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MINISTRY OF HEALTH OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY
faculty for foreign citizens' education
department medicinal chemistry

QUALIFICATION WORK

on the topic: **« DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF AZITHROMYCIN BY THIN-LAYER CHROMATOGRAPHY FOR ECOTOXICOLOGICAL MONITORING»**

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ANNOTATION

The work is devoted to the development of the methodology for the detection of azithromycin in wastewater by thin-layer chromatography for possible use in ecotoxicological monitoring. The method developed for identifying azithromycin at a certain range of its concentration in water using thin-layer chromatography makes it possible to detect it in water easily and without selecting mobile phases and developers. The qualification work includes an introduction, a review of scientific and patent literature, two experimental chapters, general conclusions, and a list of references. The work is presented on 40 pages, includes 1 table, 3 figures, 42 sources of literature.

Keywords: Pollutants, Antibiotic Resistance, Azithromycin, Thin-Layer Chromatography, Wastewater, PEC, PNEC.

АНОТАЦІЯ

Робота присвячена розробці методики виявлення азитроміцину в стічних водах методом тонкошарової хроматографії для можливого використання в екотоксикологічному моніторингу. Розроблений метод ідентифікації азитроміцину в певному діапазоні його концентрації у воді методом тонкошарової хроматографії дозволяє легко виявляти його у воді без виділення рухомих фаз і проявників. Кваліфікаційна робота містить вступ, огляд наукової та патентної літератури, два експериментальних розділи, загальні висновки та список використаної літератури. Робота викладена на 40 сторінках, містить 1 таблицю, 3 рисунки, 42 джерела літератури.

Ключові слова: забруднюючі речовини, резистентність до антибіотиків, Азитроміцин, тонкошарова хроматографія, стічні води, PEC, PNEC.

CONTENTS

LIST OF ABBREVIATIONS

EMA	European Medicines Agency
TLC	Thin-Layer Chromatography
PEC	Predicted effect concentration
PNEC	Predicted no effect concentration
DDD	Defined daily doses per 1,000 Inhabitant-Days
DIDs	Defined daily doses per 1,000 Inhabitant-Days

INTRODUCTION

Actuality of subject. Surface and wastewater water pollution with pharmaceuticals is a complex and important problem that requires comprehensive approaches in many areas of human life, such as treatment, farming, crop production, pharmaceutical production and drug circulation. Without paying attention and monitoring to at least one of these industries, it is difficult to achieve the effect of reducing the release of pollutants into water and to stop the inevitable development of polyresistance. The problem does exist, and this can be seen from our research. The high predicted ecological concentration of azithromycin in water, which is many times higher than normal, indicates that surface waters of Ukraine are polluted by it. Contaminated surface water is the main source of drinking water that cannot be completely purified from the antibiotic. Hypothetically, we are treated with azithromycin, drink it with water, and eat it with animal and plant foods. It is not difficult to guess what will happen in a few years of such intense and incessant ingestion of it. The irrational use of azithromycin can lead to another loss of an important antibiotic from a number of the necessary chemotherapeutic drugs due to bacterial resistance to it. To confirm the PEC studies, the method that allows us to determine azithromycin in water has been developed using the available TLC method. Using conventional analytical scales and universal chromatography in thin layers of a sorbent it is possible to identify azithromycin with a water concentration of $\geq$ 30 μg/ml without complex and expensive equipment, such as HPLC or LC/MS/MS.

Purpose of work. The purpose of this qualification work is to develop a technique for the detection of azithromycin in wastewater by thin-layer chromatography for possible use in ecotoxicological monitoring.

Research objectives: To achieve this goal it was necessary to solve the following **tasks:**

- to analyze and summarize the data of literary sources regarding the use of antibiotics of broad-spectrum action and environmental pollution by them;

- to develop a technique for determining azithromycin in wastewater and groundwater by thin-layer chromatography.

The object of the research: a broad-spectrum antibiotic azithromycin

The subject of the research: development of a method for determining antibiotic of macrolide group azithromycin in wastewater and surface water and groundwater by thin-layer chromatography..

Methods of the research*:* thin-layer chromatography (TLC), calculation of PEC and PEC/PNEC ratio indicators, analysis of scientific sources.

The practical value of the results. A preparative method for the detection of antibiotic azithromycin in wastewater by thin-layer chromatography was developed for possible use in ecotoxicological monitoring.

Approbation of the research results. The results of the work were presented in the form of an oral report at a meeting of the Student Scientific Society of the Department of Medicinal Chemistry within the framework of the XXIX International Scientific and Practical Conference of Young Scientists and Students "Topical Issues of Creating New Drugs" (April 19-21, 2023, NUPh, Kharkiv). The results of the conferences are presented in the abstracts:

Development of a method for the determination of azithromycin by thin layer chromatography for ecotoxicological monitoring / Sych I.V.; Ibtissam Farid, Rakhimova M.V., Perekhoda L.O. // Topical issues of creating new drugs: materials of the XXIX International Scientific and Practical Conference of Young Scientists and Students (April 19-21, 2023, Kharkiv) - Kharkiv: NUPh, 2023. - 121 p.

The structure of the work. The qualification work includes an introduction, a review of scientific and patent literature, two experimental chapters, general conclusions, and a list of references. The work is presented on 40 pages, includes 1 table, 3 figures, 42 sources of literature.

CHAPTER 1
THE STUDY OF SURFACE WATER POLLUTION WITH ANTIBIOTICS IN WORLD

Review of literature

1.1 . General characteristics, classification and mechanism of action of antibiotics

The word "antibiotics" comes from the Greek anti ("against") and bios ("life"). Antibiotics are chemotherapeutical drugs, which are produced by microorganisms, some plants and animals as well as their synthetic analogs that have the ability to inhibit the growth of infectious agents and delay the development of malignant tumors. Antibiotics are drugs that either destroy bacteria or prevent their reproduction [1,2,3]. Antibiotics that kill bacteria are called «bactericidal» and the ones that stop the growth of bacteria are called «bacteriostatic». Antibiotics should only uses when absolutely necessary, because:

- There is increasing resistance of bacteria to treatment.
- Antibiotics may have serious adverse effects in some people.

CLASSIFICATION OF ANTIBIOTICS

1. The classification according the mechanism of action of antibiotics to the bacterial cell

Inhibitors of bacterial cell wall synthesis

Inhibitors of transpeptidation reaction, halting peptidoglycan synthesis (Penicillin, Cephalosporins, Bacitracin, Vancomycin, Cycloserine).

Antibacterial agents that disrupt the structure of cytoplasmatic membrane.

Polymyxins violates the osmotic resistance of cytoplasmatic membrane. Gramicidins increase the permeability of the bacterial cell membrane. Polyene antimycotics **(**Amphotericin B, nystatin, and natamycin) bind to ergosterol in the

fungal cell membrane and promote leakiness which may contribute to fungal cell death.

Inhibitors of bacterial protein synthesis

Aminoglycosides and Tetracyclines bind to the bacterial 30S ribosomal subunit. Macrolides and Chloramphenicol bind to the bacterial 50S ribosomal subunit.

Inhibitors of transcription and synthesis of nucleinic acids (DNA and RNA).

Rifamycins inhibit of DNA-dependent RNA synthesis

2. The classification according to the spectrum.

The spectrum means the number of the organisms affected by the same drug. There are narrow and wide spectrum antibiotics. The wide spectrum antibiotics affect several types of bacteria and fungi and it is usually used where the specific type of the microorganism is unknown.

3. The classification is according to the type of the action of antibiotics.

It could be bactericidal or bacteriostatic. The bactericidal antibiotics kill the harmful microorganism while the bacteriostatic ones tend to slow down their growth and give the body the chance to use its immune system against the microorganisms.

4. The classification of antibiotics according to the route of administration of the drug.

According to the route of administration the drug devided on oral, injection, topical application.

5. The classification of antibiotics according to the origin.

According to the origin the drug devided on natural, semisynthetic, synthetic compounds.

6. The classification according the chemical structure

Antibiotics of heterocyclic structure: β-lactams (penicillins, cephalosporins, carbapenems, monobactams);

Antibiotics of alicyclic structure: tetracycline;

Antibiotics of aromatic structure: chloramphenicole;

Antibiotics of heterocyclic structure: Aminoglycosides, Macrolides, Lincozamides, Poliene antibiotics of glycoside structure (nystatin, amphotericin);

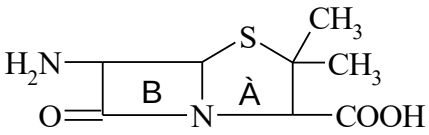
Anzamicins (Rifamycins);

Antibiotics of polypeptide structure (Gramicidins, polymixins);

Antitumor antibiotics.

Antibiotics of heterocyclic structure

This group includes penicillins, cephalosporins, carbapenems and monobactams. All are B-lactam compounds, so named because of their unique four-membered lactam ring [4]. Penicillins were discovered by Flemming in 1928. In the base of the structure of penicillin is 6-aminopenicillanic acid which consist of thiazolidine ring and B-lactam cycle.



A thiazolidine ring is attached to a B-lactam ring that carries a secondary amino group (RNH-). Substituents can be attached to the amino group. Molecules of penicillins contain three asymmetric atoms of carbon. Structural integrity of the 6-aminopenicillanic acid nucleus is essential for the biological activity of these compounds, which are unstable in acidic and alkaline medium. Hydrolysis of the beta-lactam ring by beta-lactamases yields penicilloic acid, which lacks antibacterial activity. Semisynthetic penicillins (aminopenicillins) are acetylated derivatives of the 6-aminopenicillanic acid. The general formula of penicillins:

The mechanism of action of penicyllins are connected with the disruption of synthesis of cell wall during mitosis, which are competitive inhibitors of transpeptidases (enzymes, catalyzing a cross linking of peptidoglycan). The mechanism of pharmacological action is bactericidal. There are now different categories of penicillin but the core of these categories is the natural penicillin or

what is known the penicillin G. Natural penicillin is effective against most of the gram positive microorganisms like streptococcus and staphylococcus. It is also effective against some gram negative bacteria and it is mainly used for treatment of oral cavity infections.

