

Application of a Biorelevant Dissolution Method for Intrauterine Device

by

Zhongfang Zhang

Shenyang Pharmaceutical University, BS, 2019

Submitted to the Graduate Faculty of
School of Pharmacy in partial fulfillment
of the requirements for the degree of
Master of Sciences

University of Pittsburgh

2021

SCHOOL OF PHARMACY

UNIVERSITY OF PITTSBURGH

This thesis was presented

by

Zhongfang Zhang

It was defended on

April 6, 2021

and approved by

Dr. Lisa C. Rohan, Professor, Pharmaceutical Sciences

Dr. Vinayak Sant, Associate Professor, Pharmaceutical Sciences

Dr. Sravan Kumar Patel, Assistant Professor, Pharmaceutical Sciences

Thesis Advisor/Dissertation Director: Dr. Lisa C. Rohan, Professor, Pharmaceutical Sciences

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ABSTRACT

There are currently a significant number of contraceptive options available for women. However, the unintended pregnancy rate is still high throughout the world. Low- and Middle-Income countries have disproportionate unintended pregnancy rates. Adverse health, social and economic consequences are associated with unintended pregnancy. About 5% of unintended pregnancies are due to contraceptive product failure. In contrast, the majority of unintended pregnancies are associated with incorrect and inconsistent product use. Thus, there is a desire and need for contraceptive products that limit the amount of ongoing effort required by the woman for efficient protection from unintended pregnancy. Compared with the oral contraceptives, which need to be taken daily, long-acting reversible contraceptive (LARC) methods (intrauterine devices (IUDs), implants, and injectables) offer women an option that can be effective without daily effort and decrease the burden on women's daily life. Among the LARC contraceptive options, hormonal IUDs, copper IUDs, and implants have the lowest failure rate (less than 1%). Therefore, the uptake of LARC contraception by women has significantly increased, especially in low-income countries. The majority of women in the US who are using LARC contraception use IUDs^[Citation error].

Development of IUDs requires evaluation of their drug release characteristics during the product usage time. Although some in vitro studies for IUDs have been reported, none of them simulate the biological environment of the uterus. Specifically, these non-biologically relevant

models, which have been previously applied, use organic solvents, higher temperatures, or surfactants, all of which do not simulate the uterine environment. Besides, none of these dissolution methods can simulate the in vivo dissolution rate.

The overarching objective of this work was to conduct biorelevant dissolution testing for marketed IUD products using in vitro methods that can simulate the in vivo release rate of the IUD. Hence through this project, a biologically relevant in vitro dissolution method was developed and applied to study the in vitro dissolution profiles for the levonorgestrel (LNG) containing IUDs: Mirena[®], Skyla[®], Liletta[®] and Kyleena[®]. To support this work, a liquid chromatography-mass spectrometry (LC-MS) method for the quantitation of LNG was also developed and validated.

Key words: Intrauterine devices, contraceptive, dissolution test, Liquid chromatography-mass spectrometry

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Preface

This work described in the thesis is a collaborative work under grant HHSF223201810188C. The collaborators within this grant include the U.S Food and Drug Administration (FDA), the University of Buffalo, and the Magee-Women Research Institute/University of Pittsburgh. The purpose of this thesis is to both introduce and demonstrate the development of a biorelevant in vitro dissolution method for intra-uterine devices and release profiles from these IUDs. The findings in this work will be helpful toward future development planned by the group for design of a physiologically based pharmacokinetic (PBPK) model for the female reproductive tract and also will also be useful for future development of generic IUD products. The data generated in this study may also be helpful when researching the extended use of marketed IUDs.

I would like to express my sincere gratitude to my dissertation advisor, Dr. Lisa C. Rohan, for giving me the opportunity to work on this important project. I am very fortunate and grateful to have gotten the opportunity to work with a professor who always motivates and cares for her students.

I would also like to thank Dr. Sravan Kumar Patel for his guidance and advice throughout my project. His kind guidance was important for my soft skill development and helping me to become an excellent researcher. Whenever I encountered a problem, he would always give me a lot of useful suggestions and support.

I would like to thank Dr. Vinayak Sant for serving on my thesis committee and giving me invaluable advice not only during my journal club and scientific course but also during my project.

I would like to thank Ms. Lin Wang for her help and support throughout the entire course of the project. She helped me with the experimental designs and gave me advice whenever I encountered a problem. Her support was essential in the completion of this dissertation.

I would like to take this opportunity to thank a number of other individuals as well: Dr. Alantary for all of the hard work in generating the preliminary in vitro release data, Mr. Phillip Graebing for all the training and help of LC-MS analysis, Dr. Valicherla for all of his help in method development and bioanalytical analysis, Ms. Christina Bagia for teaching me how to validate a HPLC method, Mr. Xin Tong for training me on all of the instruments throughout the laboratory, Mr. Prithivirajan Durairajan for helping me with dissolution setups and problems that arose during my projects, and Dr. Junmei Zhang for giving me valuable advice on the process of writing a thesis. I cannot thank all of the lab members in the Rohan Lab enough for their help and support. It is all because of them that I have completed my thesis and accomplished my goal.

I would also like to thank Dr. Maggie Folan, Ms. Lori Altenbaugh, and all the faculty, staff, and students at the University of Pittsburgh School of Pharmacy.

Finally, I would like to thank all my friends and family in the United States and back in China. I want to thank my parents for giving me all the support and love. Thank you.

1.0 Introduction

1.1 Unintended pregnancy and unmet need for contraception

Unintended pregnancy is defined by Centers for Disease Control and Prevention (CDC) as a pregnancy that is either unwanted or mistimed. It can carry serious consequences on the women's physical and mental health, a child's health and development issues, and financial burden to the health care system. Each year in the U.S, there are more than 3 million unplanned pregnancies, and almost half of these result in abortion².

A study also indicated that during the COVID-19 pandemic, the probability of unintended pregnancy potentially increased due to difficulties associated with accessing contraception. Nearly 12 million women in poorer countries lost access to contraception during the pandemic, leading to 1.4 million unplanned pregnancies³. Particularly, lower-income countries such as east and middle Africa have a higher risk of unintended pregnancy compared to higher-income countries such as the US and countries in Europe. Generally, women living in the poorest regions of the world are nearly three times as likely to face an unintended pregnancy as those in the richest regions ⁴.

There are 61 million U.S. women of reproductive age, and about 43 million of them are sexually active^{5,6}. A total of 885 million women of reproductive age living in low and middle income countries would like to avoid pregnancy, while 214 million of them have an unmet need for modern contraception^{7,8}. This unmet need for contraception will cause a significant amount of unnecessary medical costs. The annual medical costs of unintended pregnancy in the United States was approximately 4.6 billion dollars, and 53% of those unintended pregnancies were due to

imperfect contraception adherence¹. It is encouraging to note that the rate of unintended pregnancy has declined as more diverse contraception options became available. Furthermore, the healthcare cost decreases significantly when women switch from oral contraceptives to long-acting contraceptives (LARC). Studies demonstrated that even if a LARC is not used for the entire duration, it is still the most cost-effective contraceptive method within three years of use compared to no method or short-acting reversible contraceptive methods^{9,10}.

These findings emphasize the importance of making contraception more affordable and convenient for every woman in the world. According to the World Family Planning 2020, the number of users of traditional methods (condoms, oral contraceptive) remain around 85 million¹¹. There is not only a need to improve access to effective contraceptive methods but also a need to enhance women's knowledge of all the contraceptive methods, especially those LARC that are more effective and convenient to use.

1.2 The unintended pregnancy rate and its negative consequences

Worldwide, only 62.7% of women use any form of contraception, including 72.4% of women in developed areas, and 61.2% of women in less developed areas¹². This causes 85 million unintended pregnancies annually. These pregnancies result in 50% abortions, 12% miscarriages, and 38% unplanned births⁴.

Abortions will have many adverse effects on women's health. Women may have anxiety symptoms and lower self-esteem and life satisfaction after receiving an abortion¹³. Some unsafe abortions may even cause maternal death, especially in developing countries where people are in poverty and lack sanitation. Compared to planned pregnancy, unintended pregnancy also has a greater risk of causing miscarriage, which in turn causes elevated levels of anxiety and depression.

Major depressive disorder has been reported in 10-50% of women after miscarriage¹⁴. Psychological symptoms could persist for six months to 1 year after the miscarriage^{14,15}. Unintended pregnancy may even cause abridged educational careers, labor-market struggles, and higher crime rates in society¹⁶.

The leading cause of unintended pregnancy is the nonuse of contraception, followed by 43% due to incorrect use of contraception and 5% due to method failure¹⁷. While oral contraception is widely used among women, adherence to this method varies from 19% to 100%^{18,19}. Multiple studies have shown that routine counseling, daily text-message reminders as well as health education information will increase patients' adherence to oral contraceptive pills and contraceptive injections. Incorrect use of contraception happens mostly to condom users. Long-acting reversible contraception has the lowest failure rate due to minimal effort needed by women to follow the regimen²⁰.

