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 2:27:59 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 2:45:30 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 254nm

<Peak Table>
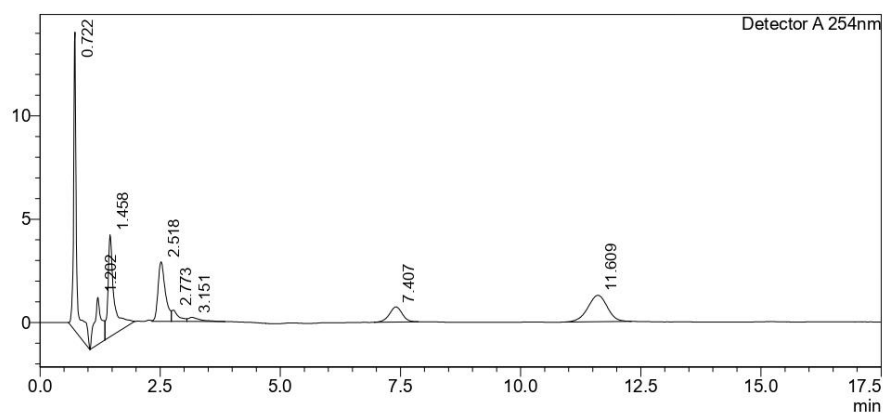
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.722	61442	14431	0.000			
2	1.202	22828	2282	0.000			
3	1.458	44144	4930	0.000		V	
4	2.518	30643	2885	0.000		V	
5	2.773	5501	545	0.000		V	
6	3.151	3457	202	0.000		V	
7	7.407	13450	729	0.000			
8	11.609	35907	1277	0.000			
Total		217372	27281				

SHIMADZU LabSolutions Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample K		
Data Filename	: Sample K.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 2:46:05 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 3:03:35 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
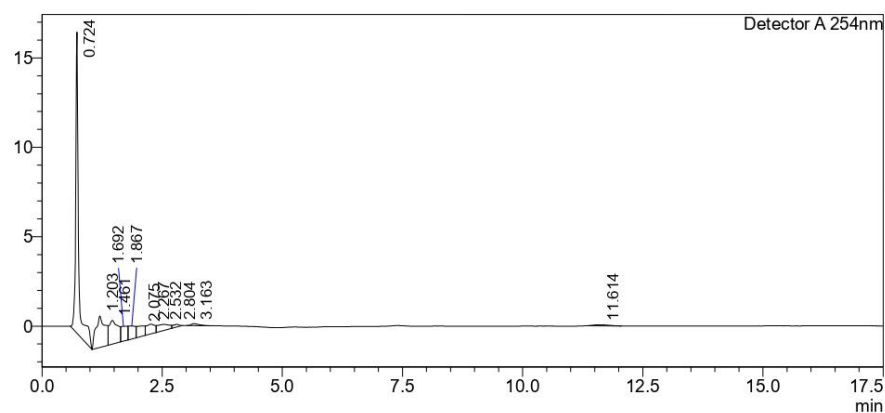
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.724	67669	16820	0.000			
2	1.203	23625	1749	0.000			
3	1.461	16801	1324	0.000		V	
4	1.692	7768	839	0.000		V	
5	1.867	7085	724	0.000		V	
6	2.075	6708	575	0.000		V	
7	2.267	6692	541	0.000		V	
8	2.532	5989	339	0.000		V	
9	2.804	1447	147	0.000		V	
10	3.163	1155	103	0.000			
11	11.614	1301	60	0.000			
Total		146239	23221				

Analysis Report

SHIMADZU LabSolutions

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample L		
Data Filename	: Sample L.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 3:04:31 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 3:22:02 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 254nm

<Peak Table>
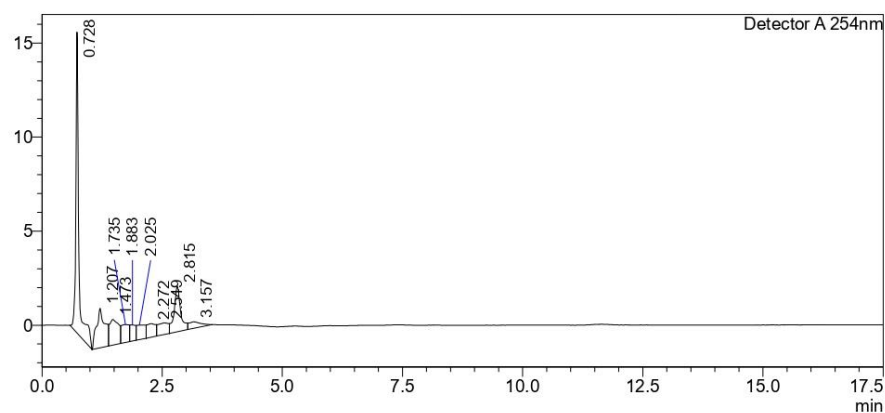
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.728	66478	15983	0.000			
2	1.207	25656	2092	0.000			
3	1.473	17736	1364	0.000		V	
4	1.735	10787	954	0.000		V	
5	1.883	6729	854	0.000		V	
6	2.025	9397	772	0.000		V	
7	2.272	8870	714	0.000		V	
8	2.549	9444	607	0.000		V	
9	2.815	23114	2404	0.000		V	
10	3.157	6012	347	0.000		V	
Total		184222	26091				

SHIMADZU
LabSolutions Analysis Report

\<Sample Information\>

Sample Name	: Sample		
Sample ID	: Sample M		
Data Filename	: Sample M.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 3:22:38 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 3:40:09 PM	Processed by	: Ayaz Khan

\<Chromatogram\>

\<Peak Table\>
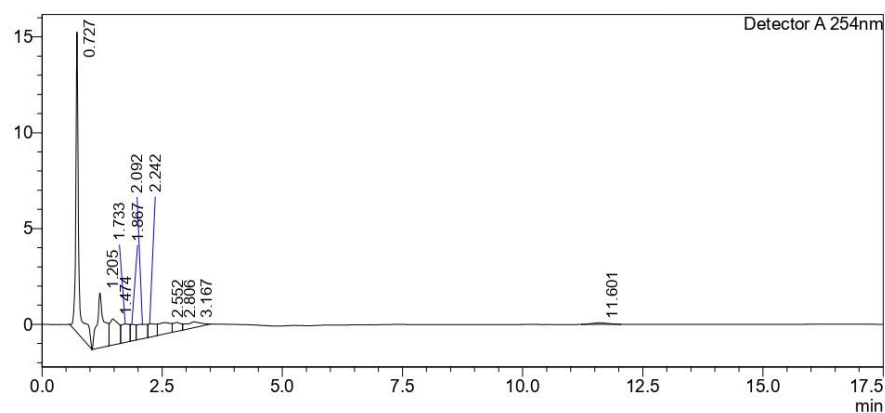
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.727	65295	15672	0.000			
2	1.205	29574	2849	0.000			
3	1.474	17173	1357	0.000		V	
4	1.733	11276	963	0.000		V	
5	1.867	6307	868	0.000		V	
6	2.092	10845	749	0.000		V	
7	2.242	7794	684	0.000		V	
8	2.552	10324	591	0.000		V	
9	2.806	5439	462	0.000		V	
10	3.167	7011	297	0.000		V	
11	11.601	1860	78	0.000			
Total		172899	24570				

SHIMADZU LabSolutions ## Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample N		
Data Filename	: Sample N.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 3:41:51 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 3:59:22 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
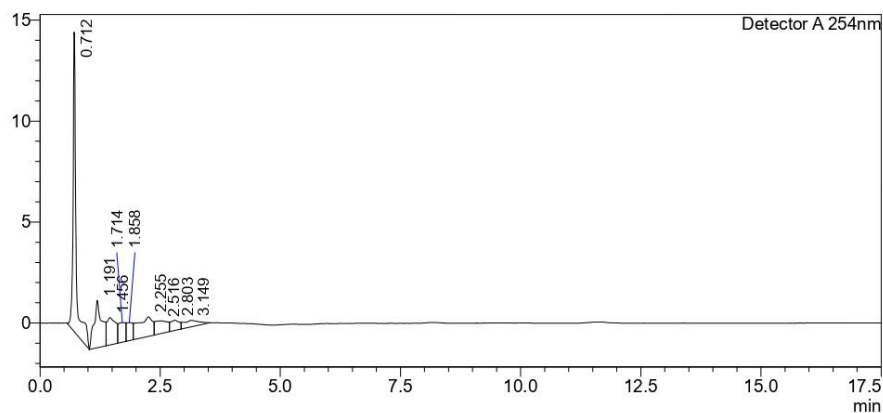
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.712	63792	14833	0.000			
2	1.191	27341	2334	0.000			
3	1.456	17005	1349	0.000		V	
4	1.714	10022	965	0.000		V	
5	1.858	7848	891	0.000		V	
6	2.255	20734	961	0.000		V	
7	2.516	11259	623	0.000		V	
8	2.803	6442	506	0.000		V	
9	3.149	7331	320	0.000		V	
Total		171774	22782				