The second category of penicillin antibiotic is penicillinase resistant antibiotics. Oxacillin and cloxacillin are prominent examples for this group. This group does not target a wide range of microorganisms like the natural penicillin but it targets a specific group of bacteria which produces the beta lactamase enzyme. The lactamase producing bacteria are resistant to natural penicillin.

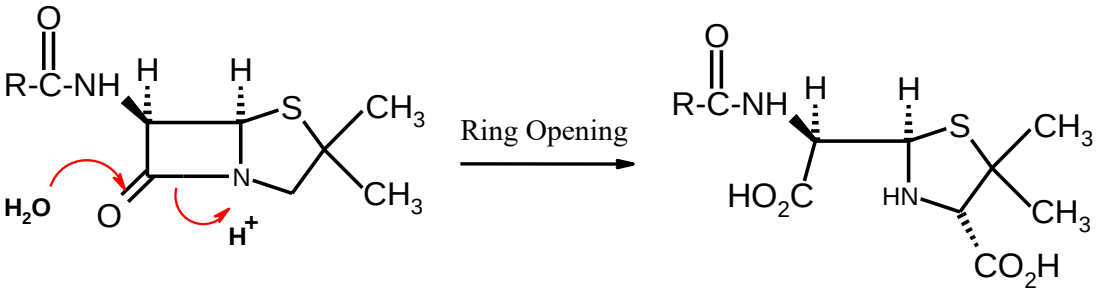
The third category of penicillin antibiotics is the aminopenicillins. Amoxicillin is the best example for this group and it is widely used today. This is group is very potent against gram negative bacteria like E-coli and Hemophelus influenza. The best feature of this group of penicillin antibiotics is that they can tolerate the gastric acid. This means that they could be administered orally without being degraded through the acid medium of the stomach. The aminopenicillins, ampicillin and amoxicillin, have identical spectrums and activity, but amoxicillin is better absorbed orally [5]. Carboxypenicillins (Ticarcillin) and ureidopenicillins (Piperacillin) have a antipseudomonal activity. A beta-lactamase inhibitors are drugs given in conjunction with a beta-lactam antibiotic. Although the inhibitor does not usually have significant antibiotic activity on its own, it inhibits activity of beta-lactamase, a protein that confers resistance of beta-lactam antibiotics to bacteria. Beta-lactamase inhibitors in clinical use include clavulanic acid and its potassium salt (usually combined with amoxicillin or ticarcillin), sulbactam and tazobactam. Natural and semisynthetic penicillins are white crystalline powders, odorless, bitter taste. Penicillins almost are not metabolized in the organism and are excreted by the kidneys. The exception is oxacillin, which is metabolized to 5-hydroxymethylderivaive, which has a moderate antibacterial activity.

Shortcomings of Penicillins.

1. Ring strain of the β- lactam ring

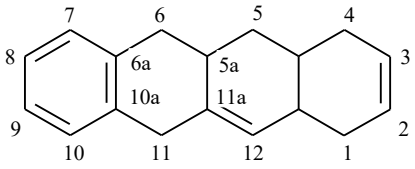
2. The ring strain is significant with contributions from angle and and torsional strain.

Ring opening relieves this strain



Antibiotics of alicyclic structure

Tetracyclines are broad-spectrum bacteriostatic antibiotics that inhibit protein synthesis. Once inside the cell, tetracyclines bind reversibly to the 30S subunit of the bacterial ribosome. They are active against many gram-positive and gram-negative bacteria, including anaerobes, rickettsiae, chlamydiae, mycoplasmas and against some protozoa (eg, amebas). The basis of their chemical structure is partially hydrogenated tetracene ring (naphthacene).



All of tetracyclines are produced by Streptomyces rimosus and Streptomyces aureofaciens. According to origin all tetracyclines are divided to:

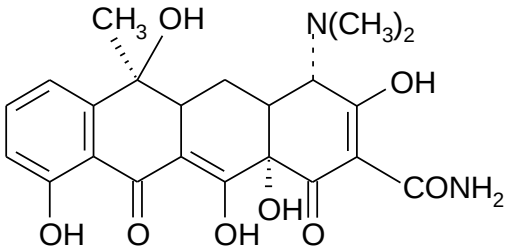
Natural tetracyclines – tetracyclines and oxytetracycline;

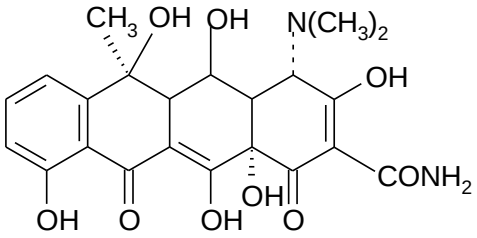
Semisynthetic tetracyclines – morphocycline, monocycline, chlortetracycline, doxycycline, metacycline.

Classification:

1. Short acting (half -life 6 hours)-Tetracycline, chlortetracycline, oxytetracycline

Tetracyclini hydrochloridum **Oxytetracyclinum**



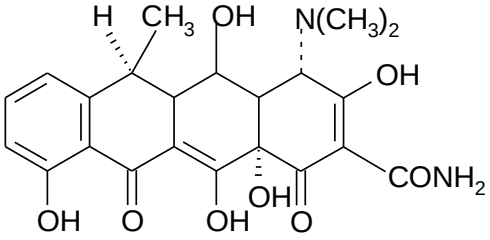


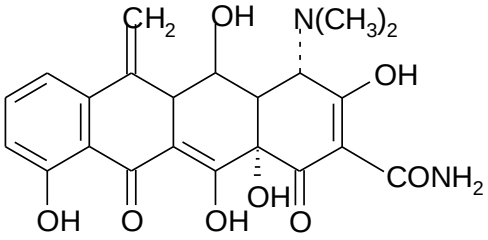
2. Intermediate acting(half -life 16 hours)- Demeclocycline

3. Long acting (half- life-18-24 hours)- Doxycycline and monocycline are more lipophilic and most active.

Doxycyclini hydrochloridum **Methacyclini hydrochloridum**

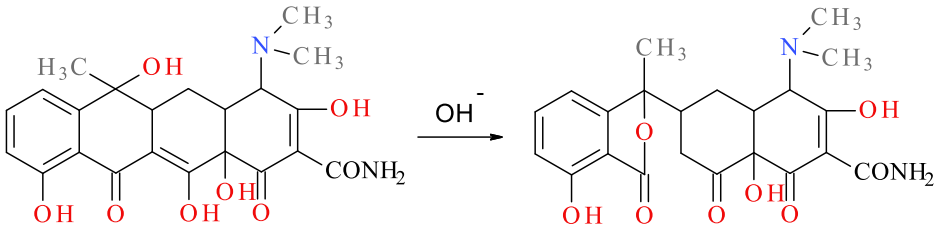
(Vibramycin) (Rondomycin)





Tigecycline - newest, longest acting (half life-36 hours).

Free tetracyclines are yellow crystalline amphoteric substances of low solubility. They are available as bases and hydrochlorides, which are more soluble. Them solutions in hydrochloric acid rotates the plane of polarized beam to the left. Due to dimethyl aminogroup in position 4 shows basic properties of tetracyclines, and acid properties are provided by phenolic hydroxyl at position 10 and enol hydroxyls in positions 3 and 12. Most acid properties has hydroxyl group in position 3 (pKa = 3,3). Tetracyclines at certain pH values can undergoes to reversible isomerization associated with changes in the configuration of asymmetrical center at the atom C-4. In aqueous acidic solutions of tetracycline an equilibrium is established during the day:



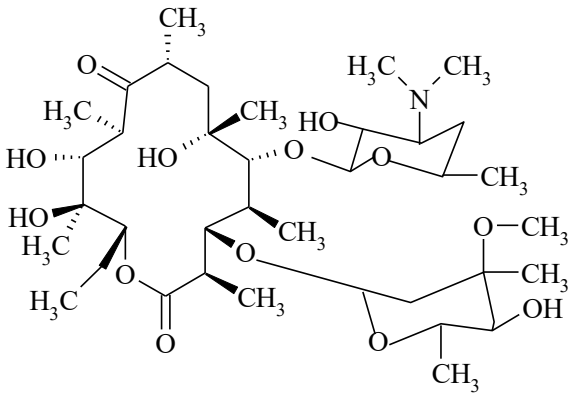
Tetracycline derivatives which contain OH-group at C-6 (tetracycline, oxytetracycline, chlortetracycline) are easily dehydrated in weak acidic medium and are converted to inactive unhydroderivatives. Unhydrotetracyclines have a nephrotoxic effect. Therefore, the most safe tetracyclines are those, which can not be dehydrated (metacycline, doxycycline). Tetracyclines chelate divalent metal ions, which can interfere with their absorption and activity. Absorption of Doxycycline and Minocycline - 100%. Food does not interfere with absorption of Doxycycline or Minocycline. Absorption is decreased by concurrent administration of dairy products (milk etc) aluminum hydroxide, calcium, magnesium, and iron salts, and bismuth subsalicylate- due to chelation of divalent or trivalent cations. All tetracyclines are concentrated in the liver and are exc. in intestine via bile and excreted in urine and feces in unchanged form.