1.3 Overview of contraceptive methods

There are multiple contraceptive methods: condoms, oral contraceptive pills, emergency contraception pills, IUDs, contraceptive implants, contraceptive injections, vaginal contraceptive film and gels, contraceptive rings, diaphragms, and sterilization. Among these different contraceptive methods, the contraceptive CHOICE project indicated that the failure rate of oral contraceptives, patches, and vaginal rings is 20 times higher than the failure rate of IUDs and implants²¹. The contraceptive methods can be classified into four types based on the mechanism of action: (1) Barrier methods: condom, diaphragm, and cervical cap; (2) Nonhormonal methods: sponge, gel, film, suppository, and copper IUD; (3) Hormonal methods: Injectables, patch, implant, and intravaginal ring, hormonal IUD; and (4) Sterilization. Among these different

contraceptive options, a broad review has confirmed the hierarchy of contraceptive effectiveness in descending order: female sterilization, LNG IUD and implant, copper IUD, other coitally independent hormonal contraceptives (oral contraceptives, patch, vaginal ring), and coitally dependent methods such as condoms, spermicides and the rhythm method²².

Some contraceptive options will be briefly reviewed below, and a more detailed description of IUDs will be provided in the next section.

Male and female condoms

The condom is the only form of contraception that affords protection from both sexually transmitted diseases (STDs) and unintended pregnancy. Male condoms are simple to use, inexpensive, and widely available but their use requires partner's involvement. A female condom is a pouch that's inserted in the vagina, which is a great tool for women to control their protection needs without having to rely on partner involvement²³. Clinical studies showed that using a female plus male condom is more effective than the use of male condom only in preventing STDs²⁴⁻²⁷.

The efficacy of female condoms is 75% to 82% under typical use and 95% under perfect use²⁸. Typical use means when usage is not always correct or consistent, whereas perfect use refers to consistent and correct usage. This method is not only hormone-free but also can be used on-demand, which provides more convenience compared with other contraceptive methods. According to the CDC data, during 2011-2015, 23.8% of women and 33.7% of men aged 15-44 used a condom at last sexual intercourse in the past 12 months. However, disadvantage of this method is that significantly reduced sexual pleasure²⁹. This indicates why condoms sometimes are not used properly during intercourse, and they are associated with a higher-than-expected failure rate for contraception.

Diaphragm and cervical cap

Diaphragm and cervical cap are on-demand contraception methods. A diaphragm is a small, flexible silicone dome-shaped cap placed inside the vagina intended to stop the sperm from entering the uterus. It can form a physical barrier to block sperm from reaching eggs³⁰. Diaphragm can be used alone or together with a spermicide. However, the presence or absence of spermicide was found to have no significant difference in the pregnancy rates or discontinuation rates³¹. For the diaphragm to take full effect, it needs to stay in place for at least six hours but no longer than 24 hours after sex. Caya[®], a single-size contraceptive device, is the first new diaphragm design to enter the U.S. market in 2015. If the user applies the diaphragm correctly, 92%-96% of pregnancies can be prevented³².

The cervical cap is a silicone cup that needs to be inserted into the vagina six hours prior to sexual activity to prevent pregnancy. It comes in different sizes to fit the users. There's only one brand of cervical cap available in the U.S. today, which is the FemCap[®]. The FemCap[®] cervical cap comes in three sizes: the small cap is for people who've never been pregnant; medium cap is for people who've had an abortion, miscarriage or a cesarean delivery; large cap is for people who've been given birth vaginally. A comparison study between FemCap[®] and diaphragm has shown that the FemCap[®] was not as effective as its comparison diaphragm.³² The failure rate was found to be 8.4% in a clinical trial, and 89% were satisfied with the method^{33,34}

Sterilization

Sterilization is the surgical irreversible means of contraception. It includes tubal sterilization in women and vasectomy in men³⁵. Tubal sterilization is performed by sealing the fallopian tubes and thus preventing contact between the ovum and sperm³⁶. Hysteroscopic tubal occlusion involves placement of micro-inserts into the fallopian tubes through a vaginal approach^{37,38}. Vasectomy is male sterilization achieved by sealing or cutting off the ductus (vas)

deferens tubes that transport the sperm cells out of the testes³⁹. The failure rate of vasectomy is 0.15%, whereas the failure rate of tubal occlusion is 0.5%⁴⁰.

Sterilization has widespread use in America. It also has a low risk of surgical complications³⁷. Even though infections, bleeding, and scarring of the fallopian tubes may occur after the surgery. Notably, a rare case after sterilization is that the tubes will heal and the fetus will implant in the fallopian tube, thus leading to an ectopic pregnancy⁴¹.

Tubal sterilization is the only permanent method of contraception, yet some women can sometimes regain fertility depending on the method of tubal occlusion, age, and other factors. Additionally, tubal sterilization has little effect on women's sexual function or known long-term adverse health effects. However, expression of regret and requests for reversal are experienced by some women.

Spermicidal vaginal contraceptive films, gels, sponges and suppositories

The vaginal contraceptive film[®] (VCF[®]) is a translucent square of material containing the active ingredient Nonoxynol-9 (N-9). N-9 is a nonionic surfactant that can immobilize sperm by disrupting the cell membrane. It is widely used in different vaginal dosage forms: suppository, gel, and film⁴². The VCF[®] is a two-inch square soft soluble film which is manually inserted into the vagina at least 15 minutes before sexual intercourse. It dissolves into a gel after placement.

One advantage of the VCF[®] is that it does not require removal by the user and thus there is no associated concerns with product disposal after use. However, repeat use of the N-9 containing vaginal film was shown to induce epithelial damage, which can increase the risk of some sexually transmitted diseases⁴³. Furthermore, VCF[®] has a relatively higher failure rate (21% after typical use and 6% in perfect use)⁴². A systematic review summarizes the literature on the effect of spermicides on prevention of gonorrhea, chlamydial infection and HIV. For gonorrhea

and chlamydial infection, researchers have found spermicide may have a potential protective effect in several observational study⁴⁴. However, further larger clinical studies need to be done to prove this conclusion.

Spermicide active agents can be incorporated into different dosage forms in order to provide more diverse choices for women. Some options include gels, inserts, and the vaginal sponge. The vaginal contraceptive gel is a semi-solid system containing spermicide. Some women find these products desirable given there is no systemic exposure to the active and gel products can provide lubrication during sexual intercourse. There are a number of vaginal inserts available which contain spermicidal agents. The oval-shaped vaginal contraceptive suppository Pharmatex[®] is a solid dosage form that can dissolve at body temperature at a pH of around 4. This non-hormonal contraception is appealing for lactating women who have concern with using hormone-containing products⁴⁵. Finally, the birth control sponge is a small piece of white plastic foam that is inserted into the vagina. It can be inserted up to 24 hours before sex. The Today sponge[®] is made of polyurethane foam and is coated with N-9.

Rhythm method

The rhythm method is also called the calendar method. Women can track their menstrual history to predict when they will ovulate. For women who are seeking a natural method for contraception, Natural Cycles is the first FDA-cleared contraceptive app. The rhythm method is hormone-free, non-invasive and women can predict their fertility by measuring their body temperature. However, the failure rate of using rhythm is relatively higher than other contraceptive methods, ranging from 15% to 18.5%⁴⁶.

Withdrawal method

The withdrawal method is also called coitus interruptus. Contraception by this method

requires removing penis from the vagina prior to ejaculation. The goal of the withdrawal method is to prevent sperm from entering the vagina. Withdrawal method is a natural and side-effect free contraceptive method but sometimes men cannot pullout in time and end up unintended pregnancy. The contraceptive effectiveness of the withdrawal method is 78%⁴⁷.

Mechanism of hormonal contraceptive agents: menstrual cycle, ovulation and hormones

The contraceptive methods discussed above are hormone free. However, the majority of contraceptive methods with a failure rate lower than 10% are all hormone-containing. Before introducing hormonal contraception methods, background information on how hormone regulated by menstrual cycle will be provided.

The menstrual cycle has four distinct phases: menstruation, the follicular phase, ovulation, and the luteal phase. Menstruation is considered as the first phase of the cycle; the follicular phase is the beginning of egg formation. The pituitary gland releases follicle-stimulating hormone (FSH) which can cause the formation of 10 to 20 follicles, during this period. Only one of the follicles will develop into a mature egg, while the other follicles will break down and be reabsorbed by the ovary. During this phase, the ovary produces estrogen, which causes the endometrium to thicken. When estrogen levels are increased in the body, the hypothalamus releases gonadotropin-release hormone (GnRH). The release of GnRH causes the pituitary gland to produce increased level of LH. This abrupt increase of LH triggers ovulation, which indicates the end of the follicular phase. During ovulation, the egg enters the fallopian tube and moves towards the uterus. If the egg is not fertilized, it will break down within 24 hours. However, sperm retains its motility in cervical mucus for seven or more days after insemination.

During the luteal phase, the remnants of follicles will release progesterone and estrogen, which thicken the lining of uterus. If fertilization does not occur, progesterone level decreases and

the corpus luteum breaks down. The shredded uterine lining flushes out of the vagina and forms menstruation.

The rapid drop in estrogen-to-progesterone ratio suggests the luteinization of the ovarian follicle. In addition, ovulation correlates with LH peak. This demonstrates how hormones control the ovulation. The increased amount of estrogen will inhibit pituitary production and secretion of FSH and LH. The decreased level of FSH and LH further leads to the inhibition of the follicular development or ovulation. Increased level of progesterone also causes inhibition of ovulation and thickening cervical mucus. These biological effects of estrogen and progesterone demonstrate the methodology of hormonal contraceptive methods.