SHIMADZU LabSolutions Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample O		
Data Filename	: Sample O.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/26/2023 4:00:12 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/26/2023 4:17:42 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 254nm

<Peak Table>
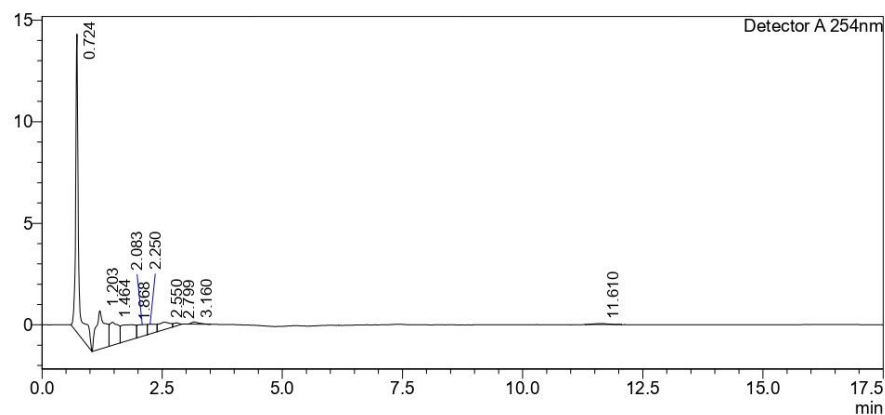
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.724	61565	14673	0.000			
2	1.203	25416	1886	0.000			
3	1.464	14178	1131	0.000		V	
4	1.868	15578	724	0.000		V	
5	2.083	7703	571	0.000		V	
6	2.250	5290	475	0.000		V	
7	2.550	6025	358	0.000		V	
8	2.799	1276	148	0.000		V	
9	3.160	1130	99	0.000			
10	11.610	1172	52	0.000			
Total		139334	20119				

SHIMADZU
LabSolutions   # Analysis Report

<Sample Information>

Sample Name	: Ceftriaxone Sodium		
Sample ID	: Standard		
Data Filename	: Standard		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 5/31/2023 3:09:12 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/31/2023 3:19:12 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 254nm

<Peak Table>
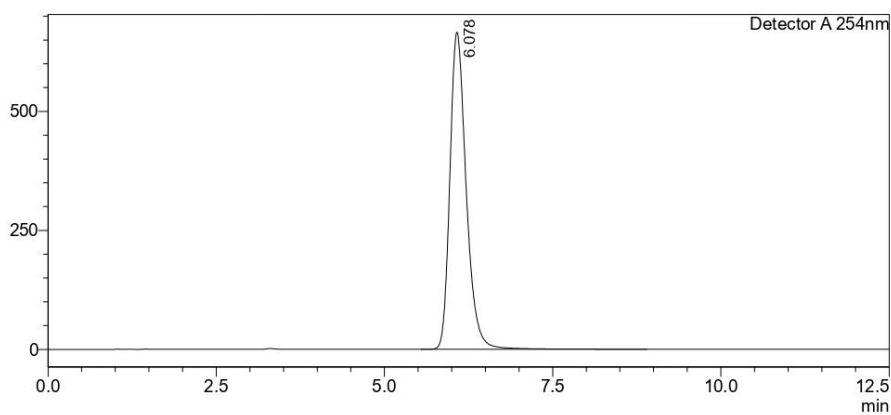
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	6.078	11239612	666035	100.000			
Total		11239612	666035				

Tailing Factor
1.296

SHIMADZU LabSolutions   Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample A		
Data Filename	: Sample A.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 5/31/2023 1:04:16 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/31/2023 1:14:16 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
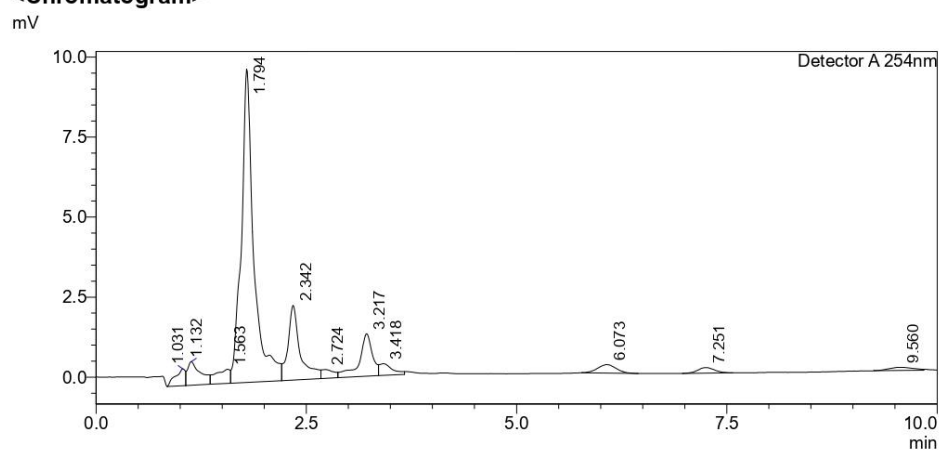
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.031	4300	526	0.000			
2	1.132	8038	737	0.000		V	
3	1.563	5291	440	0.000		V	
4	1.794	99978	9783	0.000		SV	
5	2.342	23553	2329	0.000		V	
6	2.724	2755	276	0.000		V	
7	3.217	14537	1331	0.000		V	
8	3.418	3978	366	0.000		V	
9	6.073	4026	261	0.000			
10	7.251	2381	173	0.000			
11	9.560	1935	95	0.000			
Total		170773	16318				

SHIMADZU LabSolutions # Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample B		
Data Filename	: Sample B.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 5/31/2023 1:15:14 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/31/2023 1:25:14 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 254nm

<Peak Table>
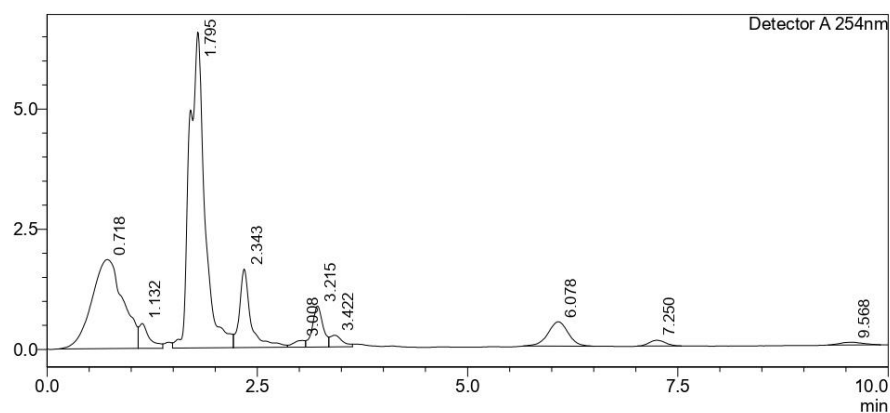
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.718	47853	1853	0.000			
2	1.132	4430	519	0.000		V	
3	1.795	82282	6565	0.000		V	
4	2.343	15106	1631	0.000		SV	
5	3.008	1347	134	0.000		V	
6	3.215	7335	852	0.000		V	
7	3.422	2462	240	0.000		V	
8	6.078	8467	505	0.000			
9	7.250	1590	119	0.000			
10	9.568	1033	55	0.000			
Total		171905	12475				

