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CHEMISTRY DEPARTMENT

AN INVESTIGATION ON THE CONCENTRATION OF NORETHISTERONE IN WASTEWATER

BY

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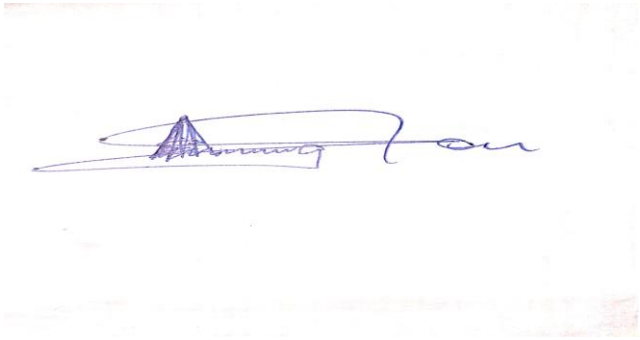

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DECLARATION

I, Konzo Brighton, hereby declare that the experimental work described in this reportwas carried out in the Bindura University of Science Education Chemistry Laboratory and in the Department of Science and Mathematics, Faculty of Education from January 2022 to September 2022. This is my original work and was not reproduced from someone else's work. Also, the work has not been submitted previously to qualify for any other academic award. Where use has been made of other people's work, it has duly been acknowledged in the text.

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DEDICATION

This research work is dedicated to my forever loving, inspiring, helpful, supportive and encouraging wife and friend, Fellisters. Without you, I would not have done it. To my children Decency,Brilliance and Vigilance- Munenyasha, parents,siblings ,Job,Ruth,Obvious and Purity thank you for your support and encouragement you are wonderful in my life. Special dedication is to my High School teachers MrMapuvire and MrAhlekiye who strongly encouraged and motivated me to pursue my studies, you are the best!

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ABSTRACT

The sudden increase in pollution to the natural environmenthas attracted global attention from researchers , governments,environmentalists and many other stakeholders.The death of aquatic animals,hormonal imbalances on fish ,reduced survivability of aquatic organisms among others triggered the research on the concentration of compounds in wastewater.Laboratory work carried out to determine the concentrations of norethisterone (norgestrel) used standards that were prepared from control contraceptive pills mixed with acetonitrile.This was done , so as to quantify it in the aquatic environment. UVabsorbance was between 200- 342 nm. The UV-spectrophotometer and electric shaker were the instruments used in the study. Grab sampling method was used ,where samples of wastewater were collected at one point in time .Three samples were collected from Mbare (Matapi),Chitungwiza (Manyame river) and Bindura river. It was then quantified by using UV-Vis Spectrophotometer instrument. Values of 9.57mg/L ,14.8 and21.13 mg/L of norgestrel were observed in wastewater samples for Bindura river,Chitungwiza (Manyame river) and Mbare (Matapi) respectively.Also, linearity was observed on calibrationgraphs .Further reasearch studies should focus on upgrading of wastewater treatment plants to remove progesterone-like pollutants.The use of highly advanced instruments like NMR and LC-MS to obtain molecular structure of standard should be prioritised.

CONTENTS

CHAPTER 1: INTRODUCTION

1.1 Introduction to the study

The technological advancement in the pharmaceutical drug industries, whose dugs are being used worldwide have lengthened the life spans ,cured deadly diseases and improved the quality of life in human beings.However,someorganic compounds which were not known to be of value in water bodies, in terms of distribution and/or concentration, arerecently being detected as analytical methods improves. Such compounds, which have the potential to cause known or suspected adverse ecological or human health effects, are often collectively referred to as emerging contaminantsEC (Shareef, Kookana and Kumar, 2008).ECs include newly synthesized substances among others .More and more compounds belonging to this group are synthetic substances, produced and introduced to the natural environment in different ways. Such compounds are called xenoestrogens (environmental estrogens).

Xenoestrogens show the ability to interact with the endocrine system, disrupting its normal operation. They are mainly related with estrogen-based drugs, such as contraceptives, and hormones used in hormone-replacement therapy. It was observed that these substances cause side effects such as impaired fertility, lack of masculine features, etc. This phenomenon is particularly observed for marine environment and water-related wildlife. Also for humans,xenoestrogens are present in drinking water, causing fertility problems in men.Phamaceuticals like norethisterone,being a common drug used by women worldwide, has ,had fair advantages to the users but a threat to environmental sanitation .The disposal of this drug and how chemical compounds end up in waste is a cause for concern and this has triggered an interest into finding the level of concentration of norethisterone as well as its effects to the surrounding environments.

The discovery of the contraceptive pill, also known as family planning tablets has significantly improved women's lives (Mastrup et al., 2005). The contraceptive pill aids the bioavailability of synthetic hormones that facilitate contraception. These hormones are usually found in insignificant amounts naturally in the body. To date, there are three categories of oral contraception that are available. The first category is the sequential type where the hormones are

taken in sequence.Firstly, oestrogen is taken alone for the first week, then a lower dosage of the oestrogen together with a progestin for the rest of the course is taken. The second type is commonly used where both an oestrogen and a progestin are present in the tablets. Lastly, in the progestin category, a progestin alone is taken (Sohrabi et al., 2010; Sohrabi&Mohammadpour, 2009).Contraceptive tablets are found in three combinations and are marketed worldwide under different generic and brand names. Combinations include that of ethniylestradiol, an oestrogenandnorgestrel, a progestin, ethniylestradiol and gestogene, a progestin and ethniylestradiolanddrospirenone, a progestin (Seifeldeen et al., 2016).
Norgestrel is a synthetic steroidal progestin and ethinylestradiol, EE2, is a semi syntheticsteroidaloestrogen (Berzas, 1997; Rathod& Captain, 2014; Zaidi et al., 2014).

1.2 Background of the study

There has been a high level of poor drug disposal and management of pharmaceautical drugs world wide.This is evidenced by a high level of chemical compounds of drugs that have been found present in many environments including air,land,and water .The presence of such chemicals have resulted in the contamination of these environments ,subsequently affecting reproduction,lifecycles,health status ,emergency of genetically altered conditions of the nontarget aquatic plant and animal life.The ecological system has been altered owing to rising concerns of climate change and environmental degradation.Scientific investigations on the concentration levels of such pharmaceautical drugs in wastewater are required to ascertain contamination levels so as to take appropriate measures to mitigate the effects of the contamination.

Most diseases have been detected in human life, withthe background of these diseases being traced back to chemicals found in food, water, air and contraceptive drugs. The development has since been a threat to aquatic animal and plant health that seeks to investigate the sources of such chemicals. To control ballooning world population, researchers have invented contraceptive drugs, having side effects and metabolic properties without being fully explained to the public. Coupled with all these ambiguous circumstances surrounding drug management and use, their disposal have become a menace to the environment, thus the desire to investigate the concentration of norethisterone levels in wastewater.

1.3 Justification of the Study

The purpose of the study is to investigate the concentration of norethisterone in wastewater, which has been a problem to both aquatic plant and animal life, an exposure to the effects of anthropogenic activites in the contamination of environment

1.4 Statement of the problem

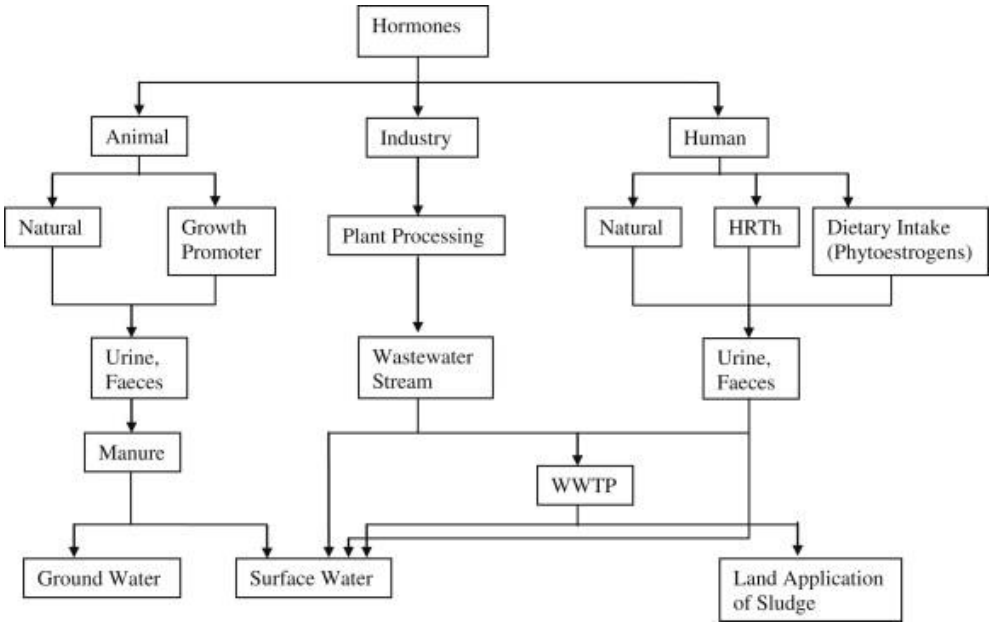
Norethisterone, being a contraceptive drug has chemical compounds which has been found in wastewater in many urban areas worldwide, this has been necessitated by poor disposal methods by manufacturers andthe users, thus resulting in its chemicals found in house hold waste, land fill effluent, septic systemic ,sewer lines and wastewater.