Macrolides

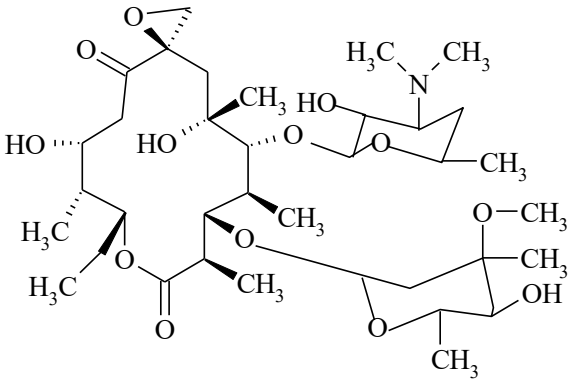
The macrolides are a group of antibiotics whose activity stems from the presence of a macrolide ring, a large macrocyclic lactone ring to which one or more deoxy sugars are attached. 12-17 Carbon atoms may be present in the molecule of macrolides. Macrolides are protein synthesis inhibitors. Macrolide antibiotics act by binding reversibly to the subunits 50S of the bacterial ribosome. This action is mainly bacteriostatic, but can also be bactericidal in high concentrations. The lactone rings are usually 14-membered (Erythromycin, Roxitromycin, Oleandomycin, Clarithromycin, Dirithromycin), 15-membered (Azithromycin), or 16-membered (Spiramycin, Midecamycin, Josamycin).

Food enterferes with absorbtion of macrolides (ecpecially erytrhromycin). Macrolides are bound with proteins and are metabolized in the liver by CYP 450. Metabolites are excreted with bile.

Erithromycinum



Oleandomycinum

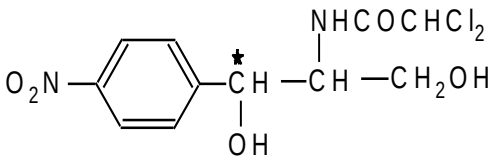


Antibiotic macrolides are used to treat infections caused by Gram positive bacteria, Streptococcus pneumoniae and Haemophilus influenzae infections such as respiratory tract and soft tissue infections. Beta-hemolytic streptococci, pneumococci, staphylococci, and enterococci are usually susceptible to macrolides. Macrolides have been shown to be effective against mycoplasma, mycobacteria, some rickettsia, and chlamydia.

Antibiotics of aromatic structure

Chloramphenicol is a bacteriostatic antimicrobial originally derived from the bacterium Streptomyces venezuelae.

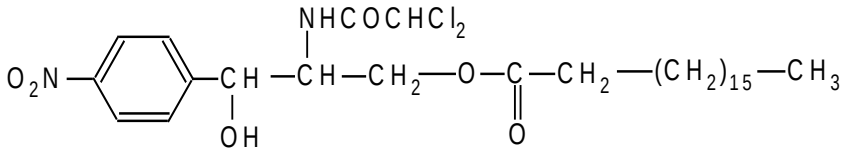
Chloramphenicole *(*Chloramphenicolum)



Chloramphenicol is a bacteriostatic broad-spectrum antibiotic that is active against both aerobic and anaerobic gram-positive and gram-negative organisms. It is active also against ricketsiae.

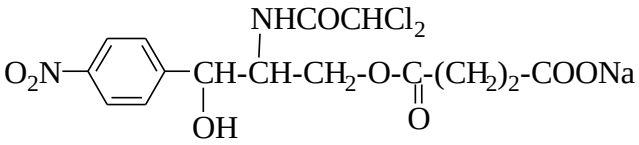
Chloramphenicol stearate (Chloramphenicoli stearas)

(Laevomycetini stearas)



Chloramphenicol succinate (Chloramphenicoli succinas)

(Laevomycetini succinas solubile)



Chloramphenicol molecule has two asymmetric carbon atoms and therefore may exist four spatial isomers: D-and L-threo-, D-and L-erythro-. Threo-and erythro-isomers differ in the spatial arrangement of functional groups.

Chloramphenicol and chloramphenicol stearate are white with a yellowish or yellowish-green tint powders, Chloramphenicol is soluble in alcohol but poorly soluble in water, chloramphenicol stearate is badly soluble in ethanol. Chloramphenicol has a bitter taste and chloramphenicol stearate is tasteless. Chloramphenicol succinate, which is used for parenteral administration, is highly water-soluble. Therefore, the intravenous preparation of chloramphenicol is the succinate ester, because pure chloramphenicol does not dissolve in water. This creates a problem: Chloramphenicol succinate ester is an inactive prodrug and must first be hydrolysed to chloramphenicol. It is mainly metabolized by glucuronidation (3-glucuronide is formed) or by reduction of nitrogroup to amino group and dehalogenation[6-7]. Active chloramphenicol (about 10% of the total dose administered) and its inactive degradation products (about 90% of the total) are eliminated in the urine. A small amount of active drug is excreted into bile and feces. Resistance of microorganisms to chloramphenicol is developed due to the

fact that microorganisms produce a specific enzyme chloramphenicol-transacetylaze. This enzyme acetylates chloramphenicol with the participation of acetyl-CoA as a donor of acetyl groups. The most serious side effect of chloramphenicol treatment is aplastic anaemia. It is common for chloramphenicol to cause bone marrow suppression during treatment, toxicity for newborn infants (gray baby syndrome). Adults occasionally develop nausea, vomiting and diarrhea.

1.2. The study of surface water pollution with antibiotics in world

Antibiotics are one of the important pharmaceuticals since they are widely used for treating infectious diseases of humans and animals, as well as the feed additives to promote the growth of farming animals. Clinic evidences have revealed that antibiotics could not be thoroughly metabolized. Most of them were excreted through feces and/or urine as the mixtures of drugs and their metabolites, and then discharged to municipal wastewater treatment plants (WWTPs) [8]. These compounds could not be eliminated completely via the traditional treatment process in WWTPs. The residues of them might contaminate the aquatic environment system [9], which may pose adverse effects on ecosystems and human health owning to the increase of allergies in humans and the spread of antibiotic resistance [10]. Due to these adverse effects, it is important to estimate the consumption and emission levels of antibiotics in a specific region or country. In the literature, a number of studies did attempt to estimate the consumption of antibiotics by different models [11]. However, these studies all had fundamental limitations due to lack of basic information, sales, and/or census data.

Pharmaceuticals and their derivatives are among the largest pollutants of the environment, and in particular water. The excessive and irrational consumption of medicines by people, waste from pharmaceutical plants, as well as wastewater from hospitals, veterinary clinics, pharmaceutical enterprises, and livestock farms are the main pollutants of surface water. Among the large number of drugs that get to water and soil, a significant share is occupied by antibiotics. Most of them are excreted unchanged by the human body, preserving their antibacterial properties;

active molecules get to aquatic ecosystems and affect them. In addition, antibiotics cannot be completely removed from wastewater during its treatment at sewage treatment plants. They penetrate surface waters, seep through the soil into underground ones, pollute all natural water storage facilities, and then get to drinking water from them.

The European Medicines Agency (EMA) noted that the impact of active pharmaceutical substances on the environment is an urgent and new environmental problem that requires proper monitoring and urgent solutions [12]. There is still no detailed assessment of how different classes of drugs affect the environment, but on the example of antibiotics, there are enough data on their negative direct impact on the environment.

Environmental pollution from pharmaceuticals, such as antibiotics, is another big problem. Antibiotic resistance is recognized as one of the most global threats to humanity [13]. According to the WHO forecast, as early as 2050, the number of deaths due to this problem in the world may reach 10 million people per year, and the annual losses for the global economy will exceed 100 trillion US dollars.

Antibiotic resistance is the development of protective properties to antibacterial drugs by microorganisms. Most often, it occurs due to the irrational and improper use of antibacterial drugs by people; the reason for it is free access to these drugs, their use without a doctor’s prescription, non-compliance with the course of treatment (the use longer than the prescribed course, use of other people’s antibiotics, failure to complete the necessary course of administration), as well as a widespread use of cheaper and not always high-quality genetic antibacterial drugs in developing countries. In Ukraine, there is a state problem with the release of antibiotics without a prescription although they are legally listed as prescription drugs, but we understand the fact that, like most other prescription drugs, they are freely sold in pharmacies.

Especially dangerous is the mass application of antibacterial drugs in animal husbandry for the treatment, control of morbidity and prevention of infectious

diseases, as well as the growth stimulation. According to the EMA, in 2018, 6.500 tons of antibiotics of various classes were sold in 31 EU countries for veterinary needs alone. The same antibiotics as for humans are often used to treat animals. When using animal products, antibiotic substances together with resistant strains get to the human body, and as known, constant exposure to small concentrations of antibiotics leads to resistance of the bacterial flora. In addition, some resistant strains of bacteria have a zoonotic potential, that is, they can spread between animal and human populations. The use of reserve antibiotics, such as polymyxins, cephalosporins of the third and fourth generation, in agriculture is of particular concern since this group of antibiotics is considered critical for medicine they are drugs of “last hope”. Only such a drug as Colistin, an antibiotic from the reserve group, has been added to animal feed without control for many years in many countries of the world. For example, in China, the MCR-1 resistance gene to colistin has already been discovered in animals and humans [14]. Moreover, some strains of Klebsiella spp. (K. pneumoniae, K. oxytoca, K. aerogenes) retain high sensitivity only to Colistin (98%) [15]. Taking this into account, antibiotics continue to be used on a global scale in animal husbandry in all countries of the world despite the fact that later infections that are usually easily treated with a course of antibiotics can become fatal for humanity.

A great problem on the part of doctors is the use of empirical antibiotic therapy without taking into account the microbiological identification of pathogens and determination of the sensitivity profile to antibiotics, inadequate prescribing and dosage, the lack of educational work with patients (in particular, the necessity to complete a full course of antibiotic therapy, which causes incomplete eradication of the pathogen).