R _D - 1-27-1 - Sample B.lcd

SHIMADZU
LabSolutions   # Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample C		
Data Filename	: Sample C.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 5/31/2023 1:25:55 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/31/2023 1:35:55 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
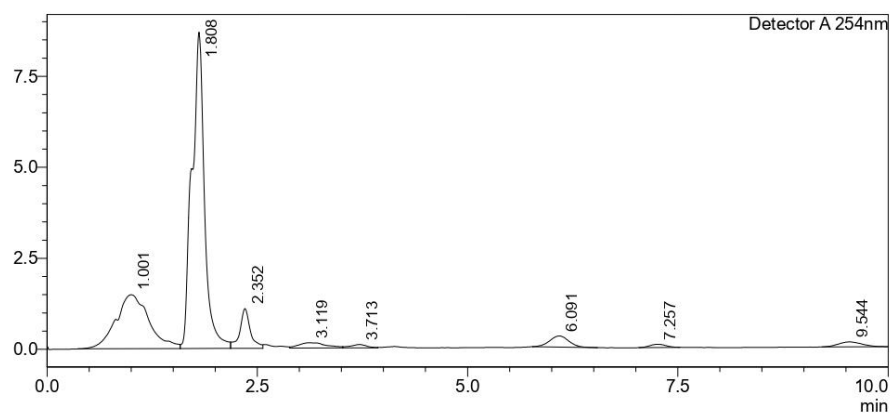
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.001	40625	1483	0.000			
2	1.808	89524	8687	0.000		V	
3	2.352	9458	1089	0.000		V	
4	3.119	3336	147	0.000		V	
5	3.713	1251	93	0.000		V	
6	6.091	4837	305	0.000			
7	7.257	1123	87	0.000			
8	9.544	2657	136	0.000			
Total		152812	12025				

SHIMADZU LabSolutions Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample D		
Data Filename	: Sample D.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 5/31/2023 1:38:55 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/31/2023 1:48:55 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
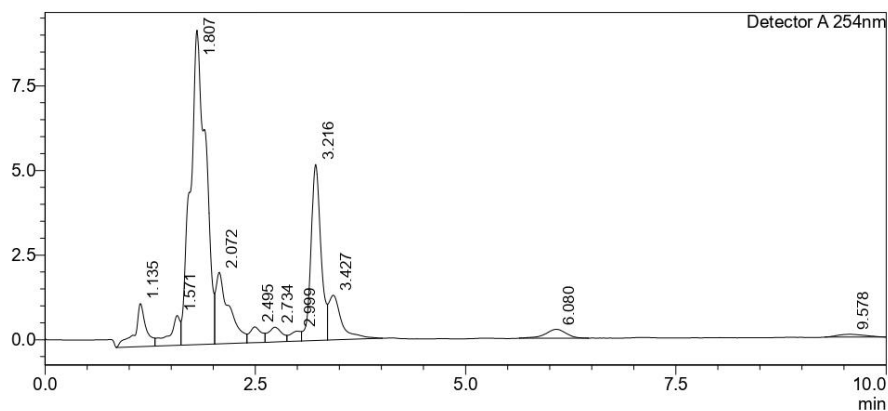
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.135	11870	1273	0.000			
2	1.571	7850	877	0.000		V	
3	1.807	116563	9289	0.000		V	
4	2.072	23228	2109	0.000		V	
5	2.495	4568	466	0.000		V	
6	2.734	4874	436	0.000		V	
7	2.999	2678	295	0.000		V	
8	3.216	42986	5201	0.000		V	
9	3.427	14133	1320	0.000		V	
10	6.080	4382	258	0.000			
11	9.578	1838	81	0.000			
Total		234968	21605				

78

5/31/2023 3:49:14 PM Page 1 / 1

![SHIMADZU LabSolutions] **Analysis Report**

<Sample Information>

Sample Name	: Sample			
Sample ID	: Sample E			
Data Filename	: Sample E.lcd			
Method Filename	: R&D.lcm			
Batch Filename	:			
Vial #	: 1-1		Sample Type	: Unknown
Injection Volume	: 20 uL			
Date Acquired	: 5/31/2023 1:52:43 PM		Acquired by	: Ayaz Khan
Date Processed	: 5/31/2023 2:02:44 PM		Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
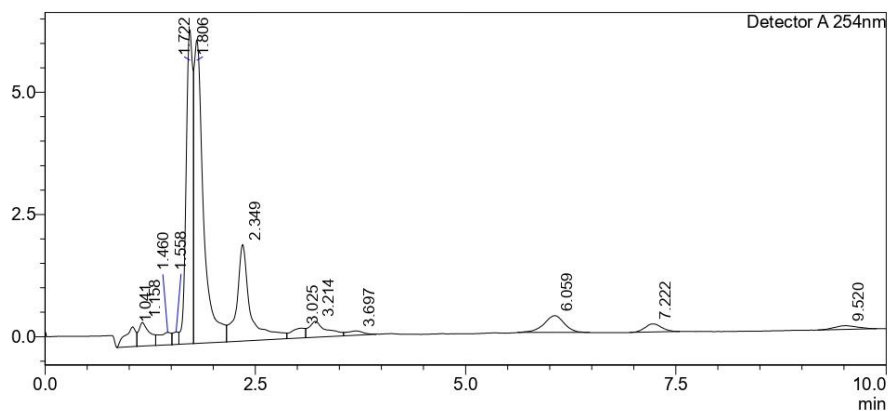
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.041	3558	404	0.000			
2	1.158	4458	484	0.000		V	
3	1.460	2635	255	0.000		V	
4	1.558	1233	255	0.000		V	
5	1.722	36808	6412	0.000		V	
6	1.806	46970	6210	0.000		V	
7	2.349	21786	1972	0.000		V	
8	3.025	2321	199	0.000		V	
9	3.214	4729	326	0.000		V	
10	3.697	1171	87	0.000		V	
11	6.059	5810	343	0.000			
12	7.222	2275	164	0.000			
13	9.520	1471	75	0.000			
Total		135226	17187				

R _D - 1-30-1 - Sample E.lcd

SHIMADZU LabSolutions   Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample K		
Data Filename	: Sample 1.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 5/31/2023 2:06:32 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/31/2023 2:16:32 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 254nm

<Peak Table>
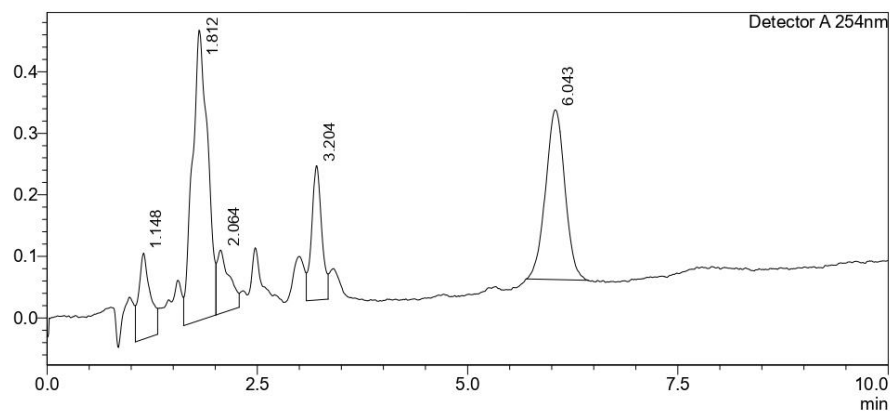
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.148	1333	139	0.000		V	
2	1.812	5931	471	0.000		V	
3	2.064	1036	103	0.000		V	
4	3.204	1801	218	0.000		V	
5	6.043	4420	276	0.000			
Total		14520	1208				