Table 1: Daily Excretion of hormones by humans and animals (Blasco and Pico, 2009)

Specie	Category	Urine excretion (µg day^{-1})	Hormones excreted
Humans	• Cycling Fema	16-35	E1,E2,E3.EE2
	• Pregnant Fema	5270-7030	E1,E2,E3.Pg
	• Noncycling Fema	7-13	E1,E2,E3
	• Mal	7-9	T,E1,E2

The main route of excretion of hormones from human is via urine. Excretion via faeces forms another route of excretion but in small fraction in humans. The hormones are conjugated to glucuronide or sulphate moieties. After excretion they undergo deconjugation resulting in free active hormones.

Fig:1 Pathways through which synthetic and natural estrogenic hormones can reach the environment (adapted from Kozlick et al, 2010)



Hormones are the most potent endocrine disruptors even at nanogram per litre levels. The presence of the estrogenic hormone, estradiol (E2), and the progestin, progesterone in aquatic environments even at low levels (0, 1-10 ng/L) has been linked with different steroidal effects in aquatic species (Eljarrat, Cruz and Farre, 2009). This is because they are able to interact with the endocrine system. As such they interfere with reproductive, growth and development systems in both humans and animals.

1.5Significance of the study

The study shall seek to determine the level of concentration of norethisteronedrug in wastewater .Its effects to aquatic life such as plants and animals and howthis can impact the aquatic ecosystems. Measures to minimize the levels shall be recommended in a bid to maintain a clean environment.The accurate measurement of norethisterone hormone concentrations in sediments is fundamental to a number of investigations, e.g. for estimating their role in the removal of

steroidal estrogens from the water column (Chen and Wu, 2012), for determining their persistence and risk of permeation to ground waters and to determine the risk to be in the organisms from exposure to estrogens (Eljarrat et al, 2009). Furthermore, in case of sediment resuspension and hormone remobilization, sediments may act as a secondary source of exposure to aquatic organisms living in the water column (Chen and Wu, 2012).

1.6 Aim

To analyse the wastewater samples for the presence of norethisterone hormone using the UV-Vis spectrophotometer

1.7 Research objectives

1.to identify the disposal methods of contraceptive drugs in Zimbabwe.

2.To examine the chemical constituents of norethisterone.

3. To establish the negative effects of Norethisterone to aquatic plants,animal and human beings.

4.To determine the concentration of norethisterone in wastewater samples.

1.8 Limitations

Due to the very low concentrations of norethisteronehormones, the analytical information is restricted only to UV-Vis analysis. Additional analysis such as NMR cannot be achieved due to low sample concentrations and the high purity required for this analysis.

CHAPTER 2: REVIEW OF RELATED LITERATURE

2.1 Introduction

Pharmaceutical drugs have been disposed lawfully and indiscriminately by both the users and manufacturers worldwide. The disposal methods applied have caused environmental contamination and toxic chemicals have been exponentially been increasing in water bodies such as dams, lakes and seas. In recent times, the main problems associated with environmental pollution are the origin of organic compounds found in wastewater. Substances coming from sewage leaks into the environment in the form of a multicomponent mixtures often increases the toxicity caused by these compounds. The contaminants present in wastewater, among the most marked are, endocrine disrupting compounds, residues of pharmaceuticals and personal care products, plastics and sunscreens.

In the recent times a new group of compounds have been detected, as already stated and is called emerging contaminants .Drugs whose availability and has been highly legalized and this has beenaxercabated by their uncontrollable use, storage and disposal have been found in wastewater. Exposure to these type of compounds, named in the literature as emerging contaminants (ECs), causes, among others, such effects as hormonal imbalance, reduction of the survivability of aquatic organisms and reproductive problems(Shareef, Kookana and Kumar, 2008) .This has left a significant number of both plant and animal lives being lost and some aquatic species are now extinct due to contamination by these drugs ,among them being norethisterone. The chemicals found in these drugs have an adverse effect on aquatic life found in waste water prompting many researchers becoming interested in investigating the effect of norethisterone levels in wastewater.

2.2 Why are pharmaceutical drugs disposed in Zimbabwe?

A wide range of drugs and chemicals are released into the environment on daily basis in huge quantities .Such drugs have been observed of late that they are a danger to the environment .Research shows that, aquatic animals had their homeostatic systems been altered in most water

bodies. Particular attention should be paid to the aquatic environment – both natural and artificial water reservoirs. (Hughes, Kay and Brown ,2013).

Detection and determination of active forms of organic compounds in aquatic ecosystems is one of the priority tasks of environmental chemistry. A key problem for assessing the organic contamination in the water samples is the fact that a large group of hazardous compounds is present in trace amounts, often almost impossible to determine. This problem over the years was a challenge for analytical chemists, who are trying to develop more appropriate procedures intended for the determination of trace amounts of a wide variety of organic compounds in water samples with complex matrices.

2.2.1 Expiration

WHO (1999) argues that there are various reasons why drugs are disposed, one among so many reasons is expiration. Items whose expiry dates have exceeded or items that have expired along the time they have been transported or stored in the supply chain network before distribution.
The drugs that have exceeded their shelf life are no longer effective as per period when they have been manufactured. For this reason, such drugs whose shelf life have exceeded can no longer be administered to human bodies as this is tantamount cause health complications to the user thus they are disposed.

2.2.2 Damaged

Items can be damaged during transportation or during storage.The delivery stage of the procurement cycle is one of the most difficult stages to manage or even with a quality assurance systems for procurement ,problems can occur and contraceptives can be exposed to the environment .(WHO,1999,2007)

2.3 Drugs disposal Methods.

Proper disposal methods of drugs are important because it helps to protect environmental and human health. Normally unwanted drugs should not be flushed down the drain .Medical drugs should be kept secure at home to prevent unlawful use and regularly disposed .Drugs which are no longer useful should be collected to various places for proper disposal methods such as incineration to destroy the chemicals within them and prevent them from entering the sources we use for drinking water(Grossman,2021).

There are quite a number of methods for disposing drugs in Zimbabwe.The disposal methods have negative impact on the land ,water and air.These methods include landfill,open uncontrolled non-engineered drugs(WHO 2011).

2.3.1Land fill

There are three general methods of landfills,which are ,area method,trench method and ramp or slope method.The area method is best suited for flat or gently sloping areas where some land depressions may exist.(Kristina,2018).Waste sent to a land fill is placed directly into a land disposal site without prior treatment or preparation .Land fills are the oldest and the most widely practised method of disposing of solid waste .There are three types of landfills.(WHO 2007)

2.3.2 Open uncontrolled non-engineered dump.

WHO (1999) observes that, it is the most common land disposal method in developing countries.However ,untreated waste discharged into an uncontrolled ,non engineered open dump does not protect the local environment and should not be used .It should be noted that discharging waste in open uncontrolled dumps with insufficient isolation from aquifers or other watercourses can lead to pollution with the risk of contamination to drinking water in the worst cases.

2.3.3 Engineered landfill

Direct deposit of pharmaceuticals is the second best option to discharge immobilisedphamaceuticals waste into a land fill .This type of land fill has some features to

prevent the loss of chemicals into the aquifers.Properlyconstructed and operated landfill sites offer a relatively safe disposal route for municipal solid wastes ,including waste pharmaceuticals.The top priority should be the protection of the aquifer.Each day's solid waste is compacted and covered with soil to maintain sanitary conditions.The term "safe sanitary landfill" refersvto such a site that is adequately situated ,constructed and managed.WHO(1999).

2.3.4 Encapsulation

It is a form of waste immobilization ,which involves immobilising the products in a solid block within a plastic or steel drum .Drums are filled to 75% capacity with solid and semi-solid contraceptives and the remaining space is filled by pouring a medium such as cement /lime mixture plastic foam or bituminous sand .Once the drums are filled to 75% capacity the mixture of lime ,cement and water in the ratio of 15:15:5 respectively (by weght) is added and the drum filled to full capacity .A large amount of water may be required sometimes to attain a satisfactory liquid consistently .Steel drums lids should then be bent back and sealed idealy by welding .The sealed drums should be placed at the base of a land fill and covered with fresh manucipal solid waste.(WHO 1999)

2.3.5Inertization

This is another form ofwaste immobilization with a variant encapsulation and involves removing the packaging materials ,paper and cardboard and plastic from phamaceuticals.For example ,pills would need to be removed from their blister packs.These are then ground and a mix of water ,cement and lime added to form a homogeneous paste .Worker protection in the form of protective clothing such as boots,masks,gloves is required as there may be dust –hazard .The paste is then transported in a liquid state by a concrete mixer truck to a land fill and then decanted into the normal urban waste .The process is relatively inexpensive and can be carried out using unsophisticated equipment.The main requirement are a grinder or road roller to crush the phamaceauticals a concrete mixer and supplies of cement ,lime and water.Theapproximaterations by weight used are pharmaceuticals (contraceptive) waste 65%,lime 15%,cement 15% and water 5% or more to form a proper liquid consistency.(WHO,1999).