Wastes from people, animals, pharmaceutical plants, hospitals and preventive institutions contain antibiotics and their decomposition products; they pollute the environment, getting into water and soil. In many rivers around the world the concentration of antibiotics is several times, and in the worst cases even 300 times, higher than the permissible norm. It is most often observed in low-

income countries in Africa and Asia. Only in the Kenyan rivers the concentration of antibiotics exceeds the indicator, which is 100 times higher than normal. The level of drugs is so high that no river animals can survive [16]. The problem lies in the technology of wastewater treatment, which often does not meet all the necessary standards. Countries with cheap production, such as India and China, are part of the global pharmaceutical market, and do not always adhere to the necessary rules for purification and recycling of recoverable resources. It contributes to a large share of environmental pollution with pharmaceutical waste. Due to the presence of antibiotics in water bodies, bacteria that live in water and in river dwellers become resistant to these drugs. When humans and animals come into contact with water and river products, resistant microbes enter their bodies and exchange genetic information about resistance with other bacteria, in particular with pathogens of severe infectious diseases. Thus, antibiotics from water gradually lead to the transformation of ordinary bacteria into resistant pathogens of deadly diseases, such as pneumonia, tuberculosis, syphilis, meningitis, sepsis, etc. As a result, in the near future, humanity risks being left without effective means of fighting infections.

Azithromycin belongs to the checklist of substances to be monitored throughout the European Union in the field of water policy (Directive 2015/495 / EC of March 20, 2015) as its content above 90 ng/L in water poses a significant risk to the aquatic environment throughout the European Union [17]. Azithromycin is an antibiotic of the macrolide group, it is characterized by a wide spectrum of activity against Gram-positive and Gram-negative bacterial pathogens, used to treat infections, most often those that cause middle ear infections, sore throat, pneumonia, typhoid fever, bronchitis and sinusitis (mild to moderate severity). In recent years, it has been widely used mainly in the treatment of bacterial infections in children and in the treatment of children with weakened immune systems [18]. Azithromycin is taken by people more often than other antibiotics. It has a convenient mode of administration (once a day), a short course of treatment (3-5 days) due to its long half-life and a small number of adverse reactions (these are

mainly gastrointestinal disorders and hypersensitivity reactions). Indeed, the antibiotic does not require any difficulties in its use despite the fact that it is an important and relatively new chemotherapeutic drug in the range of broad-spectrum antibiotics. Another impetus for its widespread use was the COVID-19 pandemic. Azithromycin is known to have documented anti-inflammatory and immune regulatory effects, which led to its prescription to patients with Covid at the beginning of the pandemic when many existing drugs were used off-label. Although the effectiveness of azithromycin when combined with hydroxychloroquine has not been confirmed by research, as evidenced by clinical data, people continue to use azithromycin for viral infections [19]. Note that broad-spectrum antibiotics, including azithromycin, are most often subject to polyresistance since they neutralize a large number of bacteria, as opposed to narrow-spectrum ones that affect certain populations. The popularity of Azithromycin in recent years and its widespread use during the pandemic was the reason for its choice as the object of our research.

Conclusions to Chapter 1

1. The presence of increasing amounts of antibiotics in water and soil poses a potential threat to all microorganisms in these environments. In addition, environmental contamination with antibiotics is one of the causes of the formation of antibiotic resistance.
2. Therefore, the development of sensitive techniques for the determination of antibiotics in wastewater and groundwater for the purposes of ecotoxicological monitoring aimed at reducing the negative impact of pharmaceutical substances present in water on the environment and human health is an urgent problem.

CHAPTER 2

ASSESSMENT OF CONSUMPTION AND POLLUTION OF SURFACE WATERS IN UKRAINE BY THE MACROLIDE ANTIBIOTIC AZITHROMYCIN

2.1. Antibiotics – Ecotoxicological effects

Pharmaceuticals are one of the largest pollutants of the environment, including water. A significant portion of the antibiotic dose is excreted by the human body unchanged, meaning that the antibacterial properties of the molecule remain largely intact, and thus affect the state of the ecosystem. The more people consume and use medicines in their lives, the more they are produced by the pharmaceutical industry and the more they end up in water and soil. Another challenging problem is that antibiotics cannot be completely removed from wastewater during the process of its treatment at sewage treatment plants[20]. They penetrate into natural water bodies and from there into drinking water sources. The high ecological risk of antibiotics in the aquatic environment has been regarded as a growing concern all over the world. Antibiotics, as active molecules, are specially used to fight against pathogenic bacteria. However, in the environment non-targeted organisms are unavoidably exposed to these residues and their metabolites. The primary producers and decomposers, which are essential for a sustainable ecosystem, are vulnerable to the presence of these emergent contaminants. Consequently, antibiotics can disrupt in the aquatic environment many vital ecosystem processes. These potential ecotoxicological effects are difficult to predict, especially in complex matrices. The antibiotics' acute or chronic ecotoxicity has been assessed by several standard ecotoxicity assays that were done on different trophic levels organisms, such as bacteria, algae, invertebrates, and fish. The antibiotics that are used in the veterinary and poultry

industry increase the reluctant mechanism of bacterial strains to survive under the antibiotic stress thus resulting in the evolution of multidrug-resistant bacteria. These resistant strains of bacteria are also reported both in the hospital environment but also in the aquatic ecosystems as well as the soil environment. Studies on Bifidobacterium, Clostridium, Escherichia coli, Enterococcus and Lactobacillus clearly showed that their structural composition and metabolic activities are severely affected by antibiotics as these compounds play a crucial role in the ecology change particularly on the niche where the genetic exchanges take place. The overuse of antibiotics leads to a number of complex and global problems that require costly and time-consuming solutions. First of all, this includes such a painful topic as antibiotic resistance. The modern pace of life forces many people to resort to self-medication, and it is usually not always balanced and responsible, especially with regard to antibiotics. Irrational human consumption and improper use of antimicrobial drugs in animal husbandry, medicine, veterinary medicine, crop production, food technology, and poor treatment of industrial waste from pharmaceutical plants leads to contamination of groundwater and surface water, which are the main sources of drinking water. Consumption of contaminated water is one of the many steps that lead to multidrug resistance, i.e., bacterial resistance to multiple antibacterial agents. Antibiotics are not found in high concentrations in water, but even a minimal amount of them can build resistance in bacteria and transfer resistance genes to many other species.

2.2. Azithromycin as an object of research

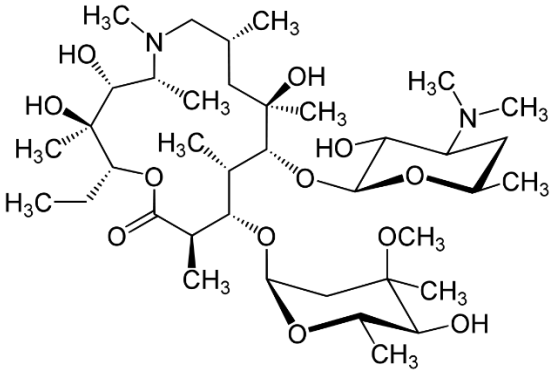
The study object:

Azithromycin, a antibiotic of the macrolide group [21]. The antibiotic is characterized by a broad spectrum of activity against gram-positive and gram-negative bacterial pathogens and is used to treat infections, most often those that cause middle ear infections, sore throat, pneumonia, typhoid fever, bronchitis and sinusitis (mild to moderate). Azithromycin is an acid-stable antibiotic, so it can be

taken orally with no need of protection from gastric acids. It is readily absorbed, but absorption is greater on an empty stomach. Time to peak concentration (T_{max}) in adults is 2.1 to 3.2 hours for oral dosage forms. Due to its high concentration in phagocytes, azithromycin is actively transported to the site of infection. During active phagocytosis, large concentrations are released. The concentration of azithromycin in the tissues can be over 50 times higher than in plasma due to ion trapping and its high lipid solubility. Azithromycin's half-life allows a large single dose to be administered and yet maintain bacteriostatic levels in the infected tissue for several days.

Formula $C_{38}H_{72}N_2O_{12}$ Molar mass 748.996 $g \cdot mol^{-1}$

Azithromycin



(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-15-oxo- 11-{[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy}-1-oxa-6-azacyclopentadec-13-yl 2,6-dideoxy-3C-methyl-3-O-methyl-α-L-ribo-hexopyranoside

Following a single dose of 500 mg, the apparent terminal elimination half-life of azithromycin is 68 hours. Biliary excretion of Azithromycin, predominantly unchanged, is a major route of elimination. Over the course of a week, about 6% of the administered dose appears as an unchanged drug in urine.

Azithromycin is partially biotransformed in the liver with the participation of cytochrome P-450 (demethylation, hydroxylation), and 50% of the dose is excreted unchanged (Fig. 2.1).