SHIMADZU LabSolutions   Analysis Report
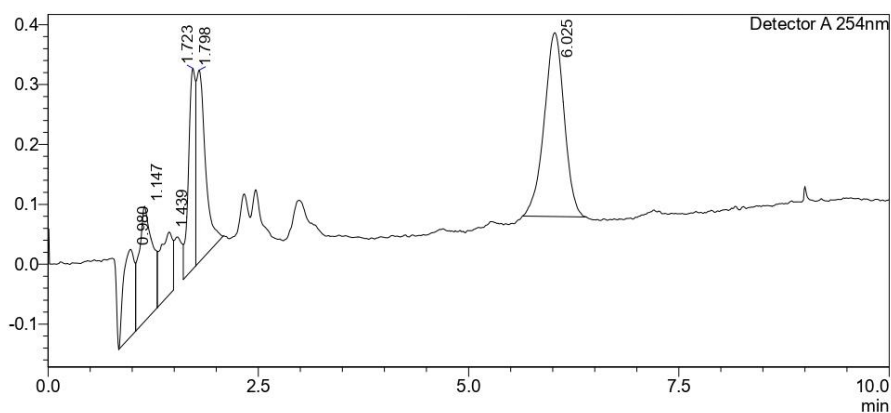
<Sample Information>

Sample Name   : Sample
Sample ID   : Sample L
Data Filename : Sample 2.lcd
Method Filename : R&D.lcm
Batch Filename :
Vial #   : 1-1   Sample Type   : Unknown
Injection Volume : 20 uL
Date Acquired : 5/31/2023 2:18:01 PM   Acquired by   : Ayaz Khan
Date Processed : 5/31/2023 2:28:02 PM   Processed by   : Ayaz Khan

<Chromatogram>

<Peak Table>
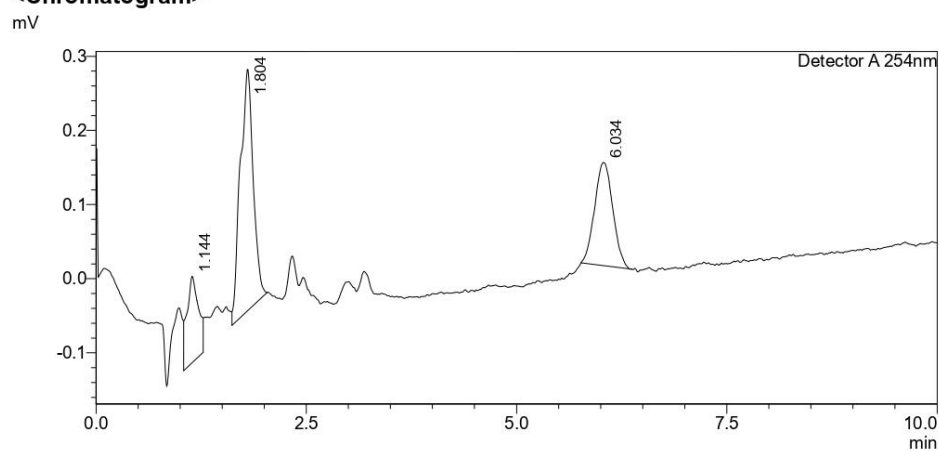
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.144	1118	116	0.000		V	
2	1.804	3474	325	0.000		V	
3	6.034	2090	139	0.000			
Total		6682	581				

SHIMADZU LabSolutions   # Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample M		
Data Filename	: Sample 3.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 5/31/2023 2:30:33 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/31/2023 2:40:34 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.980	1354	146	0.000			
2	1.147	2137	192	0.000		V	
3	1.439	1102	105	0.000		V	
4	1.723	1960	335	0.000		V	
5	1.798	2326	321	0.000		V	
6	6.025	5085	307	0.000			
Total		13965	1406				

SHIMADZU LabSolutions Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample N		
Data Filename	: Sample 4.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 5/31/2023 2:43:37 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/31/2023 2:53:37 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 254nm

<Peak Table>
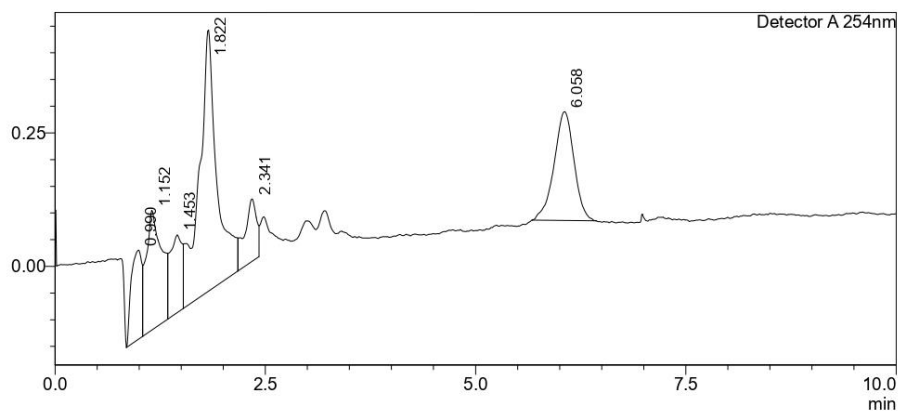
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.990	1467	167	0.000			
2	1.152	2895	224	0.000		V	
3	1.453	1445	146	0.000		V	
4	1.822	7256	489	0.000		V	
5	2.341	1180	117	0.000		V	
6	6.058	3365	204	0.000			
Total		17608	1347				

R _D - 1-34-1 - Sample 4.lcd

SHIMADZU LabSolutions # Analysis Report

<Sample Information>

Sample Name   : Sample
Sample ID   : Sample O
Data Filename : Sample 5.lcd
Method Filename : R&D.lcm
Batch Filename :
Vial #   : 1-1   Sample Type   : Unknown
Injection Volume : 20 uL
Date Acquired : 5/31/2023 2:56:11 PM   Acquired by   : Ayaz Khan
Date Processed : 5/31/2023 3:06:11 PM   Processed by   : Ayaz Khan

<Chromatogram>

<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.981	1381	152	0.000			
2	1.151	2484	199	0.000		V	
3	1.443	1302	124	0.000		V	
4	1.707	2328	331	0.000		V	
5	1.819	3731	408	0.000		V	
6	6.098	1578	103	0.000			
Total		12804	1316				

SHIMADZU
LabSolutions   Analysis Report

<Sample Information>

Sample Name	: Ciprofloxacin HCl		
Sample ID	: Standard		
Data Filename	: Ciprofloxacin HCl standard1.lcd		
Method Filename	: Ciprofloxacin HCl.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 12:42:24 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/29/2023 1:34:37 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 278nm

<Peak Table>
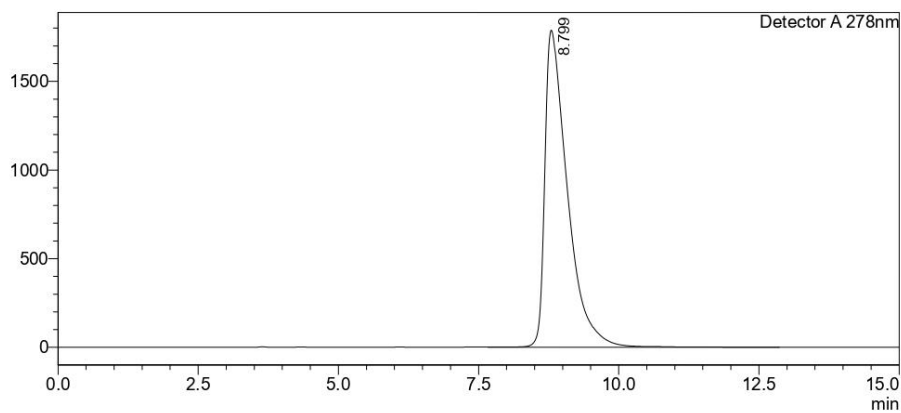
Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.799	49969612	1788017	100.000		V	
Total		49969612	1788017				

Tailing Factor
2.159

SHIMADZU LabSolutions Analysis Report

<Sample Information>

Sample Name	: Ciprofloxacin HCl		
Sample ID	: Standard		
Data Filename	: Ciprofloxacin HCl standard2.lcd		
Method Filename	: Ciprofloxacin HCl.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 12:57:55 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/29/2023 1:33:53 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>

Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.045	48239830	1867756	100.000		SV	
Total		48239830	1867756				

Tailing Factor	
	2.171

SHIMADZU LabSolutions # Analysis Report

<Sample Information>

Sample Name	: Levofloxacin
Sample ID	: STANDARD
Data Filename	: Standard
Method Filename	: R&D.lcm
Batch Filename	:
Vial #	: 1-1
Injection Volume	: 10 uL
Date Acquired	: 5/29/2023 4:53:44 PM
Date Processed	: 5/29/2023 5:07:37 PM