The oral contraceptive pill

Oral contraceptive pill is one of the most important women's health achievements of the twentieth century. They were approved for use in the USA in 1960 and meanwhile also used in many European countries. Approximately 25% of women of reproductive age currently use birth control pills, which is the most commonly prescribed form of contraception⁴⁸.

If patients can perfectly adhere to the daily regimen of oral contraceptives without missing a dose, it has the potential to be 99% effective. However, the real-world efficacy of combined oral contraceptives(COC) is 91% because of lack of perfect use by most patients⁴⁹.

Generally, there are two types of oral contraceptive pills: combined estrogen-progesterone and progesterone only pills. Combined hormonal tablets contain both progestin and estrogen. Progestin is the synthetic form of the body's naturally-occurring hormone progesterone. Progestin is primarily responsible for preventing pregnancy in three ways: (1) Prevention of ovulation: Progestin can decrease the secretion of Luteinizing hormone and follicle-stimulating hormone. In which scenario, the follicle will not be developed, and the estradiol level will not increase, resulting

in the prevention of ovulation. (2) Thickening of mucus: Women's cervical mucus varies dramatically throughout the menstrual cycle. Prior to ovulation, cervical mucus is more fluid. After ovulation, mucus becomes thicker. Progestin can increase the viscosity and cell content of cervical mucus and alters its molecular structure thus inhibits the sperm from penetrating through the cervix.⁵⁰ (3) Endometrial atrophy and decrease of tubal motility: Progestin suppresses the proliferative activity of the endometrium and decreases the frequency of uterine contractions⁵¹.

Estrogen can inhibit follicular development because of its negative feedback on the anterior pituitary but its effect is less prominent compared to progestin. It primarily controls menstrual bleeding and stabilizes the endometrium to prevent breakthrough bleeding⁵²⁻⁵⁴. Women's body can make three main types of estrogen: Estradiol, Estriol and Estrone.

Three generations of oral contraceptives have been developed to improve the efficacy for contraception and the safety for women. The contraceptive effect of progesterone was firstly investigated in 1937 when it was subcutaneously injected into a female rabbit and shown to inhibit ovulation⁵⁵. Since progesterone is poorly soluble and inactive orally, a synthetic progestational agent was developed, which paved the road for the revolution of contraceptives⁵⁶. The first combined hormonal oral contraceptive pill has 150mcg mestranol and 10mcg norethynodrel. It has significant side effects such as cervicitis, increased vaginal discharge, and dysmenorrhea⁵⁷. Since some clinical case studies found estrogen could increase the risk of arterial and venous thromboembolism, the amount of estrogen in combined oral contraceptive pills was reduced during the development. The contemporary COC always contains 10 to 35 g of Ethinyl estradiol paired with progestational agents that vary in potency and interaction with estrogen⁵⁸. The synthetic progestins used for contraception so far are structurally related to either testosterone or progesterone. Progestins derived from testosterone are usually associated with more androgenic

side effects such as acne, male pattern baldness, and weight gain while progestins derived from progesterone and spironolactone could selectively bind to progesterone receptors and minimize androgenic side effects⁵⁹.

Many factors will affect women's adherence to oral contraceptives; these can be divided into three categories: 1) patient-related factors which includes patient lifestyle, cost, side effects, and partner support; 2) health care provider-related factors including the quality of communication and information provided by physicians and guidance provided by pharmacists; and 3) health care system-related factors including insurance reimbursement and access to the private healthcare providers⁶⁰. Real-world evidence shows that many women did not receive sufficient information from their health care provider after missing doses of their oral contraceptive products⁶¹. This further points out the importance of providing comprehensive information regarding the impact of missed doses by the healthcare providers.

One of the most common side effects of oral contraceptive pills is irregular bleeding. Some studies report that more serious side effects such as venous thrombosis may even happen in older women. Besides, combined oral contraceptives with desogestrel, gestodene, or drospirenone are associated with a higher risk of venous thrombosis than the oral contraceptive containing LNG. However, progestogen-only pills were not associated with an increased risk of venous thrombosis⁶². One case-control study even illustrated that the risk of systemic lupus erythematosus was higher in women who use combined oral contraceptives⁶³. Interestingly, another case control study indicated that women using oral contraception were more likely to have recurrent candida vaginitis due to the higher frequency of intercourse⁶⁴. A recent study in the UK pointed out that hormonal oral contraceptive may have a negative effect on mental health, especially in women who are vulnerable to depression and anxiety⁶⁵. Further clinical studies are needed to investigate the

mechanism underlying this phenomenon. Moreover, the utilization of combined oral contraceptives increases the odds of overweight/obesity by two times among reproductive women after controlling for potential confounders⁶⁶. LNG half-life is significantly longer and that needs a longer time to reach steady-state in obese women due to the suppression of their hypothalamic-pituitary-ovarian system are suppressed⁶⁷. Women weighing more than 70.5kg will have 1.6 times increased risk of oral contraception failure⁶⁸.

Contraception pills can also be utilized in other diseases. A prospective study in the UK has shown that women using combined oral contraception will have lower death from ischemic heart disease, ovarian cancer, circulatory disease, and all other diseases⁶⁹. Women who use oral contraceptives will also have 50% less chance of having endometrial cancer and higher bone mineral density⁷⁰. Sexual function may also be elevated, such as orgasm frequency, arousal, and enjoyment of the sexual activity⁷¹⁻⁷⁴. Women may also use combined oral contraceptives to reduce acne, premenstrual syndrome such as mood swings, fatigue, irritability, and depression can also be relieved by oral contraception⁷⁵.

The contraceptive injection

Depot-medroxyprogesterone acetate (Depo-Provera[®]) is a highly effective contraception option that has been available in the United States for almost two decades. The perfect use of Depo-Provera[®] is 99% effective, however, the real-world efficacy is 94%⁷⁶. Injection contains the synthetic progestogen and will be applied to either in the bottom or in the upper and lower arm. The contraceptive effectiveness is up to 8 weeks or 13 weeks. Women need to inject contraceptives regularly to maintain effectiveness.

There are two formulations currently available in the United States. The standard formulation of Depo-Provera[®] containing 1mL of medroxyprogesterone acetate aqueous

suspension 150mg/mL is given intramuscularly. Another lower-dose formulation of Depo-Provera® (104 mg/0.65mL) can be given subcutaneously. Two large phase 3 studies assessed contraceptive efficacy, safety, and patient satisfaction with the lower-dose subcutaneous formulation. Zero pregnancies were reported in both studies, even in overweight or obese women⁷⁷. A pharmacokinetics study of these two formulations illustrated that the ovulation suppression effect could last longer than 13 weeks and was not affected by body mass index or race⁷⁸.

Like other hormonal contraception, the contraceptive injection may have side effects such as weight gain, headache, mood swings, and irregular bleeding. These side effects and ovulation suppression efficacy may continue up to one year after the injection. This further raises the biggest concern of having contraceptive injection: time of getting back to fertility. One clinical study has shown 68% of women can conceive after 12 months, 83% of women can conceive within 15 months, 93% can conceive after 18 months of the last injection. The median time to contraception for those who do conceive is ten months after the last injection^{79,80}. Several studies indicate that bone mineral density tends to decrease after long-term use but will finally stabilize after 4-5 years of use. The bleeding pattern will also change over one year of use of the injection. Notably, amenorrhea incidence will increase from 26% during the first three months to 55% over one year⁷⁷.

While not available in the United States, there are also combined injectable contraceptives available in Africa & Asia, Latin America under different brand names. They contain progestin and estradiol. Lunelle® was the first and only combined injectable contraceptives in the US which contained 5 mg estradiol cypionate and 25 mg medroxyprogesterone acetate. Since it contained less hormone, women would be able to return to fertility more rapidly⁸¹. However, the Lunelle® syringe was recalled by Pharmacia in 2002 due to the lack of full potency and potential risk of

contraceptive failure⁸².

The transdermal patch

Transdermal drugs are self-contained dosage forms. One transdermal patch should be applied once a week for three weeks, followed by one week without any patch. The transdermal contraceptive patch is a thin, beige piece of plastic film adhered to a user's skin, usually containing a combination of estradiol and Norelgestromin (NGMN)⁸³. It was shown to be a reliable contraceptive method, with a method failure rate of 0.7% through 13 cycles of use⁸⁴. Compared with oral contraceptives, patches have higher estrogen levels but reduced variability. The rates of breakthrough bleeding in patch users are lower compared with oral contraceptives users due to lower drug peak concentration, which makes patches a more appealing contraceptive method⁸⁵.

Side effects of the transdermal patches are primarily similar to the combined oral contraception. The most complained side effects of the transdermal patch are headache and nausea. The side effect unique to contraception patches is application site reactions, such as skin irritation. 20% of the patch users have complained about this but only led to 2.6% of discontinuation⁸⁶. Studies have indicated that heat, humidity, and exercise will not affect the adhesion of the transdermal patch⁸⁷. The rate of complete or partial detachment of contraception patch is only around 2%⁸⁸.

Ortho Evra[®] is the first transdermal patch approved for contraception. It contains 6mg of norelgestromin (NGMN) and 0.6 mg of ethinyl estradiol (EE) and releases 150 µg/day and 20 µg/day, respectively⁸⁹. It is no longer available in the U.S. after the FDA approved Xulane[®], a generic hormonal birth control patch, but still available in Europe and Canada.