2.3.6 Chemical decomposition

This method involves the removal of a formulation such as tablets from their packets or opening and emptying liquid or powder injectables into bulk containers before chemical decomposition .Because of the hazards posed by this method to operators who may be involved in these operations are carried out under controlled (Bio/chemicals safety cabinets) to avoid exposure of personel and environment to potent hormones .However, this involves the use of specialisedamity and is expensive .If the method is chosen ,the type of chemical reaction to be carried out and agents to be used would be divided based on the advice from the manufacturer(VullietandCren-Olive ,2011).

2.4 The chemical components or ingredients that make Norethisterone.

Oral contraceptives are widely used by women around the world.Norethisterone is a synthetic oral progestin used for contraception or to treat other hormone-related conditions such as menopausal symptoms and endometriosis. The drug type is synthetic oral progestin. The tablet of Norethisterone weighs about 0.35mg USP.Inactive ingredients include colloidal silica,dioxide,cor80n starched and C yellow No 10,ethyl cellulose, lactose anhydrous, magnesium stearate, microcrystalline cellulose,providone,sodium starch glycolate,methylene chloride, isopropyl alcohol and talc. Each chemical shall be examined in detail in relation to its use in the tablet of Norethisterone.(Wise,Brien, and Woodruff ,2011)

2.4.1 Silicon Dioxide(SiO_2)

Scientifically colloidal silicon dioxide is sub microscopic fumed silica prepared by the vapour-phase hydrolysis of silica compound .It is affine term of silicon that can be evenly be dispersed.It does not dissolve in water .Silicon is natural element on the periodic table ,that’s non toxic and used often in industry.In the pharmaceuatical industry ,silicon dioxide also known as colloidal

silicon dioxide has many uses in tablet-making industry as an anti-caking ,adsorbent ,disintegrant or glidant to allow powder to flow freely when tablets are processed.(Thomas ,1978).

Fig2 Sh**owing Silicon Dioxide(Thomas,1978)**

2.4.2 Corn Starch

Corn starch, maize starch or cornflour is the starch derived from corn (maize) grain .The starch is obtained from the endorsperm of the kernel .Corn starch is a common food ingredient,often used to thicken sauces or soups and to make corn syrup and other sugars.Innindustries it is used as adhesives in paper products ,as an anti-sticking agent and textile manufacturing .It has medical uses as well ,such as to supply glucose for people with glycogen storage diseases.Although mostly used for cooking as well as in household,corn starch is used for many purposes in several industries ranging from its use as a clinical additive for certain products additives for certain products to medical therapy for illness.In medical fraternity,corn starch is the preffered anti-stick agent on radical products made from natural latex including condoms ,diaphragms and medical gloves .Corn starch has properties enabling supply of glucose to maintain blood sugar levels for people with glycogen storage diseases.(Whistler,Bemiller, and Paschall,2018)

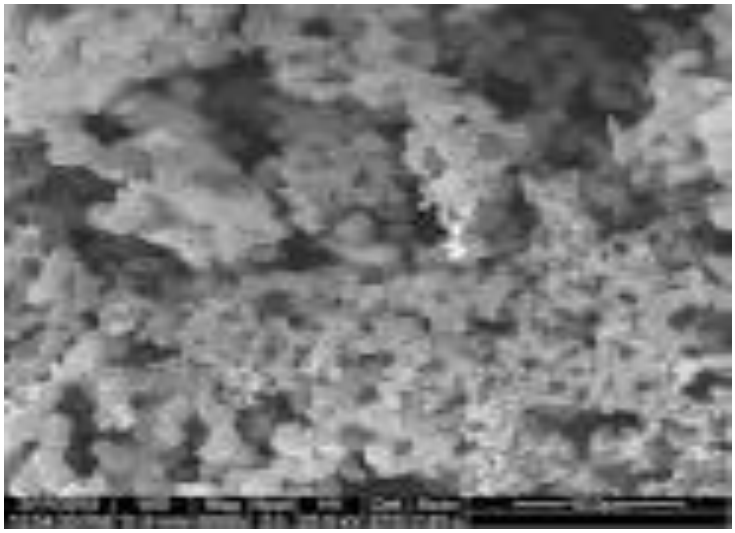
Fig3 showing Corn Starch (Wistler, Bermiller, and Paschall,2018)

2.4.3 Ethyl cellulose

It is a semi-synthetic polymer material which has good thermal stability, biocompatibility and biodegradability in vivo and has good film-forming properties. Is a derivative of cellulose in which some of the hydroxyl groups on the repeating glucose units are converted into ethyl –ether groups. The number of ethyl groups can vary depending on the manufacturer. Other names are cellulose, ethyl ether ,ethylated cellulose ,ethyl cellulose .

It is oftenly used as a thin –film coating paper ,vitamin and medical pills and for thickeners in cosmetics and in industrial processes .Food grade ethyl cellulose is one of the few non-toxic films and thickeners which are not water- soluble .This property allows it to be used as safeguard ingredients in water .Ethyl cellulose is also used as a food additive as an emulsifier (E462).In tablets ,it is used in pharmaceutical technology as a coating agent ,flavouring fixative ,binder,filler ,film –former ,drug carrier or stabilizer (Manley,1998).

Fig4 Showing Ethyl cellulose (Manley, 1998).



2.4.4 Lactose

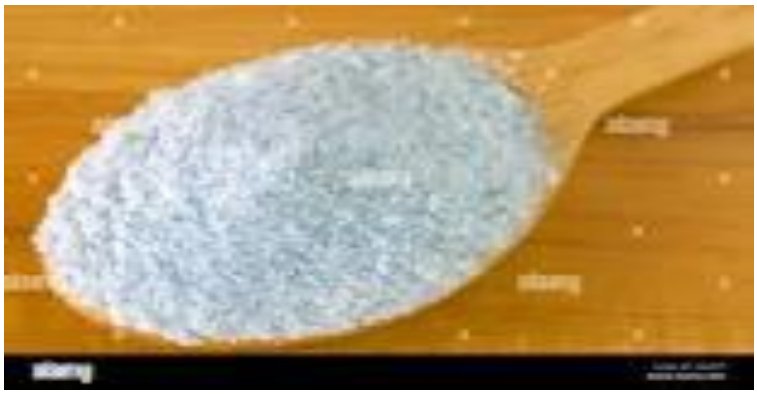
It is a disaccharide, asugar that is composed of bgalactose and glucose subunits and has the molecular formular .$C_{12}H_{22}O_{11}$.Lactose makes around 2-8% of milk .The name comes from lac,the Latin word for milk, plus the suffix –ose used to name sugars.In the phamaceautical industry ,lactose is used to help form tablets because it has excellent compressibility propertis.It is also used to form dry-powder inhalations .Its advantage over hydrous lactose is ,it does not undergo the mailard reaction which can lead to brewery and discoloration with certain drugs.(McSweeney and Fox, 1998)

Fig 5 Showing lactose (McSweeney, and Fox ,1998)

2.4.5 Magnesium Stearate

Is the chemical compound with the formula Mg_2.It is a soap consisting of a salt containing two-equivalents of stearate and one magnesium cation .It is a simple salt made up two substances ,a saturated fat called stearic acid and the mineral magnesium.Stearic acid can be found in many foods,such as chicken.Magnesiumsterate is a white ,water insoluble powder .Its application exploit its softness ,insolubility .In many solvents and how toxicity.It is a flow agent. its main job is to keep the ingredients in capsule from sticking together .It also forms a barrier between the medicines and the machines that makes them .The powder improves the consistency and quality of medication.(Bolognia,2018)

Fig 6 Showing Magnesium stearate(Bolognia,2018)



2.4.6 Microcrystalline cellulose

The chemical formular is C6 H10O5It is a white free-flowing powder. Chemically it is an inert substance it is not degraded during digestion and it has appreciable absorption. In large quantities it provides dietary bulk and may lead to a laxative effect.Is a term for refined wood pulp and is used as a texturiser ,an anti-caking agent ,a fat substitute ,an emulcifier an extender and a bulking food production .The most common form is used in vitamin supplements or tablets.It is a valuable additive in pharmaceutical food,cosmetics and other industries.Differentpropertis of microcrystalline cellulose are measured to qualify its suitability to such utilisations namely particular particle size,density and compressibility (El-Sakharwy and Hassan,2007).