Sample Type	: Unknown
Acquired by	: Ayaz Khan
Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 278nm

<Peak Table>

Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.074	1549	263	0.000		V	
2	1.475	5527	1228	0.000			
3	1.744	1113	114	0.000		V	
4	1.994	2554	282	0.000		V	
5	4.024	2305	193	0.000			
6	4.400	2211	130	0.000		V	
7	5.307	47747	2483	0.000		V	
8	6.683	36477967	1307503	0.000		V	
Total		36540973	1312196				

![SHIMADZU LabSolutions] **Analysis Report**

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample A		
Data Filename	: Sample.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 1:58:00 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/29/2023 2:13:01 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>

Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.631	5028	191	0.000			
2	1.231	2113	358	0.000		V	
3	1.400	1759	375	0.000			
4	1.721	14590	940	0.000			
5	1.984	25013	3788	0.000		V	
6	2.143	14514	1444	0.000		V	
7	2.487	7496	381	0.000		V	
8	3.129	1525	103	0.000		V	
9	4.304	2245	137	0.000			
10	5.959	80738	4234	0.000			
11	7.798	347920	17667	0.000		V	
Total		502941	29617				

SHIMADZU LabSolutions # Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample B		
Data Filename	: Sample.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 2:13:53 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/29/2023 2:28:54 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>

Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.082	2959	600	0.000			
2	1.233	2195	373	0.000		V	
3	1.412	1837	382	0.000			
4	1.738	13804	791	0.000			
5	2.022	18235	2605	0.000		V	
6	2.172	10133	1040	0.000		V	
7	2.552	1780	167	0.000		V	
8	6.148	60606	3169	0.000		S	
9	8.141	148437	7586	0.000			
Total		259987	16714				

SHIMADZU LabSolutions Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample C		
Data Filename	: Sample.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 2:30:16 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/29/2023 2:45:17 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
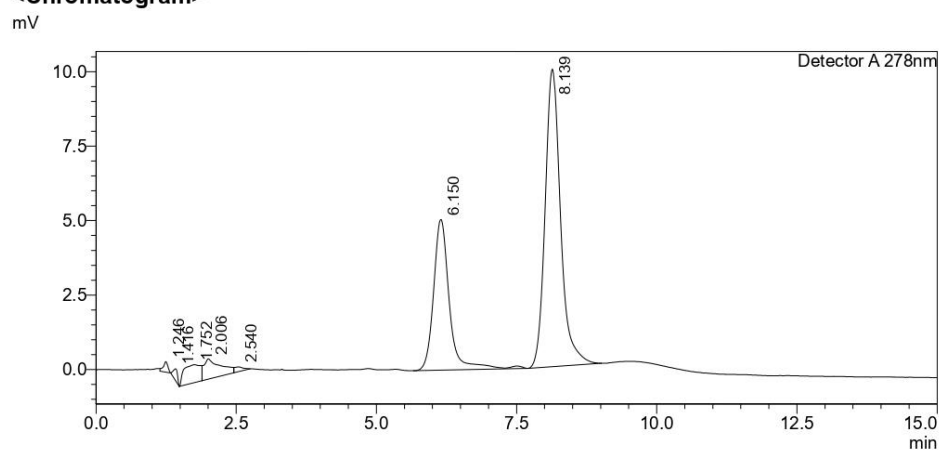
Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.246	1976	349	0.000		V	
2	1.416	1896	388	0.000			
3	1.752	12766	605	0.000			
4	2.006	13744	682	0.000		V	
5	2.540	1694	160	0.000		V	
6	6.150	97831	5059	0.000		S	
7	8.139	190674	9988	0.000			
Total		320581	17231				

Analysis Report

SHIMADZU
LabSolutions

<Sample Information>

Sample Name	: Sample			
Sample ID	: Sample D			
Data Filename	: Sample.lcd			
Method Filename	: R&D.lcm			
Batch Filename	:			
Vial #	: 1-1		Sample Type	: Unknown
Injection Volume	: 10 uL			
Date Acquired	: 5/29/2023 2:46:36 PM		Acquired by	: Ayaz Khan
Date Processed	: 5/29/2023 3:01:37 PM		Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
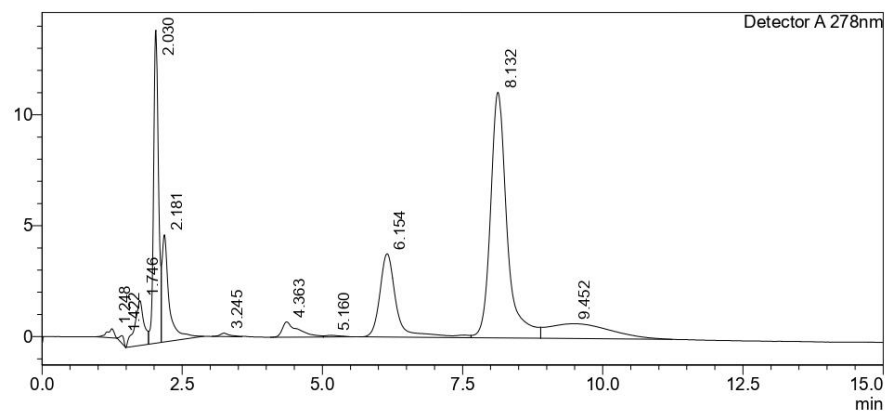
Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.248	3516	406	0.000			
2	1.422	1581	340	0.000			
3	1.746	23170	2017	0.000			
4	2.030	83980	14106	0.000		V	
5	2.181	42393	4827	0.000		V	
6	3.245	1957	155	0.000			
7	4.363	13726	691	0.000			
8	5.160	1369	76	0.000		V	
9	6.154	77018	3757	0.000		S	
10	8.132	225850	11080	0.000		V	
11	9.452	51728	670	0.000		V	
Total		526287	38123				

SHIMADZU LabSolutions   # Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample E		
Data Filename	: Sample.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 3:02:15 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/29/2023 3:17:15 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
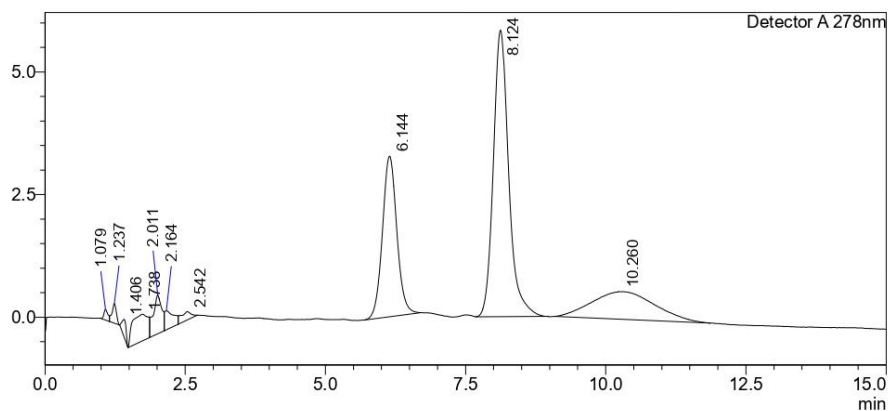
Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.079	1119	215	0.000			
2	1.237	2110	395	0.000		V	
3	1.406	1647	346	0.000			
4	1.738	10801	541	0.000			
5	2.011	8274	784	0.000		V	
6	2.164	4200	383	0.000		V	
7	2.542	2280	166	0.000		V	
8	6.144	58017	3272	0.000			
9	8.124	110856	5840	0.000		V	
10	10.260	44008	562	0.000			
Total		243312	12505				

SHIMADZU LabSolutions   # Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: sample K		
Data Filename	: Sample.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 4:05:42 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/30/2023 4:49:19 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