Xulane[®] is the only available contraceptive transdermal patch in the US. It was developed by Mylan Pharmaceuticals. Xulane[®] is a 14 cm patch containing 4.86 mg NGMN and 0.53 mg EE.

It will release approximately 150 µg/day NGMN and 35 µg/day EE⁹⁰. Pooled data from the clinical studies demonstrated that the steady-state of serum drugs are reached within two weeks of application²⁵.

The contraceptive implant

The contraceptive implant is a small, flexible rod that is placed under the skin in a woman's arm. Both insertion and removal of the implant need health care professional involvement, which usually takes 5 to 10 minutes. There is a low incidence of removal complications. Nexplanon® has a polyethylene vinyl acetate (PEVA) core and contains 68 mg of etonogestrel. The implant is 40 mm long and has a diameter of 2 mm, and the contraceptive effect can be up to three years⁹¹. The release rate of the etonogestrel is 60-70 µg/day initially after the insertion but will progressively decline to 25-30 µg/day at the end of use⁹². Serum levels of Etonogestrel (ENG) at three years are 60-65% of those levels measured firstly after insertion. ENG can inhibit the ovary from releasing the egg and thicken the cervical mucus making it difficult for sperm to enter. Nexplanon® can prevent pregnancy for three years, and the failure rate is only 0.05%. The cumulative discontinuation rate of Nexplanon® after one year is 18%, which is approximately half the discontinuation rate of oral contraceptive pills^{93,94}. Women can return to fertility immediately after removal of the implant. The one-year pregnancy rate after stopping the implant is between 76.5% to 85.6%⁹⁵⁻⁹⁸.

ENG subdermal implant is clinically effective and safe. A systematic review study has shown that 57% to 97% of women will continue to use this method, and discontinuation is mainly attributed to the disturbances in menstruation⁷⁶.

Jadelle® has been registered in more than 47 countries for five years of use; it contains 75mg of progestin. The removal problems occur less frequently with Jadelle® compared with

Norplant⁹⁹. A two-rod implant Sinoplant[®] is approved in China and registered in 19 countries. It contains 150 mg LNG. Cumulative probabilities of pregnancy with Sinoplant[®] are only 0.9% and 1.06% in two clinical trials for four-year use¹⁰⁰. Sinoplant[®] is much more cost-effective, which is 60% less than the cost of Jadelle[®].

Intravaginal ring

Intravaginal rings are non-biodegradable soft and flexible polymeric rings made of ethylene-vinyl acetate (EVA), which provide controlled release of estrogen and progestin. The ring is flexible, soft, transparent with a diameter of 54 mm, a thickness of 4 mm, and will not cause any damage to the vaginal tissue¹⁰¹. Pregnancy rates with the intravaginal ring appears to be low. The failure rate was 1.18% in a large, international and multicenter observational trials¹⁰². Clinical studies have demonstrated that there are no colposcopic or cytological changes observed in vaginal ring users even though some women have complained about the increase of vaginal discharge¹⁰³. While *Candida* yeast was shown to adhere to vaginal ring *in vitro*, a clinical study did not show an increased risk of *Candida* yeast in vaginal ring users and the vaginal ring did not change the bacterial ecology^{104,105}.

The intravaginal ring marketed in the United States is NuvaRing. It releases 120 µg etonogestrel and 15 µg EE per day, and is used for three weeks followed by one week free of the ring¹⁰². Three weeks' use of intravaginal ring will completely inhibit ovulation based on follicular diameter and progesterone concentrations¹⁰⁶. The maximum serum concentrations of ENG and EE were achieved in one week after insertion of NuvaRing. The maximum serum concentrations of ENG and EE were 40% and 30% of those achieved for the combined oral contraceptive^{78,92}. However, the absolute bioavailability of NuvaRing was higher for etonogestrel and similar for ethinylestradiol compared with oral contraceptive⁹². Another vaginal ring is Annovera[®], is a

silicone elastomer device that contains segesterone acetate and ethinyl estradiol. This vaginal ring is reusable for 13 cycles. It is inserted in the vagina for 21 continuous days and removed for seven days each cycle.

Compared with oral contraceptives, NuvaRing has better control of the menstrual cycle and also better bleeding patterns¹⁰⁷. However, it may have local adverse effects such as leukorrhea, vaginal discomfort, and vaginitis¹⁰⁸.

Recently, 3D printers are utilized to develop different vaginal systems with personalized shapes, such as “O,” “Y,” and “M” shaped vaginal rings^{109,110}. The intravaginal ring is also a good platform for multi-purpose prevention (MPT) for human immunodeficiency virus (HIV-1), Herpes simplex virus (HSV-2), human papillomavirus (HPV). Lately, an extended-release vaginal ring has incorporated antiviral drug dapivirine and contraceptive drug LNG as an MPT for combined contraception and HIV prevention¹¹¹.

Emergency contraception

There are two main types of emergency contraception (EC): oral methods and copper intrauterine devices. The most commonly used oral methods are LNG 1.5 mg Plan B[®], and ulipristal acetate (UPA) 30 mg Ella One[®] as single dose. Both oral and intrauterine methods appear to have low failure rates: ~2–3% for oral LNG EC, ~1.4% for UPA EC and 0.09% for Copper IUDs, respectively¹¹². LNG EC can inhibit the Luteinizing hormone surge thereby disrupting the ovulatory process and they do not induce an abortion or interrupt an established pregnancy. UPA EC can also prevent ovulation, besides delaying follicular rupture for at least five days¹¹³. The Yuzpe method uses a combined oral pill containing both estrogen and progestin, but it is less effective and causes more side effects than LNG or UPA EC. Clinical trials found that the

pregnancy rate among users of the Yuzpe method was 3.2% compared to 1.1% in the LNG EC group¹¹⁴.

EC's side effects are usually transient and mild, such as nausea and vomiting. The most common side effect of EC is changes in the menstrual period. If LNG ECs are taken early in the menstrual cycle, they shorten the cycle length. However, when they are taken later in the cycle, they may prolong the length of the cycle or have no effect on cycle length¹¹⁵.

1.4 IUDs

IUDs have played an essential role in the development of contraception. Nowadays, IUDs are the most widely used method of reversible birth control¹¹⁶. The distribution of IUD users worldwide is 83% in Asia, 8% in Europe, 4% in Latin America, 4% in Africa, and 1% in the United States¹¹⁷. However, IUD is especially a method surrounded by misconceptions among women. Many women think it may cause infertility or it is not indicated either for young or nulliparous women and others express great fear about the insertion procedure¹¹⁸.

Except for female sterilization, LNG IUDs and implants are the most effective among the various contraceptive options²². Unlike female sterilization, Copper LNG, LNG IUD and implants are reversible, therefore, they are more attractive to some women. A hormonal IUD works for 3 to 5 years, depending on the brand. A copper IUD works for up to 10 years and can be used as emergency contraceptive if inserted within 120 hours, while an implant works only for up to 3 years. They are all highly effective, well tolerated, and require only a one-time insertion. Once women get the copper IUD, they do not even need to worry about their birth control for up to 12 years. This is a particularly suitable option for women who do not want to get pregnant.

1.4.1 Anatomy and physiology of uterus

The uterus is the primary female reproductive organ located between the bladder and the rectum. It is connected distally to the vagina and laterally to the uterine tubes. It responds to hormones and produces changes to allow for the implantation of a fertilized egg or menstruation. In nonpregnant women, the uterus weighs between 30 and 80gm. Uterus measures 7.5cm, 5cm, 2.5cm in length, breadth, and thickness respectively¹¹⁹.

The uterus subdivided into three segments, namely: the body, the cervix and the fundus. The larger upper pear-shaped part of the uterus is called the body. The fundus is the broad curved upper body of the uterus, connected to the entry point of the uterine tubes. The body of the uterus is the site for implantation of the blastocyst and can enable the growth of the embryo and fetus. The uterus body has three layers. They are the endometrium, myometrium, and perimetrium from innermost to outermost. The endometrium is a glandular mucous membrane and forms the epithelial layer of the uterine cavity. The upper third of the endometrium lining will be shed off during menstruation. The blood supply to this layer is rich and consists of small basal arteries. The myometrium, the middle layer of the uterine wall, is a thick, smooth muscle layer. It undergoes hypertrophy and hyperplasia during pregnancy in preparation to expel the fetus at birth. Finally, the peritoneum is a double-layered membrane linked to the abdominal peritoneum.

The cervix is the lowermost part of the uterus linked with the vagina and is a fibromuscular structure 2cm in length. The endocervix is the upper portion of the cervix beneath the uterus; it can create mucus and forms a barrier to the uterine cavity. The lower ectocervix is joined to the vagina and is lined by squamous epithelium. The cervical canal is a passage through which sperm travel to fertilize an egg cell after sexual intercourse. Under the influence of estrogen, the cervix undergoes changes in position and texture during the menstrual cycle. When ovulation approaches, the cervix becomes softer and rises to open in response to the higher levels of estrogen. Glands in

the endocervix could produce 20-60mg of cervical mucus a day and increase to 600mg around the time of ovulation. Cervical mucus contains 93% of water, some proteins such as mucins, and electrolytes such as calcium, sodium, and potassium, and trace elements like zinc, copper, iron; fatty acid, and enzymes¹²⁰.

The uterus is supplied mainly by uterine and ovarian arteries. The uterine arteries are the main blood vessels that supply blood to the uterus. They give off branches that play an important role in maintaining blood supply during the menstrual cycle and during pregnancy.