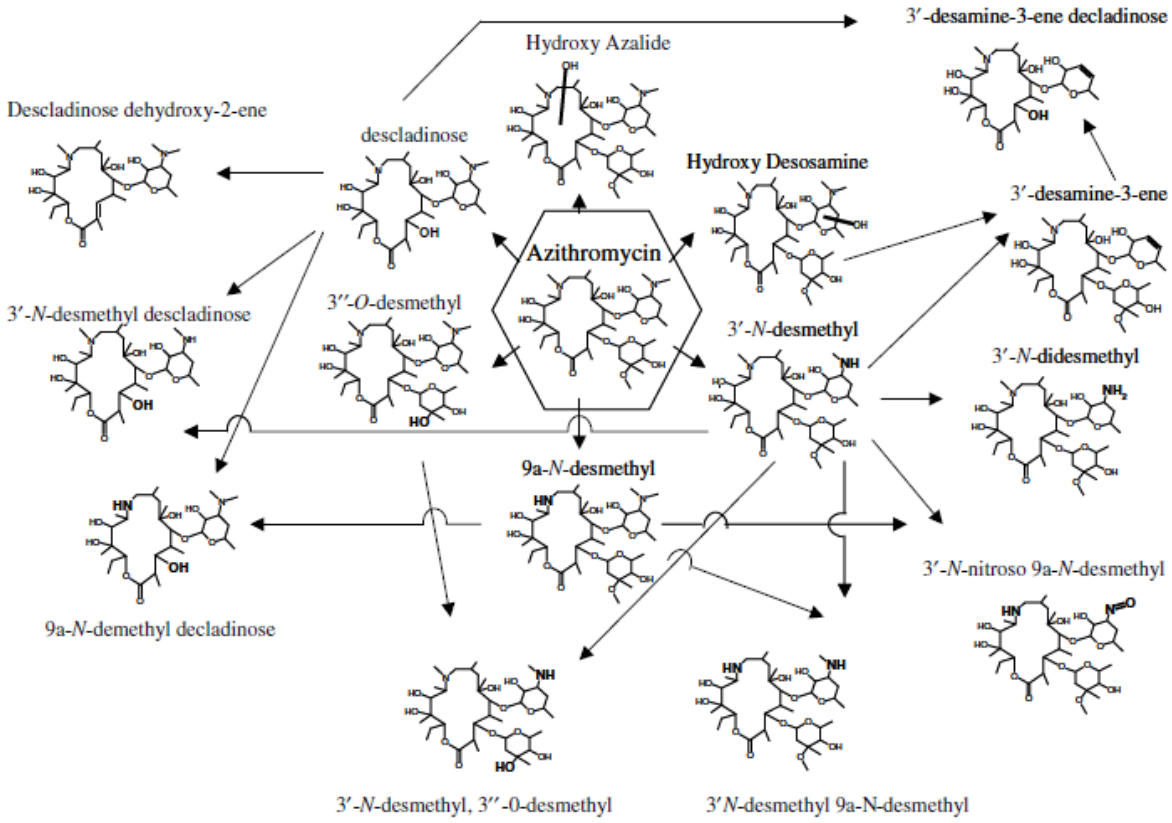


Fig. 2.1. Scheme of azithromycin metabolism

In recent years, it has been widely used mainly in the treatment of bacterial infections in children and in the treatment of children with weakened immune systems. It has well-documented anti-inflammatory and immune regulatory effects, which has led to its frequent use in clinical practice in COVID infection [22]. It has proven to be quite safe and, apart from mild gastrointestinal side effects, is usually well tolerated, which is why it is often prescribed by doctors and, unfortunately, often used for self-medication by patients. In addition, the short course of treatment (3-5 days) and the convenience of taking it (once a day) is another attractive reason for self-medication. It is interesting to note that due to the prolonged maintenance of high concentrations of Azithromycin in tissues and a

correspondingly long half-life (36-76 hours), which results in prolonged action of the drug, the incidence of resistance is much higher than that of other macrolides, such as Erythromycin. This is due to the long-term persistence of Azithromycin in the body in low concentrations that are not able to inhibit the growth of microorganisms, but can nevertheless cause mutations that contribute to the development of resistance [23]. The popularity of Azithromycin in recent years and its widespread use during the COVID-19 pandemic led to its selection as the object of my research.

2.2 Assessment of surface water pollution in Ukraine

The expansion and deepening of the concept of „ecology" led to the emergence of new independent scientific fields. These directions are in contact with each other: they have a common methodology, a conceptual apparatus, research objects and they use the same scientific results to solve their own specific problems. As an example, the chemical ecology studies the chemical changes under the influence of environmental factors. The central task is to study the behavior of anthropogenic substances: concentration in the environment, accumulation processes in the environment. The sphere of interest includes chemical processes in the environment due to changes caused by human activity. The close connection between ecological chemistry and ecotoxicologists is expressed in the fact that both fields use methods and techniques of chemical and physico-chemical analysis.

Antibiotic pollution is becoming an increasingly serious threat in different regions. The distribution of antibiotics in water sources varies significantly in time and space, corresponding to the amount of antibiotics used locally. The main source of this contamination in the aquatic environment is wastewater from antibiotic manufacturers, large scale animal farming, and hospitals. In response to the excessive antibiotic contamination in the water environment globally, environmentally friendly alternatives to antibiotics are being developed to reduce their use. Furthermore, researchers have developed various antibiotic treatment techniques for the degradation of antibiotics, such as physical adsorption, chemical

oxidation, photodegradation, and biodegradation. Among them, biodegradation is receiving increasing attention because of its low cost, ease of operation, and lack of secondary pollution. Antibiotic degradation by enzymes could become the key strategy of management of antibiotics pollution in the environment in future. Special attention is paid to their degradation by various enzymes. The adverse effects of the pollutants and need for more effective monitoring and mitigating pollution are also highlighted. Since Fleming discovered penicillin in 1929, hundreds of other antibiotics have been synthesized, which are being increasingly used to treat infections in humans and animals. Inexpensive and effective antibiotics have become the preferred antibacterial drugs used by pharmaceutical and farming industries to inhibit the growth of bacteria and eliminate pathogens. In the aquaculture industry, antibiotics are used extensively as drugs to prevent bacterial infections and parasitic diseases .Only a small portion of the antibiotics in aquatic products are actually absorbed, with most being discharged into the environment, resulting in antibiotic residues in aquaculture areas in discharged wastewaters and accumulated in the surrounding sediments through adsorption (Fig. 2.1)

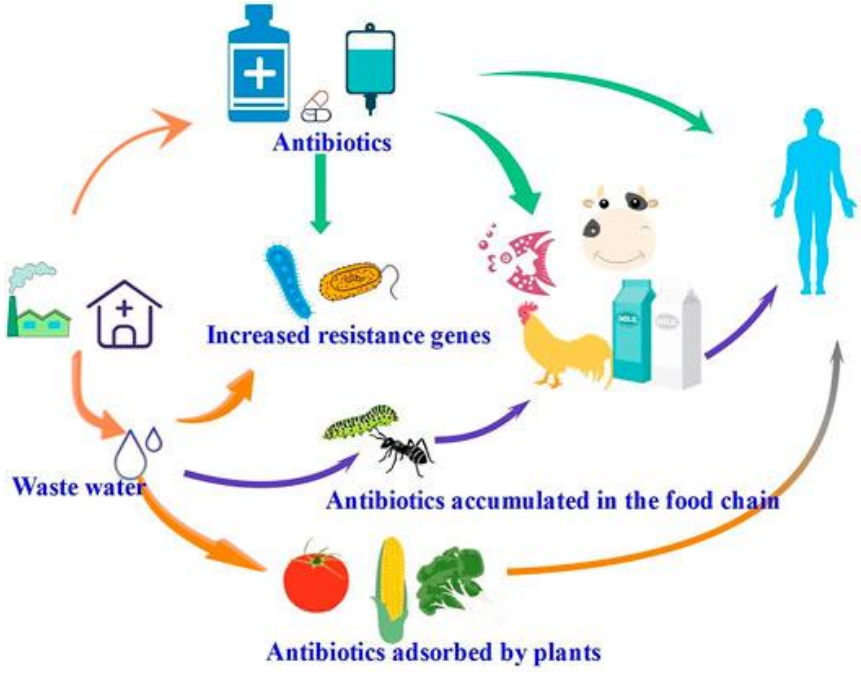


Fig. 2.1. Result antibiotic residues in aquaculture areas.

In livestock farming, antibiotics are important for the prevention of infectious diseases and their treatment as well as for promoting the growth of livestock. Antibiotics applied to livestock and poultry are not fully absorbed, with most being excreted into the environment through animal feces or urine. Residual antibiotics enter rivers and lakes through wastewater and accumulate in soil, where they are taken up by plants and animals.

The subject of ecotoxicological research is the establishment of the link between the dynamics of chemicals and the development of communities of living organisms in ecosystems. Unlike ecological chemistry, the objects of ecotoxicological research refer not only to anthropogenic substances, but also to natural substances, through which their interaction on living communities takes place, and to deciphering the role and importance of organisms in transport processes, circuitry and reducing or increasing the toxicity of chemicals. A common feature of toxicology and ecotoxicology is that both fields of research use the methodology, the toxicological conceptual apparatus and the fundamental bases regarding the toxicity.

Toxicology is mostly a compartment of medicine that studies the physical, chemical properties of poisons (harmful and toxic substances), the mechanisms of their action on the human body and develops methods of diagnosis, treatment and prevention of poisoning [24].

In order to assess the pollution of surface waters by medicinal products, such an indicator as Predicted Effect Concentration (PEC) is used. In accordance with the Guideline on the environmental risk assessment of medicinal products for human use adopted by the European Medicines Agency (EMA) [28], the following formula is used:

EMA formula

$$PEC_{surfacewarter} = \frac{DOSE_{ai} \cdot F_{pen}}{WASTERW_{inhab} \cdot DILUTION}$$

DOSEai - the maximum daily dose of the drug consumption in a particular region per capita (mg/person * number of days);

Fpen - the market penetration share;

WASTEWinhab - the volume of wastewater per inhabitant per day (L/person*day), regional data;

DILUTION - the dissolution rate (regional data).

This formula allows us to predict the impact of a pharmaceutical substance on the environment and is usually used to assess the environmental profile of new substances.