<Peak Table>
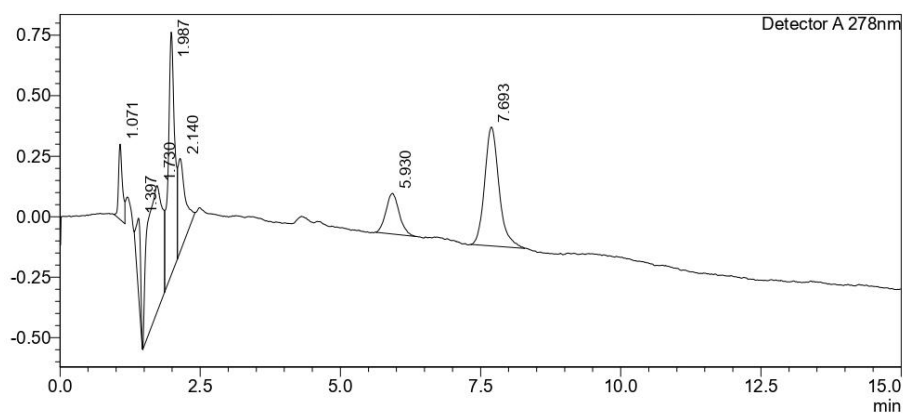
Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.071	1509	311	0.000			
2	1.397	1416	303	0.000			
3	1.730	10118	522	0.000			
4	1.987	8054	1001	0.000		V	
5	2.140	3393	387	0.000		V	
6	5.930	2761	168	0.000			
7	7.693	8799	492	0.000			
Total		36051	3183				

SHIMADZU LabSolutions # Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: Sample L		
Data Filename	: Sample.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 3:33:58 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/29/2023 3:48:59 PM	Processed by	: Ayaz Khan

<Chromatogram>

<Peak Table>
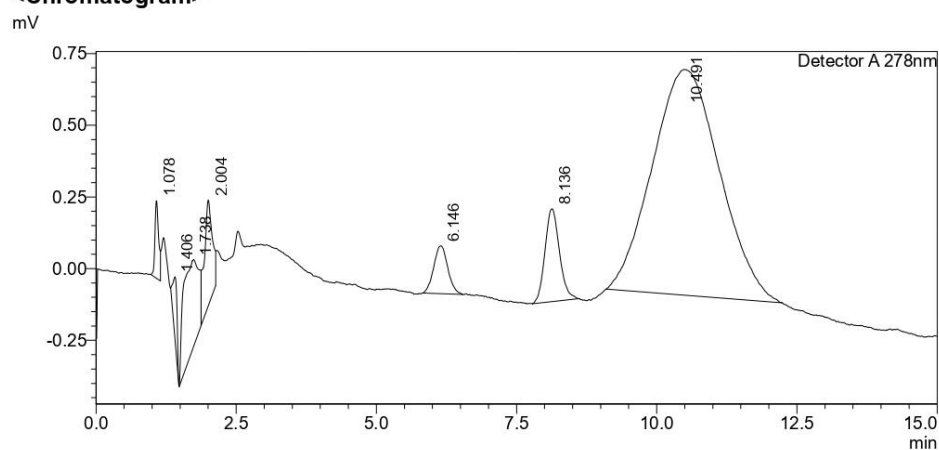
Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.078	1268	271	0.000			
2	1.406	1009	212	0.000			
3	1.738	6271	304	0.000			
4	2.004	3631	367	0.000		V	
5	6.146	2881	167	0.000			
6	8.136	5773	323	0.000			
7	10.491	65780	787	0.000			
Total		86613	2430				

**SHIMADZU
LabSolutions** # Analysis Report

\<Sample Information\>

Sample Name	: Sample		
Sample ID	: sample M		
Data Filename	: Sample.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 4:21:18 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/30/2023 4:52:28 PM	Processed by	: Ayaz Khan

\<Chromatogram\>

mV

Detector A 278nm

\<Peak Table\>
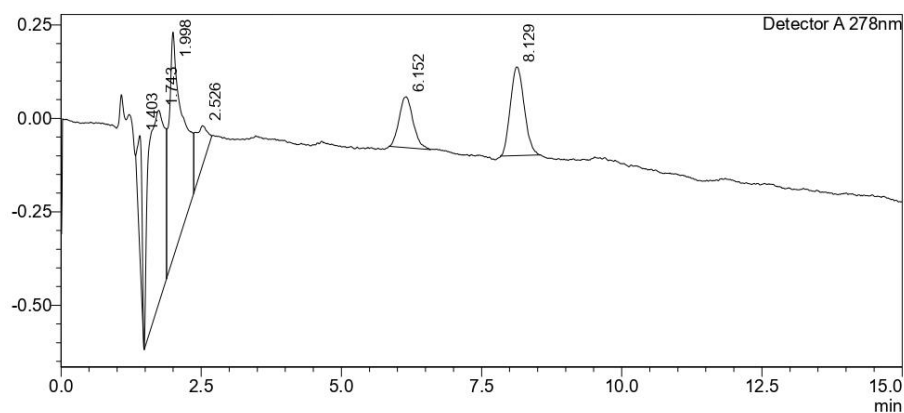
Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.403	1487	306	0.000			
2	1.743	10940	517	0.000			
3	1.998	10151	605	0.000		V	
4	2.526	1743	106	0.000		V	
5	6.152	2345	136	0.000			
6	8.129	4058	237	0.000			
Total		30724	1907				

SHIMADZU LabSolutions   # Analysis Report

<Sample Information>

Sample Name	: Sample		
Sample ID	: sample N		
Data Filename	: Sample.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 3:18:22 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/30/2023 4:48:33 PM	Processed by	: Ayaz Khan

<Chromatogram>

mV

Detector A 278nm

<Peak Table>
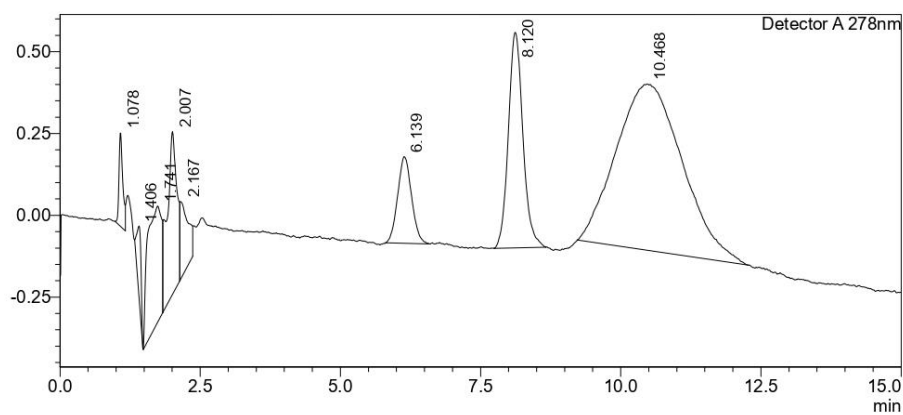
Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.078	1349	285	0.000			
2	1.406	1003	210	0.000			
3	1.741	6448	355	0.000			
4	2.007	5989	497	0.000		V	
5	2.167	2247	230	0.000		V	
6	6.139	4545	265	0.000			
7	8.120	11834	659	0.000			
8	10.468	41762	508	0.000			
Total		75178	3009				

SHIMADZU
LabSolutions   Analysis Report

\<Sample Information\>

Sample Name	: Sample		
Sample ID	: sample O		
Data Filename	: Sample.lcd		
Method Filename	: R&D.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/29/2023 3:49:40 PM	Acquired by	: Ayaz Khan
Date Processed	: 5/30/2023 4:51:04 PM	Processed by	: Ayaz Khan

\<Chromatogram\>

mV

\<Peak Table\>
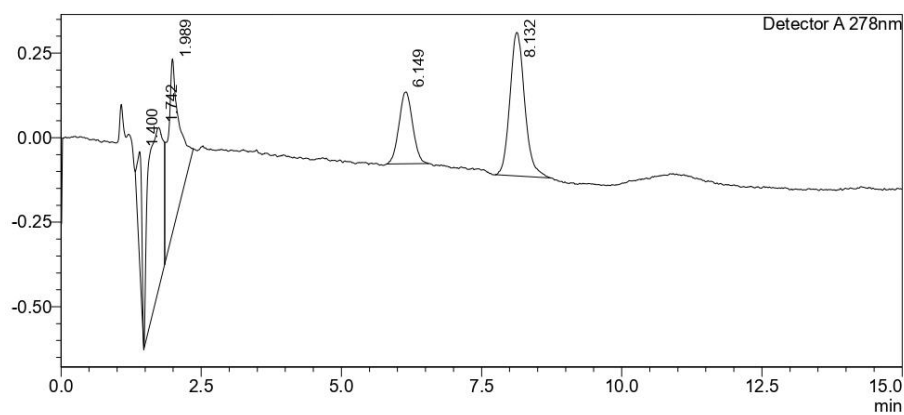
Detector A 278nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.400	1564	321	0.000			
2	1.742	9702	477	0.000			
3	1.989	7397	514	0.000		V	
4	6.149	3572	213	0.000			
5	8.132	7732	424	0.000			
Total		29967	1949				