1.4.2 History of IUDs

An IUD is a small T-shape plastic/metal polyethylene device inserted into the uterus to provide contraception.

Precursors to IUDs were first marketed in 1902. It was developed from stem pessaries and it occupied both the vagina and the uterus. This stem pessary was also known as “interuterine device”. However, this kind of device was associated with high rates of infection¹²¹.

The world’s first IUD was a ring made of silkworm gut by Richter in 1909. Dr. Graefenberg attached a silver wire to the silkworm ring in order to prevent infection, and the pregnancy rate of this device was 3%¹²². However, silver in the silver wire was absorbed by to the bodies of women and the women who inserted silkworm rings developed gingival argyrias. He then tried using “German Silver” wire which was made from a mix of metals. In 1949, Dr. Mary Halton wrapped the silkworm gut around her finger and then placed the ring into a gelatin capsule. The gelatin will liquify, and the thread will spread out. The pregnancy rate after using this modified ring was the lowest at 1.1%. Since 1960, various kinds of IUDs have been developed. In 1960, Dr. Margulies invented a new device in the shape of a coil made of polyethylene. In 1962, Dr. Jack Lippes developed the Lippes Loop. It was a simple plastic device that was pushed through an inserter and

came in different shapes. Some of them had big success, while others caused severe complications. In 1969, Dr. Howard Tatum tried to decrease the size of the IUD and made a simple plastic T, but this device had a pregnancy rate of 18%. That same year, Dr. Jaime Zipper found copper's contraceptive effect, which revolutionized the IUD effectiveness¹²³. In 1970, Dr. Antonio Scommegna devised a T-shaped device with progesterone and was approved by FDA after almost thirty years¹²⁴. In 1971, A. H. Robins Company marketed an IUD called the Dalkon Shield. However, since the IUD was not sealed on either end, the string would funnel bacteria into the uterus. More than 300,000 lawsuits were filed against A. H. Robins, forcing the company to file bankruptcy. In 1980, the Dalkon Shield IUD was recalled from the market. Subsequently, IUD usage dropped significantly, and only the progesterone T remained on the American market¹²⁵.

In 1988, a new copper IUD: ParaGard, was developed, with a long effectiveness for up to 10 years. The first levonorgestrel-releasing IUD Mirena[®] was available for use in the U.S. in 2001. In 2013, a new low-dose hormone IUD, Skyla[®] by Bayer, was approved by the FDA. This device is designed to prevent pregnancy for three years. Liletta[®] developed by Medicines 360, was firstly approved in 2015; it can be used for prevention for up to 6 years. Bayer developed Kyleena[®], which was approved in 2016. The failure rates of these IUDs approximately range from 10% to 2%^{126,127}.

1.4.3 Nonhormonal and hormonal IUDs

Hormonal IUDs release progestin, a synthetic version of the hormone progesterone. Progestin thickens the mucus in the cervix, which prevents the sperm from reaching the ovum. Progestin can also thin the lining of the uterus, thus making the ovum hard to implant in the uterus.

Copper IUDs can both disrupt the motility and morphology of sperms and also create an immune response that will decrease the number of sperm reaching the uterine cavity and fallopian tubes. They are used by more than 170 million women globally¹²⁸.

1.4.3.1 Nonhormonal IUDs

Paragard[®] is the only nonhormonal copper IUD available in the US. It is recommended to be used for those women who have a medical contradiction to progesterone (such as breast, uterine, endometrial cancer, and heart or liver disease) or women who have general concerns of hormones. With correct use, it can protect the user from pregnancy for up to 12 years with a failure rate of 0.7% (first year), 1.3% (fourth year), and 2.1% (tenth year). The failure rate will increase over 12 years because of the decreased amount of copper, but the cumulative 12-year failure rate is still low. Copper IUD works primarily by disrupting the mobility and morphology of the sperm. Copper ions released from the IUD will reach concentrations in the luminal fluid that will inhibit sperm motility and block activation of acrosomal enzymes in the sperm head needed for the sperm to penetrate through the zona pellucida. It will cause sterile foreign body reaction in the uterine cavity including biochemistry and cellular changes, which can influence the number of sperm reaching the uterine cavity and fallopian tubes. The change can also damage the sperm itself, causing head-tail separation, which will make the sperm unable to fertilize the egg.

Copper IUD can also be used as a method of emergency contraception. It can be placed within 120 hours of unprotected sex, and the pregnancy rate is < 0.1%, which makes them an excellent form of contraception but awareness of this needs to be improved^{129,130}.

One concern of copper IUD is that it may increase the risk of having systemic exposure to copper. Women may experience side effects such as menorrhagia, dysmenorrhea and metrorrhagia,

pain, and cramps. These side effects may be due to the burst release of copper ions¹³¹. They may also cause mood changes such as anxiety and cognitive impairment.

The CHOICE study found that 23%, 35.8%, and 44% of copper IUD users discontinued the use of this method after 24 months, 48 months, and 60 months, respectively. A clinical study compared the continuation rate between copper IUD and LNG IUD. At four years, LNG IUD's continuation rate is 45.1%, and copper IUD's continuation rate is 32.6%. At five years, the continuation rate was 28.1% for LNG IUD, and 23.8% for copper IUD. There was a significant difference in LNG IUD and Copper IUD at four years but not at five years¹³². The discontinuation of IUD is due to different reasons; the most common reasons are heavy menstrual bleeding, lower abdominal pain, recurrent vaginal infections, and inability to feel the string¹³³.

Another device, the Mona Lisa NT Cu380 Mini, has been marketed in Europe since 2014 and is available in Canada, Germany, France, and eight other countries. There are four different IUDs designed for women with different uterine cavity depths. In addition, their effective contraception duration differs from each other: between 3 years and 10 years.

The frameless IUD eliminates the use of the frame like that gives conventional IUDs their T-shape. This new design can reduce discomfort and expulsion, and discontinuation rates. GyneFix[®] is a small frameless copper IUD with an efficacy of five years. Compared with other conventional IUDs, GyneFix[®] does not increase menstrual blood loss.

1.4.3.2 Hormonal IUDs

LNG IUD is suitable for women who cannot take estrogen, for example, women who have blood clots, cancer, heart or liver disease, or stroke. The LNG IUD can increase the thickness of cervical mucus, thin the lining of the uterus, and inhibit sperm movement¹³⁴. LNG IUD is a

reversible contraceptive method. The majority of women can conceive during the first three months after IUD removal^{135,136}.

Given the expulsion rate and bleeding profile of copper IUD, LNG IUD is better accepted by women, especially in nulliparous women. Currently, marketed IUDs are Liletta[®], Kyleena[®], Skyla[®], and Mirena[®]. All the commercial LNG IUDs use the polydimethylsiloxane (PDMS) matrix base. A removal thread is attached to the end of the stem. A silver ring is present at the end of the device in order to be detected using ultrasound.

The effect of LNG is primarily locally but LNG will also be absorbed in the systemic circulation. Placement of IUD will cause endometrial suppression in most endometria; histological features include decidualization of stroma, atrophy of endometrial glands, inflammatory stromal cell infiltrate, and glandular metaplastic changes^{137–139}. Endometrial suppression has been observed up to 7 years after insertion¹⁴⁰. The local effects of LNG IUD include decidualization of the endometrial stromal cells and glandular surface atrophy, and increased production of insulin-like growth factor and glycodeilin A. These local effects of LNG are not affected by the systemic level of estradiol.

Maximum plasma levels are reached within the first few hours after the placement of LNG IUD and plateaued at 150 to 200 pg/mL¹⁴¹. Compared with other contraceptive methods, LNG IUD has the lowest plasma concentration^{95,142}

The effects of IUD administration on ovarian function are dose dependent. Some women will experience anovulation, and others may have a normal proliferative phase but an inadequate luteal phase. Some women's ovulatory effects are completely unaffected. The amenorrhea caused by LNG IUD is not due to central suppression but a direct effect on the endometrium. LNG will

also disturb the midcycle Luteal Phase (LH) surge, thus cause disturbance of the corpus luteum function and rupture of the follicle.

1.4.4 Advantages and disadvantages of IUDs

IUDs are highly effective and can be used by nulliparous and lactating women. Menorrhagia, dysmenorrhea, and polymenorrhagia are the major complaints and among the reasons why women discontinue this method. It will not interfere with sexual intercourse and is suitable for women who cannot use estrogen-containing contraceptives.

1.4.4.1 Advantages and characteristics of IUDs

Even though there are some disadvantages and concerns of IUDs, their advantages outweigh the disadvantages. IUDs are the longest-lasting contraceptive method and also the most cost-effective forms of contraception available today with rates of failure similar to sterilization. The benefits of IUDs include high efficacy, ease of use, reversible nature, and high patient satisfaction, especially with time commitment for long-term use and cost. In addition, they do not interfere with sexual activity and breastfeeding.

IUDs have specific advantages. Once the device is inserted, the method does not depend on any continuing action of the user. The efficacy can be up to 12 years with a failure rate smaller than 1%¹⁴³. After removal, there is rapid return to fertility, with 1-year life-table pregnancy rates of 89 per 100 for women younger than 30¹⁴². IUDs can be used in adolescents, and the LNG IUD has many noncontraceptive benefits including the treatment of dysmenorrhea, pelvic pain and heavy menstrual bleeding. In addition, the LNG IUD is an effective tool for suppression of menses. About 15% to 20% of women become amenorrheic 1 year after insertion¹⁴².