Fig 7 Showing Microcrystalline Cellulose.(El-Sakharwy and Hassan,2007)

2.4.7 Providone

Is used in the pharmaceutical industry as a synthetic polymer vehicle for dispersing and suspending drugs .It appears as white to off-white hydroscopic powderin its pure form and is readily available in water.It is soluble in cold water and mild-warm water ,ethyl alcohols ,isopropyl alcohol polythelene glycol and glycerol .Its stability in solution is much greater than that of tincture of iodine or Lugo’s solution(Fleischer,1997).

Fig 8 Showing Providone (Fleischer,1997)

2.4.8 Sodium Starch Glycolate

Is the sodium salt of carboxymethyl ether ,it is white to off-white ordorless tasteless and free – flowing powder .It is formed by crosscutting and carboxymethylation of potato starch.It can be manufactured from other starch foods such as corn wheat and rice **.**Is commonly used super-disintegrant employed to promote rapid disintegration and dissolution of IR dosage forms.It is manufactured by chemical modification of starch i.ecarboxymethylation to enhance hydrophilicity and cross-linking to reduce solubility.(Sakurai,2009).

Fig 9 Showing Sodium Starch Glycolate(Sakurai,2009)

2.4.9 Methylene Chloride

Is an organochloride compound with the chemical formular CH2Cl2 .This colourless volatile liquid with a chloroform like,sweetordour is widely used as a solvent.Although it is not miscible with water ,it is polar and miscible with many other organic solvents. It is also called dichloromethane ,is a volatile ,colorless with a chloroform like ordor.It is used in various

industrial processes in many different industries including point striping ,pharmaceutical manufacturing ,point remover manufacturing and metal cleaning and degreasing.It is used as an effective reaction and re-crystalisation solvent in the extraction of several pharmaceutical compounds and in the production of many antibiotics and vitamins. (WHO,1993).

Fig 10 Showing MethyleneChloride(WHO,1993)

2.4.10 Isopropyl Alcohol

Is a colorless ,flammable clinical compound with a strong ordor.As an isopropyl group,linked to a hydroxyl group ,it is the simplest example of a secondary alcohol,where the alcohol ,carbon atom is attached to two carbon atoms .It is a structural isomer of i-propanolethylmethyl ether.
It is mixed with water for use as a rubbing –alcohol antiseptic .It is also used in aftershave lotions ,hand lotions and other cosmetics .In industry ,it is used as an expensive solvent for cosmetics ,drugs ,shallacs and gums ,as well as for denaturing ethanol (ethyl alcohol) (Yaws,1999).

Fig 11 Showing Isopropyl Alcohol(Yaws,1999)

2.4.12 Talc

Is a hydrous silicate mineral composed of magnesium ,silicon and oxygen ,silica and water. Is a clay mineral composed of hydrated magnesium silicate with the chemical formular $Mg_3Si_4O10(OH)_2$.Talc is a powdered form often combined with corn starch ,is used as baby powder .This mineral is used as a thickening agent and lubricant .It is an ingredient in ceramics ,paint and roofing material .It is used in many industiries including paper making ,paint and coatings,rubber ,food ,electric cable,phamaceuticals cosmetics and ceramics.**(**Wistler, Bermiller, and Paschall,2018)

2.5 The negative impact of norethisterone on aquatic life.

2.5.1 The impact of Hormones in wastewater.

Freshwater ecosystems are increasingly under threat from bpth climate change and expanding human populations.Climate change has led to lower levels of rainfall in many parts of the world ,reducing freshwater stream flows,while growing populations will consume increasing amounts

of freshwater and generate more polluted wastewater .This has led to many rivers and streams becoming dorminated by processed sewage effluent.(Frank,2015)

Previous studies have shown the high ecotoxicological potential of progestogens.On the reproductive system of aquatic organisms ,yet the abiquitous presence several PGs in wastewater treatment plant effluents indicates an incomplete removal of chemical during treatment.The occurrence of pharmaceuticals in environment originating from human consumption has received increased scientific attention during the last decades due to concerns regarding their combined environmental effects in aquatic and terrestrial environment in flora and biodata and the extent in human health.Increased presence of the hermaphrodite fish populations in the reservoirs fed with purified water flowing from sewage treatment plants was observed in the US, Asia and Europe (Sumpter,Iwanowicz , Blazer, Guy,Pinkney, Mullica and Alvarez ,2009).

Synthetic oestrogen in wastewater from contraceptive pills can have effects on fish reproduction and survival that worsen over several generations ,new research has found .Studies suggest that some fish populations may not be able to recover from levels of oestrogen pollution found in many freshwater environments.One of the major challenges to water-dwelling organisms is chemical pollution through residues in effluents of wastewater treatment plants. An important group of such pollutants includes hormones like the natural estrone and 17β- estradiol (E2), and the synthetic 17α-ethinyloestradiol(EE2) that was first synthesized in the 1930s and mimics E2 in many formulations of oral contraceptives. (Vulliet E, Cren-Olive 2011)

Senior and Nakagawa(2013).The number of laboratory studies on effects of estrogen pollution is impressive and has been complemented by population models and phylogeneticanalyses.A research carried on the effects of EE2 exposure on populations of fathead minnow (pimephales promotes) fish over multiple generations.They used concentrations of EE2 in the range commonly found in the environment as aresult of wastewater effluents from approximately 3 to approximately 11 nanograms per litre(ngL^{-1} .EE2 was found to increase the chance of death in male fish by a factor of 1.7 for each $1ngL^{-1}$ of EE2 added .No males survived longer than one hundred and twenty six when exposed to the 11 ngL^{-1} .Females were less affected with around 50% surviving over the same scale .In the world fathead minnows have a life span of between one and three years.These results by researchers show that exposure to EE2 at real world levels can tdisrupt fathead minnowpopulation dynamics by affecting reproduction over multiple

generations supporting that fish populations may not be able to recover following oestrogen exposure .The findings is relevant to all fish with high reproductive rates and relatively short lifespans in environments contaminated with relevant oestrogen levels .

2.6 The UV-Vis Spectrophotometry

Samir, Kalalian, Roth, Salghi and Chakir, 2020) observed that ,in recent years, due to the advantages of high precision, high detection efficiency,nondestructive sampling, environmental protection, low cost, and portability, ultraviolet-visible (UV-Vis) technology combined with a variety of advanced technologies has developed into an excellent and effective tool for detecting pollutants in aqueous environments. Futhermore ,(Bardik, et al) observedthat,UV-Vis spectrum analysis is an important development direction for water quality monitoring, which evaluates the degree of water pollution by establishing a correlation model of absorbance, organic matter, and inorganic matter concentration PCA.

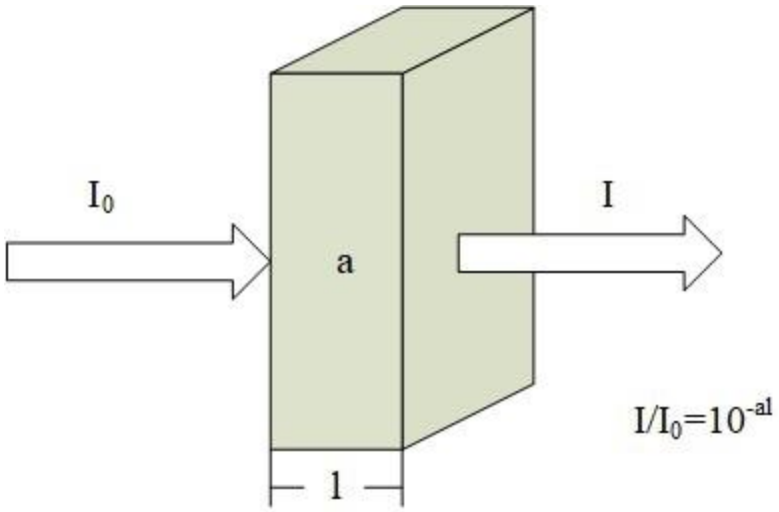
Therefore, a review is very necessary to sort out the research process and methods in this field, especially in the latest application progress of ultraviolet spectroscopy technology.This paper introduced the theoretical basis of determining various water quality parameters byUV-Vis spectroscopy and expounds the complete spectral data analysis process, including datapreprocessing extraction of the characteristic wavelength ,establishment of absorbance and relevant water quality parameter model . In addition,the research progress on the determination of COD, heavy metal ions, nitrate nitrogen, and DOC by UV-Vis spectroscopy was described in detail.

2.6.1 Basic Principles-Lambert–Beer Law

The Lambert-Beer law is the basis of quantitative analysis of water quality parameters using UV-Vis spectroscopy. The measurement principle of the Lambert-Beer law relies on a beam ofmonochromatic parallel light that irradiates the surface of the tested medium (Jiang, Zhou, and Sharma,2013). After passing through the medium with a certain thickness, the medium

absorbs part of the light energy, weakening the intensity of the transmitted light passing through the medium. The absorbance of the absorption medium is directly proportional to its thickness .