To determine PEC of medicines existing at the pharmaceutical market and entering surface water through the sewage system, the following formula is used. PEC is calculated based on the known volume of the drug consumption in a particular region, the volume of wastewater per person, and the degree of the drug elimination [25]

$$PEC_{surfacewater} = \frac{M_{ai} \times F_{excr} \times 10^9}{WASRERW_{inhab} \times N \times DILUTION \times 365}$$, де

$PEC_{surfacewarter}$ - predicted environmental concentration (PEC) of APIs in surface waters, (μg/l);

F_{excr} - the degree of API excretion from the body as a result of physiological excretion, (μg/l);

M_{ai} - sales of the drug in terms of APIs per year, tons;

N - number of residents in the region, people;

$WASTEW_{inhab}$ - volume of wastewater per capita per day (l/person*day), data for the region (by default, it is considered equal to 200 liters);

If the PEC values obtained are below 0.01 μg/L it is assumed that the drug will not carry an environmental risk after its intended use by the patient. If the PEC

value is equal to or higher than 0.01 μg/L then the PEC/PNEC ratio is calculated. The predicted concentration that does not have a harmful effect on the environment (PNEC, predicted no effect concentration) is reference data and is determined for each substance based on information about the toxic effects of substances on water bodies. If the PEC/PNEC ratio is <1, there is no risk of the negative environmental impact (at the time of determination), and if PEC/PNEC is >1, then the drug is probably found in surface waters in concentrations that are dangerous to the environment [26].

To calculate the predicted environmental concentration, data on the sale of Azithromycin in Ukraine in 2022, where 40 trade names were presented, were used. Data on the current population of Ukraine as of December 1, 2022 were taken from the website of the State Statistics Service of Ukraine, 1998-2022 [23]. The degree of Azithromycin elimination in unchanged form is 50%.

The calculation of PEC for Azithromycin were presented

$$PEC_{surface\ water} = \frac{M_{ai} \times F_{excr} \times 10^9}{WASRERW_{inhab} \times N \times DILUTION \times 365}$$

$$= \frac{5550\ \text{kg} \times 50\% \times 10^9}{200\ \text{l} \times 41629950\ \text{inhab.} \times 10 \times 365} = 9\ \text{mkg/l}$$

The PEC of Azithromycin is 9μg/L, which is 37 times higher than the norm of the API content in surface waters. The PEC/PNEC ratio is 37 [27].

The resulting PEC/PNEC ratio >1 means that the current rate of the Azithromycin consumption poses a threat to the environment. Therefore, it is appropriate to develop a method by which it would be possible to determine the permissible limits of the azithromycin concentration in surface and wastewater using the thin-layer chromatography method.

Conclusions to chapter 2

1. The PEC and PNEC for Azithromycin have calculated

2. The obtained ratio of PEC/PNEC>1 means that the current rate of Azithromycin consumption poses a threat to the environment.

CHAPTER 3
DEVELOPMENT OF A METHOD FOR THE DETECTION OF AZITHROMYCIN IN WASTEWATER WATER

Each antibiotic has its own maximum permissible concentration in water, which is usually determined using high-performance liquid chromatography. According to the European Union, the method of viscous liquid chromatography with tandem mass spectrometry (LC/MS/MS) is used to identify antibiotics in biosphere objects. This method is highly sensitive, efficient, and fairly fast, but very expensive and requires special equipment. Therefore, the use of such analysis is limited [28].

Our proposed thin-layer chromatography method is fast, simple, does not require expensive materials and equipment, and is an extremely versatile analytical method that every laboratory can afford. Therefore, the widespread and popular TLC has the right to be another alternative method for the identification of antibiotics in drinking water and to be used for ecotoxicological monitoring.

3.1 Uses Thin Layer Chromatography in Environmental Monitoring

Thin Layer Chromatography (TLC) is a method of separating substances into their individual components via two stages, the stationary phase and the mobile phase. Separating a substance into its components aids in the identification of components especially components that potentially pose a threat to humans. Thin layer chromatography is especially useful in the field of environmental monitoring in identifying components that pose a threat to human health [29]. This technique of separating substances into components is particularly useful to environmental engineers and geotechnical engineers whose work requires them to test soils and groundwater for contaminants that are toxic to humans such as pesticides, herbicides and fungicides. Thin layer chromatography is particularly efficient and low cost method in the analysis of mycotoxins in crops. Mycotoxins are particularly harmful to humans and animals if consumed.

The test was performed by thin-layer chromatography. This method has a

number of advantages: the use of inexpensive equipment, the ability to detect a compound with high sensitivity and selectivity. In addition, due to its cost-effectiveness, this method has a wide range of applications.

3.2 The development the method for determining the permissible limits of the Azitromycin concentration in surface and wastewater

We proposed a method of Azitromycin detection in a thin layer of sorbent, which is available for almost every laboratory and allows to identify antibiotic in wastewater and groundwater after their concentration.

The aim of our study was to develop the method for determining the permissible limits of the Azitromycin concentration in surface and wastewater, to select the optimal mobile phase for chromatography and a developer that would clearly detect the chromatographic zones of Azitromycin.

The following medicinal product was taken as the object of the study: "Azithromycin 500" film-coated tablets, 500 mg, manufactured by Ananta Medical Ltd.

Reagents used in the study: methanol "chemically pure", pyridine, chloroform, ethanol 99.9%, Dragendorff's solution, tap water[30].

The following equipment was used for the chromatographic study: chromatographic plates; chromatographic chamber; microcapillaries for sample application; UV lamp UVS- a device for detecting adsorption zones with UV light; glass cylindrical chromatographic chambers with a volume of 1000 cm^3. The chamber was sealed with a lapped cover glass; class A measuring and chemical glassware; analytical balance AXIS model ANG 100C.

The study was carried out in several stages.

Stage 1: Determination of chromatographic mobility of Azithromycin in thin sorbent layers

Before starting chromatography, the chamber should be saturated with

mobile phase vapors. To do this, the mobile phase prepared in advance (50 mL) was poured into the chamber, filter paper strips were passed through it, and fixed to the chamber walls. The chromatography chamber was sealed and left for further saturation for 30 minutes.

Activation and preparation of the plates for analysis

To ensure the accuracy of the analysis, as well as to clean the sorbent layer from impurities and further activate the sorbent, the plates were pre-prepared. To do this, 50 ml of methanol was added to a separate chromatographic chamber, then the plate was placed in the chamber and covered with a cover glass. After the solvent reaches the top edge of the plate, it is removed with tweezers and dried in a desiccator at 100°C for 30 minutes. If the plates are not used immediately after activation, they should be stored in a desiccator over a layer of calcium chloride or dried silica gel, or in a tightly closed plastic bag [31].

Before activation, an arrow is drawn in the upper left corner of the plate with a pencil to indicate the direction of solvent flow so that it coincides with the direction of activation during chromatography.

After the plate is activated, the markings are carefully applied to it, taking care not to damage the sorbent layer. The start line is marked at a distance of 1.5 cm from the bottom edge of the plate, and the finish line is marked at a distance of 1 cm from the top edge and 10 cm from the start line. The plate should not be contaminated, damaged, or have chips in the sorbent layer.

Two solvent systems recommended by the International Association of chemists-toxicologists were used. As mobile phases, we used a mixture of methanol-chloroform-pyridine in the ratio of 9:8:1 (System 1) and chloroform-ethanol in the ratio of 1:1. (System 2). Chromatography was performed by the ascending method on «Sorbfil» plates. The sorbent type was CTX-1BE silica gel, the fraction – 8-12 microns, the sorbent layer thickness – 100 microns, the substrate type – PETF, silicasol binding agent, additional substance – luminophore, the plate size – 10×10 cm. The mobile phase was prepared immediately before use. The necessary solvents were mixed with their constant stirring until a clear

solution was obtained. A micro capillary washed with ethanol was used to apply a sample of the substance under study. We used the following reagents: methanol, pyridine, chloroform and ethanol.

To ensure accuracy, we performed preliminary preparation of the chromatographic plates before the analysis. For this purpose, 50 ml of "b.p." methanol was added to a separate chromatographic chamber then the plate was placed in the chamber and covered with a coverslip. After the solvent reached the upper edge of the plate, it was removed from the chamber with tweezers and dried. After the plate was activated, markings were placed on it. At a distance of 1.5 cm from the bottom edge of the plate, a start line was drawn with a pencil and a finish line at a distance of 1 cm from the top edge and 10 cm from the start line [32].

Preparation of mobile phases

Prepare the mobile phases immediately before use. To prepare the mobile phases, add the necessary solvents to a conical flask with constant stirring until a clear solution is obtained. Dispense the mobile phase components using a volumetric cylinder to a final volume of 50 mL. Do not use one portion of the mobile phase twice. The ratio of solvents in the preparation of the mobile phase must be observed and their purity must be ensured. Use solvents of purity class "c.p.".

Sample application

A pre-programmed microcapillary is used to apply samples of the test substance. A separate microcapillary is used for each substance. Before use, it must be rinsed with ethanol to remove possible contaminants.

Preparation of the Dragendorff reagent

Two solutions were prepared in stages.

Solution I: 40 ml of water and 10 ml of acetic acid were added to 0.85 g of bismuth nitrate basic and shaken for 15 min.

Solution II: 8 g of potassium iodide was dissolved in 20 ml of water.

Equal volumes of solutions I and II were mixed. To 10 ml of the resulting mixture was added 100 ml of water and 20 ml of acetic acid

Step 2: Preparation of model and test solutions.

The model and test solutions of azithromycin were applied to the start line using the micro capillary method by fine application[34].

Preparation of the model solution

The model solution of Azithromycin was prepared by dissolving 0.0030 mg of azithromycin in 100 ml of methanol (Azithromycin concentration – 30 μg/ml). To prepare the test solution, 1 ml of the model solution was added to a 1 L flask, and diluted with tap water to one liter. The test solution was concentrated by evaporation under vacuum at a constant temperature of 35-40° C to the dry residue. The dry residue was diluted with 1 ml of methanol and filtered through a paper filter.

Preparation of the test solution

1 ml of model solution (with a concentration of 30 μg/ml) was added to a 1-liter flask, and tap water was added to one liter. The test solution was concentrated by vaporization at a constant temperature of 35-40ºC to a dry residue.