R _D - 1-20-2 - Sample.lcd

Turnitin Originality Report

Processed on: 04-Sep-2023 16:24 PKT
ID: 2157546699
Word Count: 12560
Submitted: 1

thesis By Saeed Ayaz Khan

Similarity Index

19%

Similarity by Source

Internet Sources: 14%
Publications: 14%
Student Papers: 4%

1% match (student papers from 16-Sep-2017)
Submitted to Higher Education Commission Pakistan on 2017-09-16

1% match (Muhammad Ashfaq, Khujasta Nawaz Khan, Muhammad Saif Ur Rehman, Ghulam Mustafa et al. "Ecological risk assessment of pharmaceuticals in the receiving environment of pharmaceutical wastewater in Pakistan", Ecotoxicology and Environmental Safety, 2017)
Muhammad Ashfaq, Khujasta Nawaz Khan, Muhammad Saif Ur Rehman, Ghulam Mustafa et al. "Ecological risk assessment of pharmaceuticals in the receiving environment of pharmaceutical wastewater in Pakistan", Ecotoxicology and Environmental Safety, 2017

1% match (chaoqiang xiao, xia zhang, Zhihua Lv, changqin hu, wei wang, Xiuyun Yang, ying sun, weifeng zhang, shuwang He, yang jie. "Developing an improved UHPLC method for impurity profile analysis of ceftriaxone using analytical quality by design", Analytical Methods, 2023)
chaoqiang xiao, xia zhang, Zhihua Lv, changqin hu, wei wang, Xiuyun Yang, ying sun, weifeng zhang, shuwang He, yang jie. "Developing an improved UHPLC method for impurity profile analysis of ceftriaxone using analytical quality by design", Analytical Methods, 2023

1% match (Internet from 18-Nov-2022)
https://at.vwr.com/m-at.vwr.com/de_AT/images/Compendium_201503_070_ProNet0811.pdf

1% match (Luqman Riaz, Tariq Mahmood, Atif Kamal, Mateen Shafqat, Audil Rashid. "Industrial release of fluoroquinolones (FQs) in the waste water bodies with their associated ecological risk in Pakistan", Environmental Toxicology and Pharmacology, 2017)
Luqman Riaz, Tariq Mahmood, Atif Kamal, Mateen Shafqat, Audil Rashid. "Industrial release of fluoroquinolones (FQs) in the waste water bodies with their associated ecological risk in Pakistan", Environmental Toxicology and Pharmacology, 2017

< 1% match (student papers from 14-Mar-2016)
Submitted to Higher Education Commission Pakistan on 2016-03-14

< 1% match (student papers from 04-Jan-2015)
Submitted to Higher Education Commission Pakistan on 2015-01-04

< 1% match (Internet from 06-Jul-2023)
https://link.springer.com/article/10.1007/s42250-023-00703-6?code=0a826106-b56b-4496-88d1-201d32e86a33&error=cookies_not_supported

< 1% match (Internet from 10-Aug-2018)
https://link.springer.com/article/10.1007%2Fs11356-018-2517-x

< 1% match (Internet from 16-Mar-2020)
https://link.springer.com/article/10.1007/s11270-019-4098-9

< 1% match (Internet from 29-Apr-2019)
https://link.springer.com/article/10.1007/s10661-017-6097-x

< 1% match (Internet from 17-Aug-2022)
https://link.springer.com/article/10.1007/s10661-020-8151-3?code=177723ef-4991-4609-a7ec-adb0016856d9&error=cookies_not_supported

< 1% match (Internet from 01-May-2023)
https://link.springer.com/article/10.1007/s10661-023-11002-5?code=09114210-5999-48d9-a15d-3d52860dbbe6&error=cookies_not_supported

< 1% match (Internet from 22-Nov-2022)
https://link.springer.com/article/10.1007/s10653-022-01421-7?code=ad58be7a-393c-4a99-8437-05ef0f547fac&error=cookies_not_supported

< 1% match (Internet from 13-Sep-2022)
https://link.springer.com/article/10.1007/s10653-021-00828-y?code=35273d6a-464f-40ca-8dec-12e9289fd33b&error=cookies_not_supported

< 1% match (Internet from 29-Jan-2023)
https://www.researchgate.net/publication/236086964_Global_risk_of_pharmaceutical_contamination_from_highly_populated_developing_countries

< 1% match (Internet from 14-Feb-2023)
https://www.researchgate.net/publication/43135942_Evaluation_of_the_interaction_between_soil_and_antibiotics

< 1% match (Internet from 02-Oct-2022)
https://www.researchgate.net/publication/338976805_Antibiotics_in_coastal_water_and_sediments_of_the_East_China_Sea_Distribution_ecological_risk_asses

< 1% match (Internet from 12-Jun-2022)
https://www.researchgate.net/publication/354380794_Impacts_of_Plastic_Pollution_on_Ecosystem_Services_Sustainable_Development_Goals_and_Need_to_Fo

< 1% match (Internet from 29-Jan-2023)
https://www.researchgate.net/publication/272835551_Predicting_concentrations_of_cytostatic_drugs_in_sewage_effluents_and_surface_waters_of_Catalonia_N

< 1% match (Internet from 21-Feb-2022)

https://www.researchgate.net/publication/358700233_11th_International_Meeting_Steroids_and_Nervous_System_-_Torino_2022_Abstract_book

< 1% match (Internet from 06-Oct-2022)
https://www.researchgate.net/publication/364118434_Pharmaceutical_and_Microplastic_Pollution_before_and_during_the_COVID-19_Pandemic_in_Surface_Water_Wastewater_and_Groundwater

< 1% match (Internet from 25-Feb-2020)
https://www.scribd.com/document/63624709/USP-32

< 1% match (Internet from 16-Apr-2023)
https://www.science.gov/topicpages/p/potential+ecological+risks

< 1% match (Internet from 14-Jun-2023)
https://www.science.gov/topicpages/i/industrial+wastewater+discharges

< 1% match (Internet from 14-Jan-2023)
https://www.science.gov/topicpages/f/fosfomycin+pharmaceutical+wastewater

< 1% match (Internet from 15-Jul-2023)
https://www.science.gov/topicpages/e/especializadosthe+pharmaceutical+virtual

< 1% match (Manvendra Patel, Rahul Kumar, Kamal Kishor, Todd Mlsna, Charles U. Pittman, Dinesh Mohan. "Pharmaceuticals of Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods", Chemical Reviews, 2019)
Manvendra Patel, Rahul Kumar, Kamal Kishor, Todd Mlsna, Charles U. Pittman, Dinesh Mohan. "Pharmaceuticals of Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods", Chemical Reviews, 2019

< 1% match ("Handbook of Microplastics in the Environment", Springer Science and Business Media LLC, 2022)
"Handbook of Microplastics in the Environment", Springer Science and Business Media LLC, 2022

< 1% match (Internet from 09-Aug-2023)
https://www.deskera.com/blog/impact-of-chemical-manufacturing-on-the-environment/amp/

< 1% match (Internet from 29-Mar-2019)
https://umu.diva-portal.org/smash/get/diva2:1085088/FULLTEXT01.pdf

< 1% match (Internet from 06-Aug-2020)
http://etheses.whiterose.ac.uk/26744/1/Giorgis_202054524.pdf

< 1% match (Internet from 13-Apr-2016)
http://etheses.whiterose.ac.uk/5283/1/S%20R%20Hughes%20-%20Corrected%20PhD%20Thesis%20%28Oct%202013%29.pdf

< 1% match (Internet from 21-Sep-2022)
https://etheses.whiterose.ac.uk/24589/1/Occurrence%20and%20effects%20of%20pharmaceuticals%20in%20estuaries.pdf