Figure 12.Schematic diagram of the Lambert-Beer law



$$A = \log(1/T) = Kal$$

In Equation (1): A is the absorbance; T is the transmittance that the ratio of the intensity of the outgoing light (I) to the intensity of the incident light (I0); K is the molar absorption coefficient related to the nature of the absorbing substance and the wavelength of the incident light; a is the concentration of the absorbing substance in mol/L; and l is the thickness of the absorbing layer in cm.

2.6.2 Basic Principle of UV-Vis Spectrophotometry

UV-Vis spectroscopy is based on the fact that pollutant molecules in the water can absorb UV-Vislight of a specific wavelength. Light of specific wavelength is absorbed by electron movement fromthe ground state to an excited state, which reduces the amount of transmitted light. According tothe Lambert–Beer law, the absorption spectrum and the concentration of the substance have a strongcorrelation, which is the principle of UV-Vis spectroscopy being used to detect water pollutants .(Maisano, Majolino,Migliardo, Venuto,Aliotta andMagazú1993),pollutants discovered by absorption characteristics and yielded spectral curves

but most of them absorblight in the UV-Vis region. Therefore, by using the Lambert–Beer law as the theoretical basis, we can selectively detect the concentration of pollutants in water. Various parameters commonly used forwater quality detection, such as the spectral absorption range of NO_3-N and NO_2-N, are 200–250 nm.Organic matter and turbidity are s selectively absorbed within the range of 380–750 nm.

Table 2.Absorption spectrum range and substance characteristics of various substances.

Wave length (nm)	**Material Characteristics**
200-220	Nitrate, nitrite
220-250	Conjugated diene, unsaturatedaldehyde, unsaturated ketone
250-380	Organic matter
380-750	-

2.7. Hormones

Steroid sex hormones such as estrogens and progestin constitute a group of environmental emerging toxicants because of their endocrine disrupting properties (Eljarrat et al., 2009). They are chemical messengers naturally produced by the endocrine system of humans and animals. They are also known as C18 steroids as they are made up of 18 C atoms which are distributed in the 3 hexagonal (A-C) and 1 pentagonal (D) rings (Racquet, 2012). It is the different substitution in the cyclopentane-phenanthrene nucleus which differentiates these molecules.These hormones find their application in human medicine as well as in animal farming. Two hormones under study include progesterone and estradiol.

2.7.1 Norethisterone

Is a progestin medication used in birth control pills,menopausal hormone therapy and treatment of gynecological disorder .It is found in the drug class progestogen.Its chemical components include 17 α-ethyl-19-known or 17 α-ethynylestra-4-en-17 β-01-3-one.It is a synthetic estrane

steroid and derivative testosterone.Its drug type is synthetic oral progestin.(Berzas, 1997; Rathod& Captain, 2014; Zaidi et al., 2014).

2.7.2 How norethisterone works

Norethisterone mimics the actions of endogeneousprogesterone ,albeit with greater potency and is used on its own or in combination with estrogen derivatives in a variety of applications including contraceptionand hormone replacement therapy .It was originally intended for use as a remedy for irregular menstruation and endometriosis and was not marketed for use as a oral contraceptive until 1962.(Seifeldeen et al., 2016).

2.7.3 How it is administered.

Norethosterone is indicated as an oral concentraceptive ,when given as monotherapy or in combination with an estrogen component such as ethinylestradiol or estradiol .In combination with an estrogen component ,oral norethisterone is also indicated as a hormone replacement therapy in the treatment of post menauposal osteoporosis and moderate –to-severe vasomotor symptom arising from menopause,when applied via transdermal patch ,the combination of norethisterone and estradiol is indicated for the treatment of hyperstrogenism ,vulvovaginal atrophy and moderate –severe vasomotor symptom .(Sohrabi et al., 2010; Sohrabi&Mohammadpour, 2009).

2.7.4 Mechanism of Action.

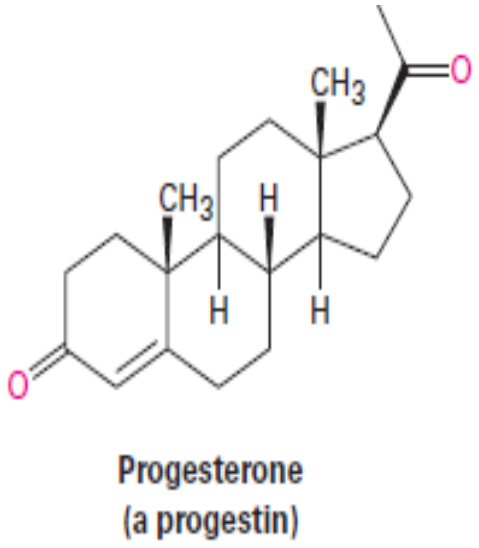
On molecular level, progestins like norethisterone exert their effects on target cells via binding to progesterone receptors that result in downstream changes to target genes. Target cells are found in the reproductive tract ,breast ,pituitary, hypothalamus skeletal tissue and central nervous system .Contraceptive efficacy is derived mainly from changes to the cervical mucus ,wherein ,norethisterone increases the cell content and viscosity of the mucus to impede sperm transport and migration.Norethisterone also induces a variety of changes to the endometrium,including atrophy .irregular secretion and suppressed proliferation ,that make it inhospitable for implantation .Working on via negative feedback loop,norethisterone also acts on both the hypothalamus and interior pituitary to suppress the release of follicle –stimulating hormone(FSH) and luternising Hormone from the anterior pituitary.Suppression these of these

hormones prevents follicular development,ovulation and corpus luteum developments.(Berzas, 1997; Rathod& Captain, 2014; Zaidi et al., 2014).

2.7.5 Root of elimination

Following administration of radio-labellednorethisterone ,slightly more 50% of the administered dose was eliminated in the urine and 20-24% was eliminated in faeces .(Jelić et al., 2012; Ying et al., 2002).Furthermore,norethisterone is excreted in the urine of mammals and also in faeces. It enters waterways through the effluent of sewage treatment plants or through run off of sewage sludge which is mainly used for agriculture. This sequence of events may lead to reproductive and developmental alterations in aquatic organisms such as feminisation and hermaphroditism (Barceló et al., 2006)

Fig13Norethisterone (Barceló et al, 2006)



2.7.6 Endocrine Disrupting Effects

Anthropogenic sources of natural hormones result in concentrations that can cause endocrine disrupting effects even at ngL^{-1} levels. Natural hormones present the greatest potency, and exposure to certain concentrations can cause disruption to endocrine systems that control development in aquatic organisms and wildlife (Damstra et al., 2002)

For example, the exposure of fish to 2 ng L^{-1} concentrations of E2 under laboratory conditions has been shown to induce measurable reproductive effects (Snyder et al., 2003). In addition the effects of these hormones has been shown to be additive. Exposure to such trace levels can harm early life stages of fish populations, including embryonic or larval development and these effects can only be detected in adulthood or in subsequent offspring. Other effects investigated include increased sex reversal, reduced hatch/birth rate, and disrupted breeding behaviour in wildlife.

2.7.7 Theoretical Basis of UV-Vis Spectrum Data Processing

The accuracy of the UV-Vis method used to detect water quality parameters is highly dependenton the established mathematical model. Therefore, the selection of an appropriate mathematicalmodel to improve the prediction accuracy has become the focus of water quality parameter studyin UV-Vis detection. The UV spectrum data processing is divided into four steps: spectrum datapreprocessing , characteristic wavelength extraction water quality parametermodel establishment , and model performance evaluation. In recent years,researchers have improved and innovated based on various algorithms in the field of UV-Vis spectrumdetection of water quality parameters and established a high-precision prediction model between waterquality spectrum data and water quality parameters. These studies have significantly contributed torapid, accurate detection of water quality parameters (Atamas,

Bardik,Bannikova, Grishina, Lugovskoi, Lavoryk, Makogonenko, Korolovych, Nerukh, Paschenko,2017)