Since 1990, the LNG IUD has played an essential role in the development of contraception; there are LNG IUDs under four different brand names. Nowadays, IUDs are the second widely used contraceptive method. Mirena[®] was first marketed in Finland in 1990 and is now available in over 120 countries. Mirena[®] has been studied in a lot of clinical trials that have demonstrated efficacy, acceptability, and safety in both parous and nulliparous women. The overall continuation of Mirena[®] is over 90% at the first year, which further confirms its tolerability. The contributing factors for discontinuation of Mirena[®] include side effects, such as irregular bleeding, a decrease in menstrual bleeding, abdominal pain, breast tenderness, and acne. Even though most women will experience amenorrhea after inserting IUD, they actually consider this as a positive effect. Mirena[®] is the only LNG IUD that is prescribed for Menorrhagia. As the first-line treatment option for heavy menstrual bleeding, Mirena[®] can subsequently improve the hemoglobin and ferritin level. Perforation is a severe side effect but rarely happens. Mirena[®] will cause morphological changes such as glandular atrophy and pseudo-decidualization after application. Mirena[®] has 52 mg of LNG that is designed to be released at 20 µg/day for five years. Until 2020, FDA has approved the supplemental New Drug application for Mirena[®] in order to extend the indication for up to 6 years of contraception¹⁴⁴. Currently, the Mirena[®] Extension Trial is ongoing to study the efficacy and safety of using Mirena[®] for up to eight years. As the first LNG IUD introduced to the market, Mirena[®] has paved the way to developing other IUDs with lower doses.

Liletta[®] (Levosert) is a new LNG IUD designed to release the same daily amount of LNG as Mirena. A one-year clinical study compared the performance between these two IUDs, and there were no differences between menstrual blood loss, ferritin, and hemoglobin, and endometrial thickness¹⁴⁵. Liletta[®] is known as Levosert[®] in Europe. Similar to Mirena, Liletta[®] can also be used to treat heavy menstrual bleeding¹⁴⁶. The main difference between Liletta[®] and Mirena[®] is

the T-frame. The buds at the end of the horizontal arms of Liletta[®] are more flattened, and its vertical stem is thinner. The loop at the end of the vertical stem and the hole in it are both smaller. The thread of Liletta[®] is also different in composition compared with Mirena[®]. The thread is made of polypropylene dyed with phthalocyaninato (2-) copper for Liletta[®], and polyethylene with 1% ferric oxide for Mirena[®]¹⁴⁷. Compared to Mirena[®], Liletta[®] is more affordable because it was developed by Medicine360, which is a nonprofit organization that aims to provide more affordable medicinal care for women.

Skyla[®] and Kyleena[®] are the smallest IUDs. Given their smaller size, they can make the insertion easier for those women who have smaller uterus, such as adolescents and perimenopausal women.

Skyla[®] is indicated to prevent pregnancy for up to 3 years and only has 13.5 mg LNG. Its arm is 28 mm, and the stem is 30 mm long. The T-shape frame is made of polyethylene. The monofilament tail-string is the same as Mirena[®]. The outer diameter is 3.8 mm. Currently, there is no data on the efficacy that illustrates the extended use of Skyla[®].

Kyleena[®] is the same size as Skyla[®] but releases 17.5 µg/day of LNG. Kyleena[®] is different from the other IUDs by a blue-colored removal thread and a visible silver ring on the ultrasound. Notably, Kyleena[®] has a higher frequency of vulvovaginitis (24%) and ovarian cyst (22%) compared with Mirena[®] (10.5% and 7.5%), respectively¹⁴⁸.

Summary of differences between these LNG IUDs are listed below:

Table 1 Comparisons between different brand of LNG IUDs

	Mirena[®]	Skyla[®]	Liletta[®]	Liletta[®]
Hormone	LNG	LNG	LNG	LNG

Active ingredient (dose)	52mg	13.5mg	19.5mg	52mg
Frame size(mm)	32 x 32	28 x 30	28 x 30	32 x 32
String color	Brown	Brown	Blue	Blue
Dose per day	20µg/day	14µg/day	17.5µg/day	18.6µg/day
Approved period of usage	5-7 years	3 years	5 years	6 years
Short term side effects	Spotting and irregular bleeding for 3-6 months			

1.4.4.2 Disadvantages of IUDs

The biggest concern of insertion of LNG IUD is how long it will take for women to return to fertility. The current literature shows that 70-75% of women will conceive within one year after the removal of IUD. However, the rate of infertility for IUD users may be twice as high as the rate of infertility in the general population¹⁴⁹. Factors such as age, semen parameters, and history of pelvic inflammatory disease will also play an important role in fertility potential. Studies that examine women's fertility are currently insufficient or lacking.

The most common side effect of IUD is changing the menstrual bleeding pattern, probably an increase in irregular and or prolonged spotting after the initial use of IUD, but this tends to decrease after long-term use. Women usually experience a decrease in bleeding over time; more than 50% of women might experience amenorrhea and oligomenorrhea. Pelvic pain is another adverse effect reported in IUD users. Women who experience pelvic pain will have a higher risk

of discontinuation of IUD. Generally, the expulsion rate of IUD ranges from 2% to 10% for all IUD users¹⁵⁰.

There are concerns about upper-genital tract infection related to the IUD. Patients who have gonorrhea and pelvic inflammatory disease should avoid the IUD placement. Fifty years ago, a study showed that insertion of IUD might contaminate the endometrial cavity with bacteria.¹⁵¹ The monofilament of IUD might facilitate the ascent of bacteria as well. However, some clinical trials compared the infection rate between IUDs with or without a string; there is no increase of risk associated with the appendage^{152,153}. Women with gonorrhea or chlamydial infection having an IUD inserted have a higher risk of salpingitis than those uninserted women¹⁵⁴. However, further study has shown that there is no substantial increase in the risk of pelvic inflammatory disease after insertion of IUD¹⁵⁴. The relationship between the risk of having pelvic inflammatory disease (PID) and insertion of IUD varies in different publications. But an observational study indicated that women who have a lower risk of STD also have a lower risk of PID.

Expulsion rates were 2.2% for LNG IUD users and 5.5% for copper IUD users because the copper IUD is more likely to cause heavy menstrual bleeding and cramping compared with LNG IUD users¹⁴¹. However, LNG IUD users and copper users have similar psychological and sexual functions. IUD use by women with HIV infection is also under investigation. Copper IUD does not increase cervical viral shedding of HIV. The combination of condoms and IUDs should be an appropriate method for HIV-infected women¹⁵⁵. IUD has also been found to have no effect on HIV transmission from female to male¹⁵⁶.

In addition, one study indicates that copper-IUD users are more likely to have abnormal vaginal flora and Bacterial Vaginosis (BV) but, another study showed that the occurrence of

abnormal vaginal flora, BV, Candida Vaginitis did not increase after IUD insertion¹⁵⁷. Even though some women reported that vaginal discharge has increased, this is not due to infection.

Malposition is one of the severe side effects for women who want to have an IUD. The overall incidence of IUD malposition is only 10% to 25%. Ultrasound is the diagnostic tool to determine the location of IUD, and copper IUD is easier to detect since they have strong echoes with ultrasound. Women who have mispositioned IUDs will have more complaints about pain and bleeding.

Another side effect is a migration of IUD: IUD is present outside of the uterine cavity, such as migration to other areas of the pelvis or abdomen. There are also cases that IUD perforated into the bladder and thus caused urinary tract infections.

Uterine perforation during the insertion of an IUD is uncommon. A pelvic X-ray is usually performed for confirmation of uterine perforation. It can occasionally result in sepsis or hemorrhage. However, the majority of uterine perforations do not cause any significant long-term damage.

1.4.5 Prevalence of current use and future use of IUDs

The number of women relying on female sterilization has increased from 195 million to 219 million between 1994 and 2019, and the number of women used IUD as a contraception method has risen from 133 million to 159 million. The use of IUD varies significantly between different countries. It is especially higher in Western Europe. There is increasing use of IUDs by women aged between 30-34 years¹⁵⁸.

In the U.S, the IUD market size was worth 1.5 million dollars in 2020 and is estimated to grow to 1.9 million dollars by the end of 2025. Since the rising awareness of IUD and increasing inclination towards planned pregnancy, the IUD market is expected to have an appealing market

expansion in the future¹⁵⁹. Based on the Global Market Insight, there will be a 2.7 billion dollars market share until 2027¹⁶⁰.

1.4.6 Applications of IUDs in other indications

Besides contraception use, IUDs are also be used to treat heavy menstrual bleeding, menorrhagia, endometriosis, and endometrial cancer. They are also considered as a tool to suppress menses, which is especially important for disabled women who have less tolerability of menses and those who face hygiene problem during menses.

Menorrhagia

Menorrhagia is heavy menstrual bleeding lasting for longer than seven days, which happens in about 30% of women¹⁶¹. The LNG IUD can reduce the menstrual blood flow by 86% to 97%. One study found that 64% of the IUD users are satisfied with this treatment for idiopathic Menorrhagia, and 77% of them would like to continue¹⁶². According to the PLAM-COEIN algorithm, Menorrhagia is attributed to a variety of causes, including structural and non-structural pathologies. Mirena[®] is licensed for the treatment of “idiopathic Menorrhagia,” which means, heavy menstrual bleeding without identified pathology. 30% of hysterectomies are performed to alleviate heavy menstrual bleeding; LNG IUD serves as a more conservative treatment compared with hysterectomy. Besides, the costs for the hysterectomy were three times higher than the IUDs¹⁶³.