Step 3. Upward chromatography and processing of chromatograms (display of adsorption zones and calculation of Rf values)

In accordance with the method of upward elution described in the State Pharmacopoeia of Ukraine (Vol. 3, Issue 2 - X, 2015. - Vol. 1. - 1128 p.), the room must be provided with all necessary conditions before the test. The temperature of the laboratory room should be 20-21°C. The chromatography chamber should be placed on a surface resistant to vibrations. The chamber lid is slightly pushed back and the plate with the samples applied to it is inserted into the chromatography chamber with tweezers[35]. Insert the plate into the chamber quickly and carefully. To do this, the lid is carefully pushed back and the plate is inserted without touching the inner walls of the chamber. The starting line should be higher than the solvent layer in the chromatography chamber. The chamber lid is then closed tightly, ensuring that the chamber is stationary and chromatography is performed until the solvent front is reached, which should not cross the finish line or the top edge of the plate. Next, the plate is removed from the chamber and placed in a

desiccator to remove any residual mobile phase.Application of the model (with a concentration of 30 μg/ml) and the studied solutions (with a concentration of 30 μg/ml after concentration) of Azithromycin to the start line of the chromatographic plate by the micro capillary method by fractional application [36].

The test samples were applied to the start line by fractional microcapillary application in such a way that the internal diameter of their spots did not exceed 0.5 mm. The distance between the applied samples was at least 2 cm.

The sample plates were dried and placed in chromatographic chambers with two systems. The plates were lowered into the chambers so that the mobile phase did not cross the start line on which the test substances were applied. After chromatography, the plates were air dried at room temperature and the samples were treated with freshly prepared Dragendorff reagent and the color of the interaction products was determined.

In thin-layer chromatography Rf is calculated as the ratio of the distance from the start line to the spot center to the distance traveled from the start line by the solvent system front

$$R_f = \frac{a}{b}$$

where, a – is the distance from the start line to the center of the spot;
b – the distance traveled from the start line by the solvent system front.

The chromatogram of the test solution should show an adsorption zone in position, size and color intensity corresponding to the adsorption zone on the chromatogram of the model solution. The test results are considered reliable if two clearly separated adsorption zones are observed in the chromatogram of the solution [37-38].

After chromatography, the plates were dried, and the samples were treated with Dragendorff's reagent and UV radiation (the color of the interaction products was determined or the appearance of spots in UV light at a wavelength of 254 nm was observed). When the chromatogram was treated with Dragendorff's reagent, the spots of the model and test solutions appeared quite clearly.

Clear spots appeared identical in size and staining intensity when the chromatograms in both proposed systems were introduced into UV light at a wavelength of 254 nm. Using UV light as a chromatogram developer Azithromycin spots of the same size and staining were clearly visible [39-42]. It was found that chromatography in both systems proposed was optimal since there was a fairly high mobility of azithromycin in these systems, and Rf was in the range from 0.2 to 0.7 (Rf = 0.54 in System 1, Rf = 0.39 in System 2) (Fig. 3.1)

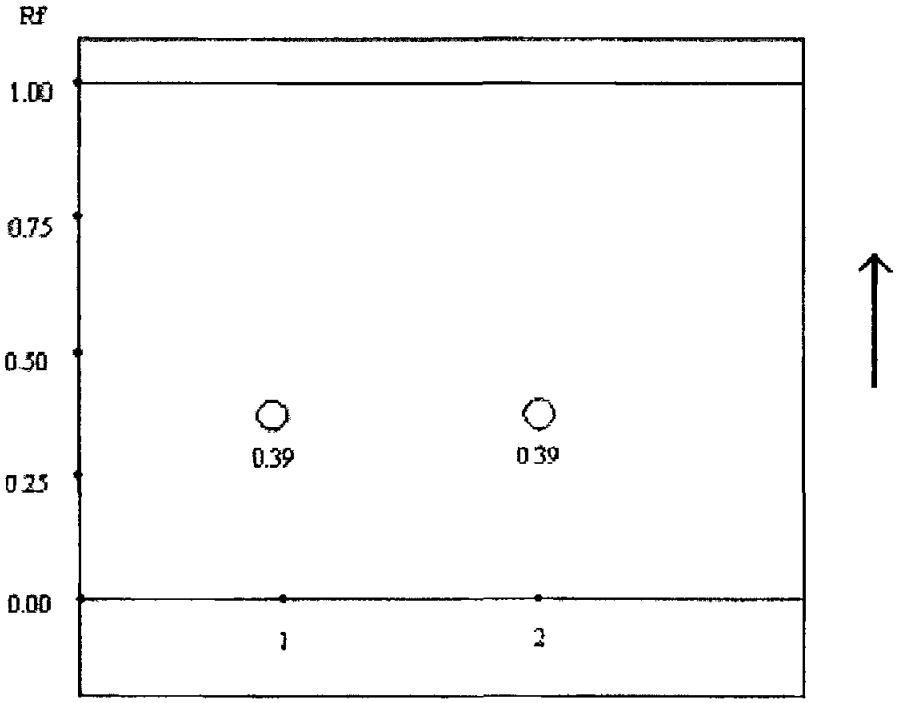


Fig. 3.1 Chromatogram of azithromycin in system 2

1- sample of model solution.

2- sample of the test solution

When treating chromatograms with Dragendorff's reagent the spots did not develop clearly. When using UV light as a chromatogram developer the identical azithromycin spots of the same size were clearly visible; therefore, we suggested that UV light should be preferred as a chromatogram developer (Table 3.1).

Tabl 3.1

Results of azithromycin chromatography

SYSTEM	Rf	DETECTION RESULTS	
		Dragendorff's reagent	UV light (254 нм)
SYSTEM № 1 (methanol-chloroform-pyridine)	0.54	fuzzy	fluorescence
SYSTEM № 2 (chloroform -ethanol)	0.66	fuzzy	fluorescence

Surface water pollution with pharmaceuticals is a complex and important problems that requires comprehensive approaches in many areas of human life, such as treatment, farming, crop production, pharmaceutical production and drug circulation. Without paying attention and monitoring to at least one of these industries, it is difficult to achieve the effect of reducing the release of pollutants into water and to stop the inevitable development of poly resistance. The problem does exist, and this can be seen from our research. The high predicted ecological concentration of azithromycin in water, which is many times higher than normal, indicates that surface waters of Ukraine are polluted by it. Contaminated surface water is the main source of drinking water that cannot be completely purified from the antibiotic. Hypothetically, we are treated with azithromycin, drink it with water, and eat it with animal and plant foods. It is not difficult to guess what will happen in a few years of such intense and incessant ingestion of it. The irrational use of Azithromycin can lead to another loss of an important antibiotic from a number of the necessary chemotherapeutic drugs due to bacterial resistance to it. To confirm the PEC studies, the method that allows us to determine Azithromycin in water has been developed using the available TLC method. Using conventional analytical scales and universal chromatography in thin layers of a sorbent it is possible to identify azithromycin with a water concentration of ≥ 30 μg/ml without complex and expensive equipment, such as HPLC or LC/MS/MS.

Conclusions to chapter 3

1. The chromatographic mobility of Azithromycin in thin sorbent layers was studied. The system of solvents providing the best chromatographic mobility of the studied compounds was selected.
2. A technique has been developed for the detection and identification of Azithromycin in water; the optimum mobile phases for chromatography have been selected and Dragendorff's reagent and UV-light have been offered as demonstrators; thanks to them Azithromycin chromatographic zones were clearly detected.
3. The proposed method of Azithromycin identification can be used for its detection in wastewater and groundwater after preliminary concentration.

CONCLUSIONS

1. The presence of increasing amounts of antibiotics in water and soil poses a potential threat to all microorganisms in these environments. In addition, environmental contamination with antibiotics is one of the causes of the formation of antibiotic resistance.
2. The development of sensitive techniques for the determination of antibiotics in wastewater and groundwater for the purposes of ecotoxicological monitoring aimed at reducing the negative impact of pharmaceutical substances present in water on the environment and human health is an urgent problem.
3. The obtained ratio of PEC/PNEC>1 means that the current rate of Azithromycin consumption poses a threat to the environment.
4. Using conventional analytical scales and universal chromatography in thin layers of a sorbent it is possible to identify azithromycin with a water concentration of ≥ 30 μg/ml without complex and expensive equipment, such as HPLC or LC/MS/MS.

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APPENDICES

APPENDIX A

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ГРАМОТА

нагороджується

Фарід Ібтіссам

у секційному засіданні студентського наукового товариства кафедри медичної хімії

XXIX Міжнародна науково-практична конференція молодих вчених та студентів

«Актуальні питання створення нових лікарських засобів»

В.о. ректора
Національного фармацевтичного університету

Алла КОТВІЦЬКА

19-21 квітня 2023 р.
м. Харків

Ф А 2.2.1–32-366

National University of Pharmacy

Faculty for foreign citizens' education
Department medicinal chemistry

Level of higher education master

Specialty 226 Pharmacy, industrial pharmacy
Educational program Pharmacy

APPROVED
The Head of Department
Lina PEREKHODA
"August 22", 2022

ASSIGNMENT
FOR QUALIFICATION WORK
OF AN APPLICANT FOR HIGHER EDUCATION

Ibtissam FARID

1. Topic of qualification work: «Development of a method for the determination of azithromycin by thin-layer chromatography for ecotoxicological monitoring», supervisor of qualification work: Lina PEREKHODA, Head of Medicinal Chemistry Department, professor.

approved by order of NUPh № 35 from "06" of February, 2023

2. Deadline for submission of qualification work by the applicant for higher education: April 2023.

3. Outgoing data for qualification work publications on wastewater contamination with antibiotics; results of the research work of the Department of Medicinal Chemistry on the development of methods for identifying antibiotics and assessing their contamination of surface waters (computer forecasting).
4. Contents of the settlement and explanatory note (list of questions that need to be developed):
-To analyze the literature on wastewater pollution by medicines, in particular antibiotics, and to investigate the ways in which antibiotics enter wastewater;
- To calculate the predicted environmental concentration of azithromycin in the surface waters of Ukraine;
- To propose a method for the determination and identification of azithromycin in wastewater by thin-layer chromatography.
5. List of graphic material (with exact indication of the required drawings):
Tables – 1, pictures – 3