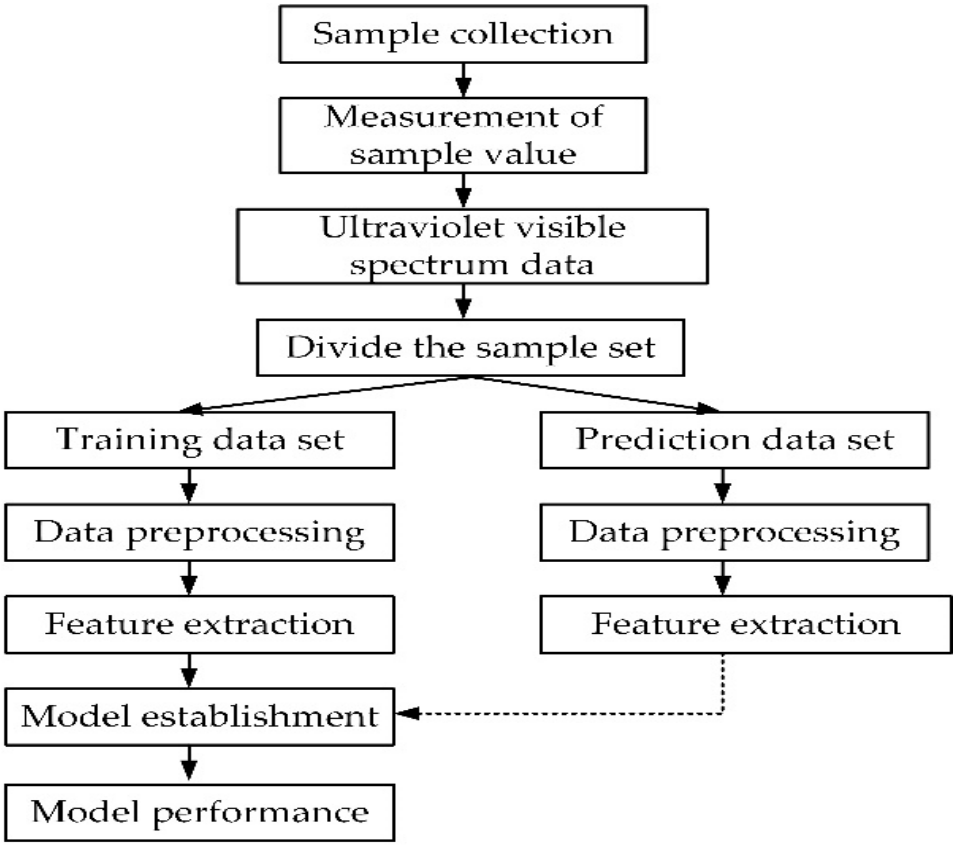
2.7.8 Data Preprocessing

During data acquisition, due to high-frequency noise, personnel operation, external environmental other factors of the instrument and equipment, some undesirable factors are often involved in the establishment of the model, which act as its prediction accuracy. This interference information cannot completely depend on the equipment modification and can be eliminated. Therefore, before the qualitative and quantitative analysis of spectral data, selective preprocessing shouldbe completed. The prediction accuracy of the model can be improved by selecting the appropriate reprocessing method.SinceSavitzky and Golay published their research results in 1967, the Savitzky-Golay (SG)smoothing filter has gradually become a broadly used method to improve the signal-to-noise ratio of most signals (Quan and Cai,2012) Savitzky, A.; Golay, M..Therefore, the SG smoothing filter plays an important role as a data preprocessing method for the UV-Vis spectrum. If the window size changes, the smoothing will be different.The larger the window is, the smoother the spectral curve becomes. However, with the increase in the window size, the spectral data also loses some information. (Quanet al.2012) proposed a time-domain method to analyze the estimation performance of SG filters, which included estimation performance of SG smoothing filters and SG differentiation filters

2.8 Method of Establishing Data Model

According to Lambert-Beer's law, the amount of spectral absorption has a strong correlation.With the concentration of water quality parameters, such that a high-precision prediction modelbetween water quality spectral data and water quality parameters can be established (Jing , L.; Yi, F 2018). The stepsof establishing the prediction model of water quality parameters was shown in Figure 4. Currently, the main methods of model analysis include partial least squares regression (PLSR) and support vectormachine regression (SVR).

Figure 14. Th**e steps of establishing a prediction model of water qualityYuchen**



CHAPTER 3: RESEARCH METHODOLOGY

3.1 Materials used

Three samples of waste water,250 ml of de-ionised water, Stopwatch/clock, electronic balance, filter paper,7 beakers,acetonitrile,pipette,84 ‘control’ pills,pestel and dish.

3.2 Instruments

Thermo Scientific Genesys 10S UV-VIS and electronic shaker,

3.3 Sampling site

Collection of samples was done at three different locations ,these were Mbare,(Magaba) ,Chitungwiza and Bindura. From each location two samples were collected at different sites. The first site is located in Chitungwiza along Seke road. The second is Mbare (Magaba) area adjacent to Matapi Flats where urban wastewater represents a significant input to the river water.The third site is a river in Bindura along Mt Darwin road .

3.4 Sample collection

Bill,Danny and Bsobby (2013) observed that,safety precaution procedures are to be followed when collecting samples .Special care should be followed not to contaminate the sample.A clean

pair of new ,non-powdered disposable gloves will be worn each time a different location is sampled.Sample collection activities shall proceed progressively from the least suspected contaminated area to the most suspected contaminated area. Wastewater samples were taken by directly filling the sample container by using a beaker. Samples were placed in appropriate labeledcontainers.Sample collection is necessary in order to obtain reliable data that can support compliance or enforcement activities.

3.5 Sampling techniques and Equipment

The sampling techniques and equipment are designed to minimize effects on the chemical and physical integrity of the sample. The sample should be collected where the wastewater is well mixed .Therefore the sample should be collected near the centre of the flow channel. at approximately 40 to 60 % of the water depth where thenturbulence is at the maximum and the possibility of solids settling is minimized.

3.6 Sampling type

3.6.1 Grab Sampling

Wastewater sampling is generally performed by one of two methods namely grab sampling and composite samplingNorethisterone is excreted in the urine of mammals and also in faeces. It enters waterways through the effluent of sewage treatment plants or through run off of sewage sludge which is mainly used for agriculture. This sequence of events may lead to reproductive and developmental alterations in aquatic organisms such as feminisation and hermaphroditism (Barceló et al., 2006).Grab sampling is just what it sounds like ,all of the test material is collected at one time.As such ,a grab sample reflects performance only at the point in time that the sample was collected and then only if the sample was properly collected.

3.7 Laboratorysetup

Family planning contraceptive tablets, ‘control’, were procured from a local Bindura Pharmacy. Distilled water used was from the Bindura University of Science Education Chemistry laboratory. The solvents used were acetonitrile ,HPLC grade by Merck, Germany.The Thermo Scientific Genesys 10S UV-VIS Spectrophotometer, equipped with 1cmquartz cuvettes from Beckmann Instruments Inc was used.

3.8 Solvent Solution

The solvent was prepared by mixing 40ml of acetonitrile and 60ml of distilled water.

3.9 Preparation of Stock Solution

Eighty-four ‘Control’ contraceptive tablets with norgestrel hormone were weighed and crushed to a fine powder using amortar and pestle and transferred to a 30 ml conical vial and 40ml of acetonitrile was added.After mechanical shaking for about 10 minutes ,the mixture in the beaker was put on the electrical laboratory shaker to increase the rate of dissolving the tablets for four hours. After having observed that dissolving was almost complete,the mixture was filtered into a clean beaker and residue was weighed and recorded using an electronic balance.

3.10 Standards Preparation

Appropriate and correct dilution of the standard stock solution was done when 50ml of the stock solution was transferred into a conical flask and was labeled as ‘stock solution.’25ml of the stock solution was pipetted into a beaker and was labeled 5 ,with the highest concentration.The solvent was pipetted into the beaker from where the first sample was extracted to the mark.Shaking was done to ensure that there was homogeneity of the solution.It was from this solution that 25ml of the solution was pipetted into a beaker labeled 4 .The procedure was consistently repeated until five samples were prepared using serial dilution method .The standard solutions were then

scanned in the spectrum mode of the instrument.The absorbance of norgestrel at different concentrations has been recorded on table 2.

3.11 Development of an analytical method for determining the quantities of norethisterone (norgestrel) from Water samples.

Collection of samples was done at three different locations ,these were Mbare,(Matapi) ,Chitungwiza and Bindura river. From each location one sample was collected at different sites. The first site is located in Chitungwiza along Seke road. The second is Mbare (Matapi) area where urban wastewater represents a significant input to the river water.The third site is a river in Bindura along Mt Darwin road .Raw water samples from the stated sites were collected in polythene bottles and were stored in the dark that had around temperature of around15^{o}C for one week until the time it was analysed.All the three water samples were filtered before analytical procedure started.Standards were put in cuvettes one at a time and the absorbance of each standard at different concentration were analysed in the Uv-Vis spectrophotometer until all standards were finished.About 60ml of raw sample water were measured and mixed with 40ml of acetonitrile.Three samples were set and analysed in the Uv-Vis spectrophotometer and results were recorded.

3.12 Quantification of aquatic environmental sample

Norgestrel in samples were quantified by the use of Uv-Vis spectrophotometer. UV Vis Calibration standards in the range of 1.25-20.00 mg/l wereprepared, their absorbances read on a UV-Vis spectrophotometer at a wavelength of 240nmand a calibration curve established by plotting concentration versus the absorbances. Theamount of norgestrel in the water samples collected were then quantified by using theinformation obtained from the calibration curve. Calibration standards of norgestrel in the range of 1.25-20.00 mg/l were prepared and read on the Spectrophotometer.A calibration curve was established by plotting concentrations versus mean area and theextracted water samples quantified by the use of the calibration curve. Blank samples were alsoread on the instrument.