Adenomyosis

Adenomyosis is a condition in which endometrial tissue exists within and grows into the uterine wall. It will have a negative impact on women’s fertility. Since LNG IUD can cause decidualization and atrophy of the endometrium, it can down regulate the estrogen receptors in endometrial stromal tissues. The LNG IUD is a more effective treatment for adenomyosis

compared with a combined oral contraceptive pill and in addition, improves the pain and bleeding over time¹⁶⁴. The use of IUD can also be helpful in reducing the recurrence of endometriosis.

Treatment of pelvic pain associated with endometriosis and adenomyosis

Endometriosis is the presence and proliferation of endometrial tissue outside the uterine cavity¹⁶⁵. It is often associated with painful periods, painful intercourse and infertility, and excessive menstrual bleeding¹⁶⁶. Oral contraceptives are considered as the first-line treatment of endometriosis¹⁶⁷. LNG IUD is also a potential option for long-term treatment for endometriosis patients, and it can also relieve noncyclic pelvic pain^{168,169}. One study showed that the women using LNG IUD would feel a significant reduction in the recurrence of the painful period after having surgery for endometriosis¹⁷⁰. A continuing study including patients with chronic pelvic pain indicated that the score of pain and ratio of severe pelvic pain decreased significantly compared with after the placement of LNG IUD¹⁷¹.

Endometrial hyperplasia

Endometrial hyperplasia thickens the uterus lining that is considered to increase the risk of endometrial carcinoma. Women develop endometrial hyperplasia due to estrogen stimulation but not enough progesterone¹⁷². LNG IUD is highly effective in treating endometrial hyperplasia, and women can experience the beneficial effect within one year. In addition, 96% of women can experience endometrial regression¹⁷³. A comparison study between oral medroxyprogesterone and LNG IUD indicates that LNG IUD has a success rate of 84% in treating endometrial hyperplasia, which is more effective than oral pills with a 50% success rate¹⁷⁴. It should be noted that a clinical study has shown 13.5 mg LNG IUD was proven to be an excellent therapy option for low and medium-risk endometrial hyperplasia but not for high-risk endometrial hyperplasia¹⁷⁵.

Endometrial cancer

Endometrial cancer is the most common gynecologic malignancy among women in the US¹⁷⁶. Women who have endometrial cancer are usually treated with surgery and the survival after five years is around 70%¹⁷⁷. LNG IUD also has a protective effect on endometrial cancer risk. The insertion of IUD can cause inflammatory actions on women's body thus eliminate the hyperplastic endometrial epithelial cells. In this case, it decreases the hyperplasia of the endometrium, a known risk factor of endometrial carcinoma. LNG IUD also demonstrated the feasibility of using IUD in the early stage of endometrial cancer. Several case studies have shown that IUD appears to eradicate some endometrioid cancer in women who have perioperative morbidity^{178–180}.

Adjunct to estrogen replacement therapy

Hormone replacement therapy (HRT) is indicated for relieving menopausal symptoms. Women during menopause will experience hot flashes and vaginal dryness because of reduced estrogen levels. Estrogen is usually prescribed to relieve the symptoms, and progesterone is used to counteract the proliferative effects of estrogen on the endometrium¹⁸¹. There are different types of hormone therapy that provide a diverse combination of hormones: 1) estrogen-only HRT, which is only recommended for women who have their uterus and ovaries removed. In this case, progesterone is not necessary; 2) cyclical HRT, which is usually given before menopause; 3) continuous HRT: a combination of estrogen and progesterone prescribed after menopause; and 4) local estrogen: Vaginal tablets, creams, or rings which can relieve urogenital symptoms¹⁸². LNG IUD is usually used as an endometrial protectant and a good alternative for the progestin part. One clinical practice also used transdermal estradiol combined with LNG IUD to treat menopausal symptoms¹⁸³.

Adjuvant treatment with tamoxifen for Breast Cancer

Tamoxifen is an essential treatment for breast cancer and also the first cancer chemopreventive approved by the FDA in 1977¹⁸⁴. Oophorectomy, hypophysectomy or adrenalectomy is usually used for breast cancer patients to reduce estrogen secretion. With the introduction of tamoxifen, the need for those surgical procedures was reduced.¹⁸⁵ However, women who use tamoxifen for more than two years always have a greater risk of endometrial cancer. A randomized clinical trial found that the LNG IUD prevented the development of endometrial polyps and hyperplasia in patients who receive tamoxifen for over one year¹⁸⁴. Furthermore, another study confirmed that the use of the LNG IUD would not increase the risk of breast cancer.¹⁸⁶

Structure and composition of LNG IUD

LNG IUD is a controlled release drug system. The structure of LNG IUD makes it a combination of monolithic and reservoir systems. The LNG is dispersed in the PDMS matrix and is surrounded by a PDMS membrane. There are three different mechanisms that the drug can be released from the matrix: drug diffused from a non-degraded polymer, drug release due to polymer degradation and erosion, enhanced drug diffusion due to polymer swelling, erosion, precipitation, or degradation¹⁸⁷. For LNG IUD, there are three steps that contribute to the drug release rate from the IUD; the first step is drug dissolution upon contact with the release media. The second step is drug diffusion through the PDMS matrix; The third step is drug diffusion through the outer membrane. Among these different mechanisms, drug diffusion is the main mechanism¹⁸⁸. The mechanical strength and the young's modulus of the PDMS membrane are thickness-dependent¹⁸⁹. The complex structure along with different release mechanisms makes the drug release rate vary during the product usage time. For example, the release rate of Mirena[®] is reduced by 50% after five years of use, the release rate of Skyla[®] is reduced by 64% after three years, and Kyleena[®]'s release rate is reduced by 57% after five years of use¹⁸⁸.

1.4.7 Drug release mechanism and kinetics of IUDs

The release rate of IUD is determined by the amount of LNG and the characteristic of the hormone-containing membrane. Given the complex structure of LNG IUD, the release rate of LNG IUD declines during the product usage time. From the clinical study, the initial release rate releases rate is 20 $\mu\text{g/day}$. After one year, the release rate decreases to 18 $\mu\text{g/day}$. After five years of usage, the release rate decreases to 10 $\mu\text{g/day}$ ¹⁴⁴. Kyleena[®] in vivo release rate is 17.5 $\mu\text{g/day}$. After one year, the release rate is 9.8 $\mu\text{g/day}$. After five years, the release rate is 7.4 $\mu\text{g/day}$ ¹⁹⁰. For Skyla[®], at first, in vivo release rate is 14 $\mu\text{g/day}$ in the first year. But, one year later, the release rate reduces to 6 $\mu\text{g/day}$. At the end of the approved year of use, the release rate is 5 $\mu\text{g/day}$ ¹⁹¹. Liletta[®] has the same dose as Mirena[®], but the release rate is slower than Mirena[®]. The initial release rate is 19.5 $\mu\text{g/day}$ followed by 17 $\mu\text{g/day}$ after one year. The average release rate in vivo is 14.3 $\mu\text{g/day}$ over a period of 6 years¹⁹². The release mechanism is not well established because it is difficult to sample with human subjects. Furthermore, the clinical studies also have a limited number of samples, so they might not represent the true release profile of the IUD. The total amount of drugs released by the end of the lifetime of all three IUD products is 40%-60%. Therefore, it is critical to develop a reliable in vitro model to mimic the in vivo drug release profile.

Drug release from IUDs can be fitted with various kinetics model, which will enable understanding of predominant release mechanism at each time period.

1: Zero-order release kinetics refers to the process of constant drug release from a drug delivery device such as oral tablet, intravaginal rings and osmotic pumps. Constant release is defined as the same amount of drug release per unit time. Since Zero-order drug delivery systems can release drug at a constant rate, the release profile can be used to limit adverse side effects, reduce dosing frequency and improve patient compliance.

When drug release is zero-order, the drug release rate is independent of concentration. The release of the drug can be represented by this equation: $Q_t = Q_0 + K_0t$

Where Q_t is the amount of drug that dissolved in time t , Q_0 is the initial amount of drug, K_0 is the zero-order release constant expressed in the units of concentration/time.

2: First order release: The amount of drug Q decreases at a rate that is proportional to the amount of drug remaining, i.e., the greater the concentration, the faster the release. The drug release rate is dependent on the drug concentration. There are several first-order release systems that widely used such as microneedles, implantable devices and hydrogels¹⁹³.

$$\log C = \log C_0 - Kt / 2.303$$

where C_0 is the initial concentration of the drug, K is the first-order rate constant, and t is the time. The data can be plotted as a straight line with a slope of $-K/2.303$, Y-axis is the cumulative log percentage of drug remaining, and X-axis is time.

3: Higuchi model: Besides the above two, the most widely used drug release model is the Higuchi model developed in 1963.

This model is based on the hypotheses that 1) initial drug concentration in the matrix is much higher than drug solubility; 2) drug particles are much smaller than system thickness; 3) perfect sink condition is maintained in the release environment; 4) drug diffusivity is constant; 5) matrix swelling and dissolution are negligible; 6) drug diffusion takes place only in one dimension.