< 1% match (Internet from 02-Jul-2020)
http://etheses.whiterose.ac.uk/23960/1/Occurrence%20and%20Effects%20of%20Pharmaceuticals%20in%20Rivers.pdf

< 1% match (Internet from 01-Nov-2022)
https://www.mdpi.com/2079-6382/11/11/1461/htm

< 1% match (Internet from 23-Jan-2023)
https://www.mdpi.com/1660-4601/18/4/1683

< 1% match (Internet from 19-Dec-2022)
https://www.mdpi.com/1420-3049/24/9/1728

< 1% match (Internet from 09-Mar-2023)
https://www.mdpi.com/2079-6382/11/7/862

< 1% match (Muhamad Sharafee Shamsudin, Syahida Farhan Azha, Suzylawati Ismail. "A review of diclofenac occurrences, toxicology, and potential adsorption of clay-based materials with surfactant modifier", Journal of Environmental Chemical Engineering, 2022)
Muhamad Sharafee Shamsudin, Syahida Farhan Azha, Suzylawati Ismail. "A review of diclofenac occurrences, toxicology, and potential adsorption of clay-based materials with surfactant modifier", Journal of Environmental Chemical Engineering, 2022

< 1% match (Internet from 12-Dec-2022)
https://www.space-elements.in/wp-content/uploads/2021/12/Pharmaceutical_Wastewater_Treatment_Technologies_Concepts_and_Implementation.pdf

< 1% match (Rabeea Zafar, Shanza Bashir, Deedar Nabi, Muhammad Arshad. "Occurrence and quantification of prevalent antibiotics in wastewater samples from Rawalpindi and Islamabad, Pakistan", Science of The Total Environment, 2020)
Rabeea Zafar, Shanza Bashir, Deedar Nabi, Muhammad Arshad. "Occurrence and quantification of prevalent antibiotics in wastewater samples from Rawalpindi and Islamabad, Pakistan", Science of The Total Environment, 2020

< 1% match (Internet from 02-Jan-2023)
http://bjp.sagepub.unboundmedicine.com/medline/citation/31254831/Occurrence_spatial_variation_and_risk_assessment_of_pharmaceuticals_and_personal_c

< 1% match (student papers from 19-Jun-2023)
Submitted to Stanmore College on 2023-06-19

< 1% match (Internet from 24-Aug-2023)
https://dokumen.pub/advanced-oxidation-processes-for-micropollutant-remediation-1032162910-9781032162911.html

< 1% match (Internet from 26-Dec-2022)
https://dokumen.pub/environmental-metabolomics-applications-in-field-and-laboratory-studies-to-understand-from-exposome-to-metabolome-1nbsped-0128181966-9780128181966.html

< 1% match (Internet from 09-Apr-2021)

https://dokumen.pub/green-analytical-chemistry-past-present-and-perspectives-9811391041-978-9811391040.html

< 1% match (Internet from 09-Oct-2022)
http://repository.stikim.ac.id/file/21-07-1755.pdf

< 1% match (Internet from 08-Sep-2015)
http://www.epa.gov/ppcp/pdf/Main-Listing-of-Literature-References.txt

< 1% match (O.F.S. Khasawneh, P. Palaniandy. "Occurrence and removal of pharmaceuticals in wastewater treatment plants", Process Safety and Environmental Protection, 2021)
O.F.S. Khasawneh, P. Palaniandy. "Occurrence and removal of pharmaceuticals in wastewater treatment plants", Process Safety and Environmental Protection, 2021

< 1% match (student papers from 19-May-2023)
Submitted to Nottingham Trent University on 2023-05-19

< 1% match (student papers from 25-May-2023)
Submitted to Nottingham Trent University on 2023-05-25

< 1% match ("Non-Steroidal Anti-Inflammatory Drugs in Water", Springer Science and Business Media LLC, 2020)
"Non-Steroidal Anti-Inflammatory Drugs in Water", Springer Science and Business Media LLC, 2020

< 1% match (Internet from 05-Sep-2021)
https://digital.library.unt.edu/ark:/67531/metadc1199423/m2/1/high_res_d/6703499.pdf

< 1% match (Internet from 20-Aug-2021)
https://ourspace.uregina.ca/bitstream/handle/10294/9187/Xin_Xiaying_PHD_ESE_Spring2020.pdf

< 1% match (Internet from 05-Oct-2022)
https://www.aprh.pt/rgci/pdf/rgci_16_3_screen.pdf

< 1% match (Pascale Champagne. "Personal Care Products", American Society of Civil Engineers (ASCE), 2009)
Pascale Champagne. "Personal Care Products", American Society of Civil Engineers (ASCE), 2009

< 1% match (student papers from 20-Jul-2023)
Submitted to University of Northampton on 2023-07-20

< 1% match (Internet from 02-Jan-2023)
https://pubmed.ncbi.nlm.nih.gov/33097270/

< 1% match (Internet from 06-Dec-2022)
https://www.preventionweb.net/files/2298_GEO4ReportFullen.pdf

< 1% match (Internet from 25-Jan-2022)
https://www.scielo.br/j/ocr/a/zWVNJ8YdNrbsSb87yFZKGVC/

< 1% match (Internet from 23-Jul-2023)
https://www.scielo.br/j/alb/a/sWntNZtpdcgdJrXRyRN6xRn/?format=html&lang=en

< 1% match (student papers from 05-Nov-2017)
Submitted to Campbell University on 2017-11-05

< 1% match (Internet from 31-Oct-2022)
https://pharmaegg.com/esomeprazole-magnesium-pellets-22-5-raw-material-testing/

< 1% match (Keisuke Ikehata, Naeimeh Jodeiri Naghashkar, Mohamed Gamal El-Din. "Degradation of Aqueous Pharmaceuticals by Ozonation and Advanced Oxidation Processes: A Review", Ozone: Science & Engineering, 2006)
Keisuke Ikehata, Naeimeh Jodeiri Naghashkar, Mohamed Gamal El-Din. "Degradation of Aqueous Pharmaceuticals by Ozonation and Advanced Oxidation Processes: A Review", Ozone: Science & Engineering, 2006

< 1% match (Tauxe-Wuersch, A.. "Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment", Water Research, 200505)
Tauxe-Wuersch, A.. "Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment", Water Research, 200505

< 1% match (student papers from 17-Sep-2021)
Submitted to University of Mines and Technology on 2021-09-17

< 1% match (Internet from 01-Aug-2022)
https://ircgov.legistar.com/View.ashx?GUID=83A0C98D-94B4-414C-8EFF-C35A57575540&ID=6179811&M=F

< 1% match ("Interaction and Fate of Pharmaceuticals in Soil-Crop Systems", Springer Science and Business Media LLC, 2021)
"Interaction and Fate of Pharmaceuticals in Soil-Crop Systems", Springer Science and Business Media LLC, 2021

< 1% match (student papers from 07-May-2022)
Submitted to American University of Beirut on 2022-05-07

< 1% match (Len Ritter, Keith Solomon, Paul Sibley,. "SOURCES, PATHWAYS, AND RELATIVE RISKS OF CONTAMINANTS IN SURFACE WATER AND GROUNDWATER: A PERSPECTIVE PREPARED FOR THE WALKERTON INQUIRY", Journal of Toxicology and Environmental Health Part A, 1/1/2002)
Len Ritter, Keith Solomon, Paul Sibley,. "SOURCES, PATHWAYS, AND RELATIVE RISKS OF CONTAMINANTS IN SURFACE WATER AND GROUNDWATER: A PERSPECTIVE PREPARED FOR THE WALKERTON INQUIRY", Journal of Toxicology and Environmental Health Part A, 1/1/2002

< 1% match (Rajendran Neha, Srikanth Adithya, Ramesh Sai Jayaraman, Kannappan Panchamoorthy Gopinath et al. "Nano-adsorbents an effective candidate for removal of toxic pharmaceutical compounds from aqueous environment: A critical review on emerging trends", Chemosphere, 2021)
Rajendran Neha, Srikanth Adithya, Ramesh Sai Jayaraman, Kannappan Panchamoorthy Gopinath et al. "Nano-adsorbents an effective candidate for removal of toxic pharmaceutical compounds from aqueous environment: A critical review on emerging trends", Chemosphere,