Chapter 4 :Results and Discussion

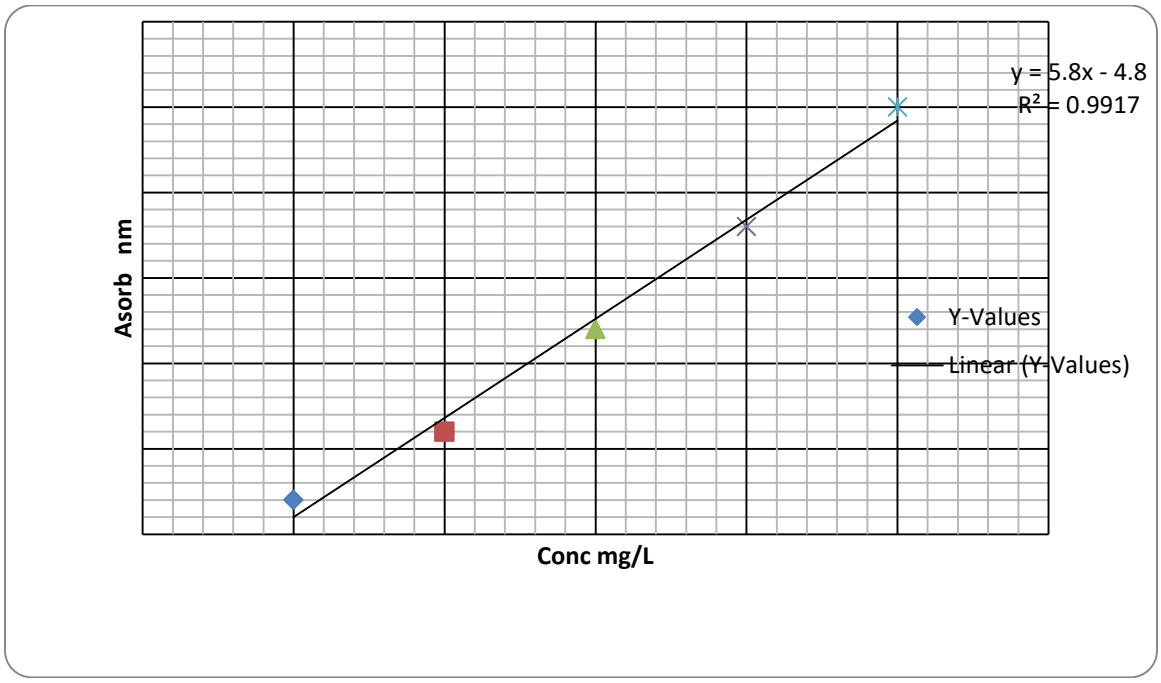
4.1 UV determination of standards

The norgestrel standards were determined using the uv-vis spectrophotometer and results obtained are shown on table 3 .The first standard used had concentration of 1.25mg/L ,it had an absorbance of 2.0nm ,the second standard whose concentration was 2.5mg/L had absorbance of 6.nm ,the third standard had 5.00 mg/L had absorbance of 12,the fourth standard had concentration of 7.50nm and had absorbance of 18 and the fifth standard had concentration of 10.0 and the absorbance was 25nm.The results shows that as concentration increases the Uv spectrum also increased,because of increased quantities of compounds in standards which absorbed infrared light, thus increased the absorbance.

Table 3: Linearity data of Norgestrel standards prepared.

Std	**Conc mg/L**	**Abs (nm)**
1	1.25	2
2	2.50	6
3	5.00	12
4	7.50	18
5	10.00	25

Fig 15 Calibration graph for Norgestrel standards



The correlation coefficient of 0.99 is positive. It is a strong positive correlation .From the above solutions of the standards prepared, 25ml were pipettedand concentration were made dilution by distilled water. Absorbance was checked for these concentrations for linearity.

4.2 UV determination of sample extracts

There were three samples of wastewater that were examined. The first sample was from the Bindurawhich had a maximum absorbance 0.033 nm with a concentration of 9.57 mg/l.Thesecond sample from Chitungwiza had 0.054 nm absorbance with a concentration of 14.82 mg/l. The third sample from Matapihadan absorbance of 0.077 nm witha concentration of 21.13 mg/l .There was considerable noisebeyond 250 nm, implying there were impurities .The

results of UV-Vis determination from the samples shows that the norethisterone(norgestrel) hormone is present in wastewater.The presence of norgestrel is in low concentrations as indicated by results obtained from the three samples analysed.The sample from Bindura had the least concentrations of norgestrellevels,followed by Chitungwiza sample and the highest concentration of norgestrel was found on the Matapi(Mbare)sample.The variations of concentrations from one city to the other indicates different levels of wastewater contamination by different population of people in these three cities.The more densely populated the city is ,the more the use contraceptive pills is ,hence the higher concentration is on wastewater.Matapi sample had higher concentration levels due to illegal squatters around the river and contribute to the high level of contamination in the river water through direct or defecation into the waters and along the banks of the river.The disposal of gabbage and pharmaceutical drugs increases contamination levels ,the contamination is even exacerbated by people from other surrounding residential areas who dump garbage along Mukuvisi River . The continued bursting of sewer pipes further expose the untreated sewer to the river banks just half a kilometer from Matapi.

Table 4.Showing Data of water samples using UV- Vis Spectrophotometer.

Sample	**Abs (nm)**	**Conc(mg/L**
1	0.033	9.057
2	0.054	14.820
3	0.077	21.130

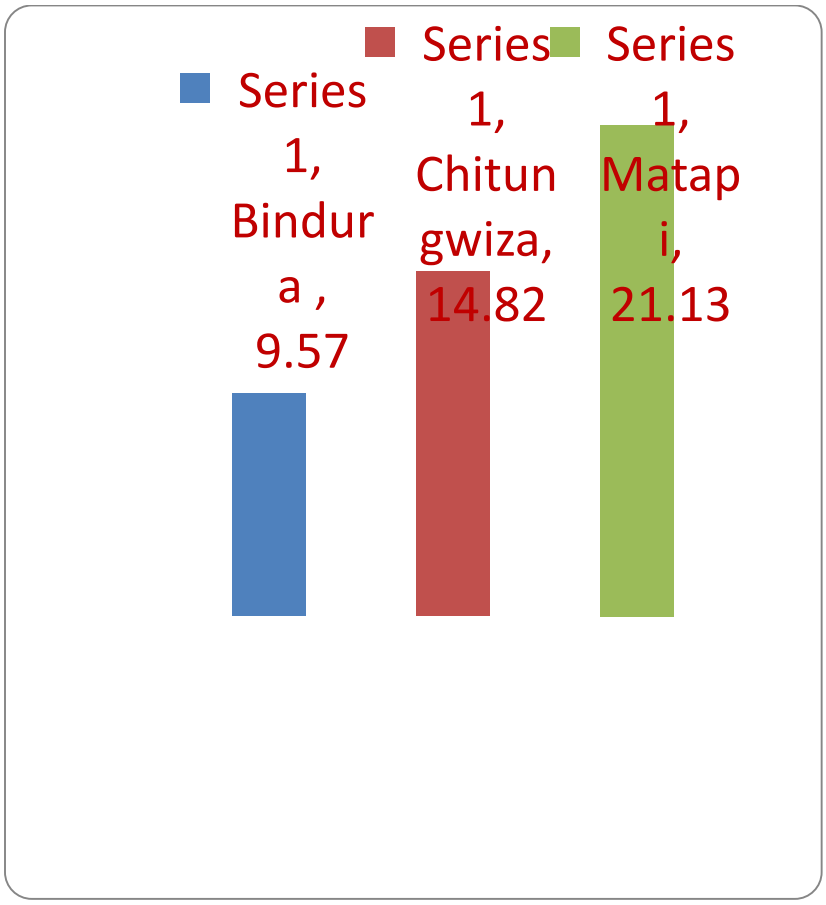
4.3 Validation

From the sample results shown on the 25ml was pipetted out concentrations were made,with dilution of distilled water.Absorbance was checked of these concentration for linearity .Norgestrel in sample 3 showed the maximum absorbance at wavelength of 0.077nm.

4.4 Wastewater samples results.

The results from the raw water from Bindura town river,Chitungwiza city and Matapi(Mbare) water samples were quantified and the results are shown in Fig. . It was observed that waste water from these sites had substantial amounts of norgestrel. However, the concentration of norgestrel varied with location, with Matapi water sample having the highest concentration.The concentration levels of norgestrel from the Bindura town river was low as compared to that of Chitungwiza and Matapi. The concentration levels of norgestrel from Chitungwia river was almost double the concentration levels of Bindura town river,this is because Chitungwiza is densely populated and the amount of discharging domestic raw sewage into rivers is very high.The concentration of norgestrel from Matapi was almost three times more than the concentration from the Bindura town river .The highest concentration of norgestrel was observed from Matapi water sample ,due to the fact that the residential area is densely populated and sanitation levels are very low,thus raw sewage is flushed into the river in large quantities from a densely populated area.Furthermore ,when people take medications,these drugs and their metabolites can be excreted and make their way to wastewater and this will subsequently increase their concentration and thereby negatively impact on the lives of aquatic plants and animals over a period of time.