6. Consultants of chapters of qualification work

Chapters	Name, SURNAME, position of consultant	Signature, date	
		assignment was issued	assignment was received
Chapters 1	Lina PEREKHODA, head of department medicinal chemistry	22.08.2022	23.08.2022
Chapters 2	Lina PEREKHODA, head of department medicinal chemistry	30.11.2022	30.11.2022
Chapters 3	Lina PEREKHODA, head of department medicinal chemistry	30.02.2023	30.02.2023

7. Date of issue of the assignment: "August 22, 2022.

CALENDAR PLAN

№ з/п	Name of stages of qualification work	Deadline for the stages of qualification work	Notes
1	Writing a literature review	Oct.-Nov. 2022	**done**
2	Assessment of azithromycin contamination of Ukrainian surface waters (forecasting).	Nov. 2022 – Jan. 2023	**done**
3	Development of a methodology for the determination and identification of azithromycin in wastewater	Jan. – March 2023	**done**
4	Formalization of the qualification work	April 2023	**done**

An applicant of higher education _____ Ibtissam FARID

Supervisor of qualification work _____ Lina PEREKHODA

ВИТЯГ З НАКАЗУ № 35
По Національному фармацевтичному університету
від 06 лютого 2023 року

нижченаведеним студентам 5-го курсу 2022-2023 навчального року, навчання за освітнім ступенем «магістр», галузь знань 22 охорона здоров'я, спеціальності 226 - фармація, промислова фармація, освітня програма – фармація, денна форма здобуття освіти (термін навчання 4 роки 10 місяців та 3 роки 10 місяців), які навчаються за контрактом, затвердити теми кваліфікаційних робіт:

Прізвище студента	Тема кваліфікаційної роботи		Посада, прізвище та ініціали керівника	Рецензент кваліфікаційної роботи
• **по кафедрі медичної хімії**				
Фарід Ібтіссам	Розробка методики визначення азитроміцину методом тонкошарової хроматографії для цілей екотоксикологічного моніторину	Development of a method for the determination of azithromycin by thin-layer chromatography for ecotoxicological monitoring	проф. Перехода Л.О.	проф. Коновий О.М.

Підстава: подання декана, згода ректора

Ректор

Вірно. Секретар

Ф А2.8-47-110

ВИСНОВОК

Комісії з академічної доброчесності про проведену експертизу

щодо академічного плагіату у кваліфікаційній роботі

здобувача вищої освіти

№ 112574 від « 25 » квітня 2023 р.

Проаналізувавши випускну кваліфікаційну роботу за магістерським рівнем здобувача вищої освіти денної форми навчання Фарід Ібтіссам, 5 курсу, _____ групи, спеціальності 226 Фармація, промислова фармація, на тему: «Розробка методики визначення азитроміцину методом тонкошарової хроматографії для цілей екотоксикологічного моніторингу/ Development of a method for the determination of azithromycin by thin-layer chromatography for ecotoxicological monitoring», Комісія з академічної доброчесності дійшла висновку, що робота, представлена до Екзаменаційної комісії для захисту, виконана самостійно і не містить елементів академічного плагіату (компіляції).

Голова комісії,
професор **Інна ВЛАДИМИРОВА**

0%

29%

Ф А 2.2.1-32-353

REVIEW

of scientific supervisor for the qualification work of the master's level of higher education of the specialty 226 Pharmacy, industrial pharmacy

Ibtissam FARID

on the topic: « Development of a method for the determination of azithromycin by thin-layer chromatography for ecotoxicological monitoring»

Relevance of the topic. The development of sensitive and affordable methods for the determination of antibiotics in wastewater and groundwater for the purpose of ecotoxicological monitoring is an urgent issue of modern science. These methods should not require expensive equipment and should be accessible to different analytical laboratories.

Practical value of conclusions, recommendations and their validity. The chromatographic mobility of azithromycin in thin layers of sorbent was studied. Two solvent systems were selected that provide the best chromatographic mobility of azithromycin. A optimal mobile phases for chromatography were selected, and UV light was proposed as a developer, which clearly detected the chromatographic zones of azithromycin.

Assessment of work. The work is performed at a high scientific level, the results are reliable, the conclusions are logical and reasonable. The overall assessment of the work is positive.

General conclusion and recommendations on admission to defend. The qualifying thesis of Ibtissam FARID meets the requirements for master's theses in terms of the relevance and scope of the research, the novelty of the results, their theoretical and practical significance, and can be recommended for defense by the Examination Commission.

Scientific supervisor prof. Lina PEREKHODA

« 7th» of April 2023

Ф А 2.2.1-32-356

REVIEW

for qualification work of the master's level of higher education, specialty 226 Pharmacy, industrial pharmacy

Ibtissam FARID

on the topic: « Development of a method for the determination of azithromycin by thin-layer chromatography for ecotoxicological monitoring»

Relevance of the topic. Antibiotic contamination of wastewater and groundwater is one of the key ways to develop antibiotic resistance, and thus to lose their effectiveness in treatment. Therefore, the development of sensitive and affordable methods for the determination of antibiotics in wastewater and groundwater, which would not require expensive equipment and would be available to various analytical laboratories, is an urgent issue of modern science.

Theoretical level of work. In this work, a method for the identification of azithromycin is proposed for its determination in wastewater by thin-layer chromatography using two solvent systems and Dragendorff's reagent and UV light as developers. The work was performed at a high scientific level, the results obtained are reliable, the conclusions are logical and reasonable.

Author's suggestions on the research topic. To use the selected conditions for the identification of azithromycin in experimental studies of wastewater pollution aimed at reducing the negative impact of pharmaceuticals present in water on the environment and human health.

Practical value of conclusions, recommendations and their validity. The system of solvents that provides the best chromatographic mobility of azithromycin was determined. A methodology was developed that allows its determination in water using the TLC method. Using conventional analytical balances and universal chromatography in thin sorbent layers, azithromycin with a concentration in water of ≥ 30 μg/ml can be identified without resorting to complex and expensive equipment such as HPLC or LC/MS.

Disadvantages of work. There are some inappropriate expressions.

General conclusion and assessment of the work. The qualification work of Ibtissam FARID meets the requirements and can be recommended for defense at the Examination Commission due to the relevance and scope of the research, the novelty of the results obtained, their theoretical and practical significance.

Reviewer _____ Oleh KOSHOVYY

«14th» of April 2023

Ф А2.2.1-91-287

ВИТЯГ
з протоколу засідання кафедри медичної хімії
№ 10 від 21 квітня 2023 р.

ПРИСУТНІ:
проф. Ліна ПЕРЕХОДА
доц. Вадим ЗУБКОВ, доц. Ірина СИЧ, доц. Віталій ЯРЕМЕНКО, доц. Ілля ПОДОЛЬСЬКИЙ, доц. Наталія КОБЗАР, доц. Марина РАХІМОВА, доц. Маргарита СУЛЕЙМАН, ас. Олена БЕВЗ, ас. Ольга ВІСЛОУС.

ПОРЯДОК ДЕННИЙ:
Звіт про стан виконання кваліфікаційної роботи Ібтіссам ФАРІД на тему: «Розробка методики визначення азитроміцину методом тонкошарової хроматографії для цілей екотоксикологічного моніторину»

СЛУХАЛИ: доповідь здобувача вищої освіти факультету підготовки іноземних громадян Ібтіссам Фарід на тему: «Розробка методики визначення азитроміцину методом тонкошарової хроматографії для цілей екотоксикологічного моніторину», керівник зав. каф. медичної хімії, д.фарм.н., проф. Ліна ПЕРЕХОДА.

УХВАЛИЛИ: рекомендувати кваліфікаційну роботу Ібтіссам Фарід до офіційного захисту в Екзаменаційній комісії.

Зав. кафедри медичної хімії,
професор **Ліна ПЕРЕХОДА**

Секретар кафедри медичної хімії,
доцент Марина РАХІМОВА

Ф А2.2.1-32-042

НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ПОДАННЯ
ГОЛОВІ ЕКЗАМЕНАЦІЙНОЇ КОМІСІЇ
ЩОДО ЗАХИСТУ КВАЛІФІКАЦІЙНОЇ РОБОТИ

Направляється здобувач вищої освіти Ібтіссам ФАРІД до захисту кваліфікаційної роботи за галуззю знань 22 Охорона здоров'я
спеціальністю 226 Фармація, промислова фармація
освітньою програмою Фармація
на тему: «Розробка методики визначення азитроміцину методом тонкошарової хроматографії для цілей екотоксикологічного моніторину».

Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Світлана КАЛАЙЧЕВА /

Висновок керівника кваліфікаційної роботи

Здобувач вищої освіти Ібтіссам ФАРІД виконала кваліфікаційну роботу у повному обсязі у відповідності до виданого завдання та у встановлені терміни.

Керівник кваліфікаційної роботи

_____ Ліна ПЕРЕХОДА

«07» квітня 2023 р.

Висновок кафедри про кваліфікаційну роботу

Кваліфікаційну роботу розглянуто. Здобувач вищої освіти Ібтіссам ФАРІД допускається до захисту даної кваліфікаційної роботи в Екзаменаційній комісії.

Завідувачка кафедри
медичної хімії

_____ Ліна ПЕРЕХОДА

«21» квітня 2023 р.

Qualification work was defended

of Examination commission on

«__ » of June 2023

With the grade _____

Head of the State Examination commission,

DPharmSc, Professor

_____ / Oleh SHPYCHAK /