Fig 16.A bar graph showing the variation on concentration in samples analysed by UV- Vis Spectrophotometer.



The presence of hormonal steroids in waste water indicates a serious threat to aquatic life. However ,this is a subject for further studies to determine the impact of hormonal steroids in aquatic life. .The levels of norethisterone (norgestrel) levels found were lower than those found by Nasuhoglu et al (2012),who obtained concentrations above 480mg/L .Furthermore the results obtained in the study were higher than those obtained by Labadie and Budzinski (2005) which were around 5ng/l.**Fig. 17**: UV Spectra of norgestrel obtained from (a) this study and (b) literature from Wyethstandard material.

Fig 17(a)

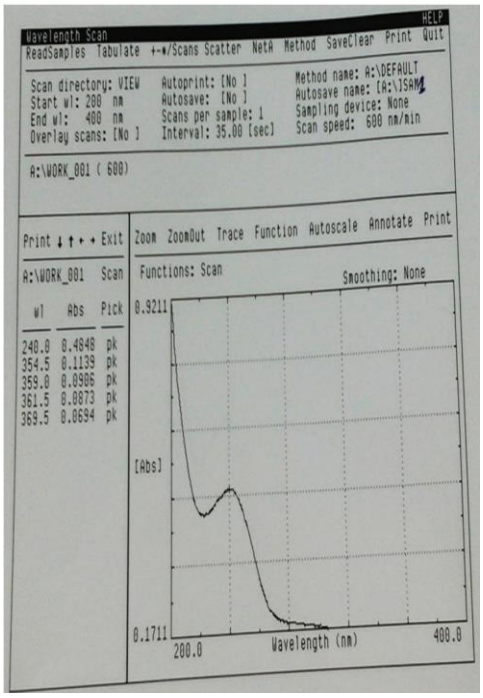
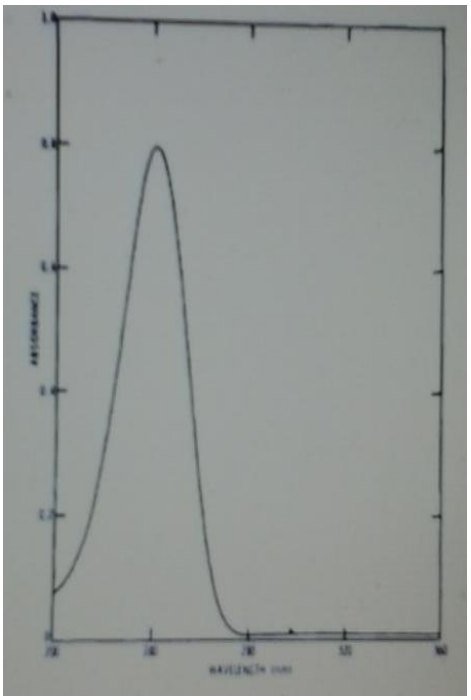


Fig 17(b)



The figures 17(a) shows a uniform pattern with the standard of material by Wyeth 17(b), an indication that the results obtained were in line with standards already established ,this vindicates the validity of the analysis carried out.

Chapter: 5 Recommendations and Conclusion

5.1 Recommendations

Although the analysis of hormones in Chitungwiza,Bindura and Mbare,was central to this study, the high deficiency of specific litrerature focusing on treatment of wastewater plant and analysis methods in Zimbabwe,has prompted the project to rely on international literature,devoid of local based examples. The following recommendations can help acquire information on occurrence of emerging organic contaminants in Zimbabwe.

From this study, it is recommended that:

- Further research is needed so as to be able to confirm that the standard obtained wastruly(norethisterone) norgestrel. Some instruments like NMR and LC-MS can be used to obtain moreinformation on the molecular structure.
- The additional compounds observed under the UV-Vis spectrophotometry should be identified by type and quantified.
- The persistence of these kind of hormones in environmental contamination should be studied by exposing norethisterone (norgestrel) to various methods of degredationlike microbial degradation, photolysis,photocatalysis and many other processes.
- Upgrading wastewater treatment plants to remove oestrogen –like pollutants .

- Conserving breeding and rearing habitats in unpolluted areas and maintaining connections between and within rivers and streams.

- The level of steroid hormonal accumulation on aquatic life ,particularly fish should further be studied.

5.2 CONCLUSIONS

The determination of norethisterone(norgestrel) levels in waste water was successful. The use of UV-Vis Spectrophotometer produced results which are similar to those in literature and the variation of concentration from one location to another indicates that pharmaceutical drug disposal differ from one residential area to another. It is therefore possible to develop standards of norethisterone(norgestrel) using the method stated above for the purpose of quantification of norethisterone in the environment. Researchersand other organizations in countries that are still developing like Zimbabwe can carry out research on detection and quantification of norethisterone in wastewater at a minimum cost without going through a bureaucratic procedure to get standards. During the research, it was observed that norethisterone levels vary in different environments and the highest concentration was evident in densely populated urban environments.

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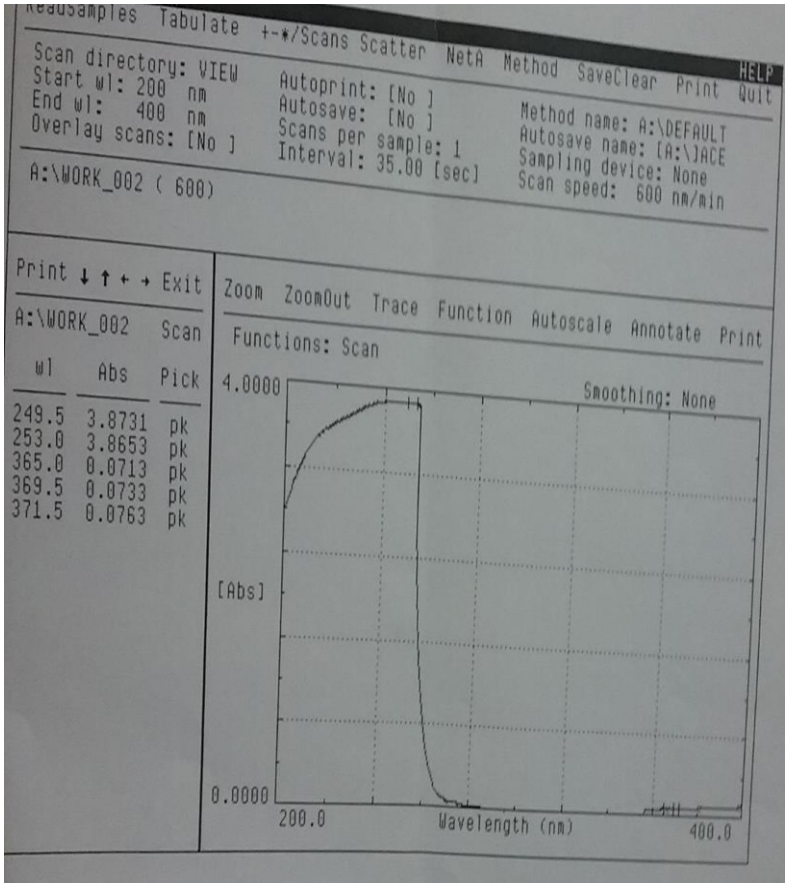
APPENDICES

Appendix 1 Sample results of norgestrel Standards.



Appendix 2

Test result for Norgestrel standards

TEST SETUP
GENESYS 10S UV-Vis v4.003 2L9Q129001

Standard Curve 13:28 29Jun22
Test Name NRM2018_KONZO
Date Standards Measured 29Jun22
Wavelength 342.0nm
Ref. Wavelength Correction Off
Curve Fit Linear Through Zero
Number of Standards 5
Units mg/L
Sample Positioner 1-Cell Platform
ID# (0=OFF) 1
Low/High Limits -9999/9999
Statistics Off
Auto Print Off
Auto Save Data On
Data File Name N

Std #	Conc. mg/L	Abs 342.0nm
1	1.250	0.002
2	2.500	0.006
3	5.000	0.012
4	7.50	0.018
5	10.00	0.025

Curve Fit Linear Through Zero
Slope 0.00364
Std Dev 0.009

Appendix 3

The Test results for the wastewater used.

ID#	Abs 342.0nm	Result mg/L
1	0.033	9.057
2	0.054	14.82
3	0.077	21.13

Appendix 4

Sample of spectra from Wastewater