Drug release is described as a diffusion process based on Fick's Law which is square root time dependent. The simplified equation of the Higuchi model is $Q = KH \times t^{1/2}$

Where KH is the Higuchi dissolution constant.

In vitro accelerated release data of IUD can be fitted in a two-phase model: zero-order release followed by the Higuchi model¹⁸⁸. In vitro release experiments need to be conducted firstly

in order to evaluate the validity of the theoretical predictions of the polymeric-controlled device. Computer simulations can then be used to predict the drug release kinetics, so the required size and shape of diffusion-controlled systems can be calculated. However, the whole in vitro release profile of IUD has been identified in accelerated conditions, such as elevation of temperature, organic solvent, and surfactants¹⁹⁴, and the in vitro release medium in those studies is not biologically relevant. Therefore, the release rate obtained under those experimental conditions cannot represent the theoretical release rate in vivo. Therefore, there is an urgent need to develop a dissolution method that can simulate the uterine fluid and the in vivo release rate of IUD. This biorelevant dissolution method can be used to study the extended use of on-market IUDs and to build a PBPK model for the female genital tract.

2.0 LC-MS quantitative method to analyze LNG content

2.1 Rationale

A simple, rapid and sensitive analytical assay is necessary for determining LNG concentrations in the dissolution study. Analytical method development and validation is critical to pharmaceutical development. A reliable analytical method could detect the analyte of interest and separate it from process impurities, degradation products, and excipients.

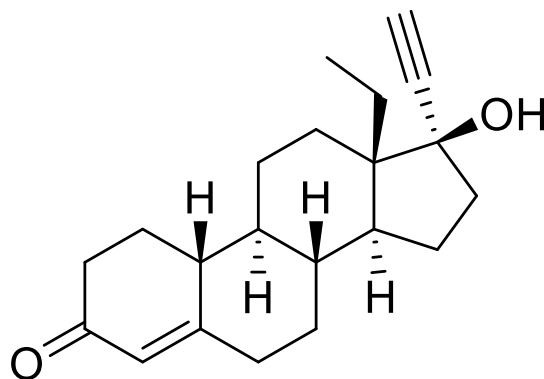


Figure 1 Chemical structure of LNG

The API in hormonal IUD is LNG, a small molecule with an average molecular weight of 312.4456 g/mol. LNG is a synthetic progesterone approved by the FDA in 1982, having a high level of efficacy but fewer estrogenic adverse effects compared with the older contraceptive regimens. It is used for hormonal contraception, emergency contraception (also known as plan B) and hormone therapy. The chemical structure of LNG is shown in figure. Its physiochemical properties are summarized in the Table 2.

Table 2 Physiochemical properties of LNG

Form	powder
Color	white
Molecular Weight (g/mol)	312.446 ¹⁹⁵
Density at 25 C° (g/cm ³)	1.1±0. *
Melting Temperature (°C)	235-237 ¹⁹⁶
Log K _{ow}	3.7 ¹⁹⁷
Solubility in water (mg/ml)	< 0.01 ¹⁹⁸
Solubility in 0.5% w/v, PBS/Tween-80 (mg/ml)	0.0105 ¹⁹⁹
Solubility in octanol (mg/ml)	4621.4 ²⁰⁰

* Calculated value from ACD/labs

LNG administered through an IUD device is absorbed in the endometrium¹³⁴. Most of the LNG is bound to sex hormone-binding globulin (SHBG) and a lower proportion is nonspecifically bound to albumin; the elimination half-life is 10-24 hours. The remaining 1% to 2% of the serum LNG is present as a free steroid. After administration, LNG is metabolized by liver CYP3A4 and CYP3A5 hepatic enzymes followed by sulfate conjugation²⁰¹.

LNG is the second-generation progestin. The first generation of progestin was developed mainly for anti-gonadotropic effect. Newer generations of progestin were developed to have potent progestational and antiestrogenic effects on the endometrium. The fourth generation of progestin was developed to bind specifically to progesterone receptor. Based on the hormones they are derived from, progestins can be classified into three families: 19-nortestosterone, C-21 progesterone, and 17 α -spironolactone. LNG is derived from 19-nortestoster.

Table 3 Different generations of progestin

Generation	Progestin name
First generation	Northindrone
	Northindrone acetate
Second generation	Ethinodiol Diacetate
	Levonogestrel
	Norgestrel
Third generation	Desogestrel
	Norgestimate
Fourth generation	Drospirenone

Chromatography is a physical separation method in which the components to be separated are selectively distributed between a mobile phase and a stationary phase. It can be used for quantitation of samples. For reverse-phase liquid chromatography, the stationary phase is composed of the long-chain alkyl group and the mobile phase consists of water and polar solvents. When a sample is injected into a column, the molecules get separated depending on their relative partition between the mobile phase and stationary phase of the column. It is the most frequently used technique in the field of bioanalysis. HPLC was used for determining LNG content in the previous accelerated in vitro release study and reliable results were generated¹⁹⁴.

Mass spectrometry is widely used because of its high selectivity, high sensitivity, and capability for providing molecular mass and structural characteristics based on the mass-to-charge ratio of ions and fragments. The mass analyzer sorts according to the mass-to-charge ratio. The separated ions are detected by an ion detector in the space or time.

Liquid chromatography-mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation power of liquid chromatography with mass spectrometry detection specificity. LC-MS has a significant impact on drug development over the past decade.

A rapid, sensitive LC-MS method was developed and validated for the determination and quantification of LNG in the dissolution study. Given the low daily release amount of LNG and low concentration of dissolved samples, LC-MS is more suitable for quantification in this study.

2.2 Methods

2.2.1 Instrument method

An LC-MS method was developed for measuring LNG in the dissolution media. An Acquity UPLC BEH C18 1.7 μm 2.1 $\times$ 50 mm column was employed to obtain the chromatographic separation at a flow rate of 0.4 mL/min. Mobile Phase A was 0.1% formic acid in Milli-Q[®] water and Mobile phase B was acetonitrile. The mobile phase composition was a mixture of 60% A and 40% B. The column temperature was 35 ° C and sample temperature rack was maintained at 10° C. The run time for each sample was 4 min. The injection volume is 5 μL . The mass spectrometer was operated in the positive mode. The selected reaction monitoring (SRM) scan settings are listed in Table 4.

Table 4 MS scan settings

Parent (m/z)	Product (m/z)	Dwell (s)	CE (V)	Cone (V)
313.100	245.160	0.160	18	48
468.100	142.800	0.160	37	35

The calibration standards used for the calibration curve were between 20 to 500 ng/mL for LNG. The method was validated for linearity, repeatability, precision, specificity, limit of quantification (LOQ), and limit of detection (LOD).

2.2.2 Sample preparation method

To prepare a 1000 µg/mL LNG standard stock solution, 10.00 ± 0.25 mg of LNG reference standard was weighted and quantitatively transferred to a 10 mL volumetric flask. LNG was dissolved by the addition of approximately 8 mL methanol and vortexed. Methanol was then added to the volumetric flask and adjusted to 10 mL. The stock solution was then stored at 4-8° C. The LNG working solution is 10 µg/mL. 100 µL of the 1000 µg/mL standard stock solution was added to 10 mL volumetric flask with 8 mL of acetonitrile, and then volume was adjusted to 10 mL by acetonitrile and vortexed. To construct a standard calibration curve, the 10 µg/mL LNG working solution was diluted with 50% MeOH to a range of 20 ng/mL to 500 ng/mL (Table 6). The concentrations of the quality controls were 400 ng/mL, 150 ng/mL, and 40 ng/mL (Table 7). The linearity of the calibration curve was obtained by plotting the concentration of LNG versus the area under curve (AUC). The amount of LNG in each sample was then back-calculated based on this calibration curve.

Table 5 Calibration curve samples

Level	Conc (ng/mL)	Total Volume (mL)	Stock conc (ng/mL)	Stock volume (µL)	Diluent volume (µL)
6	500	2.0	10000	100	1900
5	300	2.0	10000	60	1940
4	200	2.0	500	800	1200
3	100	2.0	200	1000	1000
2	50	2.0	100	1000	1000
1	20	2.0	50	800	1200

Low, middle and high-quality control samples were also diluted from the stock solution as follows:

Table 6 Quality control samples

Level	Conc (ng/mL)	Total Volume (mL)	Stock conc (ng/mL)	Stock volume (μ L)	Diluent volume (μ L)
HQC	400	2.0	10000	80	1920
MQC	150	2.0	300	1000	1000
LQC	40	2.0	400	200	1800

2.3 Materials and equipment

HPLC grade or equivalent Formic acid was purchased from Sigma-Aldrich. Acetonitrile and Methanol are HPLC grade or equivalent and purchased from Fisher scientific. Ultrapure water was obtained from an in-house Milli-Q[®] water purification system (Millipore Sigma Advantage A10). All the materials used for LNG quantification are listed in Table 7.

Table 7 Materials used for LNG quantification study

Formic acid (FA)	HPLC grade or equivalent
Water	>18 M Ω cm deionized water
Acetonitrile (ACN)	HPLC grade or better
LNG	Current lot of test substance
Methanol	HPLC grade or equivalent
HPLC vials and caps	
Assorted standard laboratory glassware	
Waters Acquity UHPLC system and Xevo TQ-S MS detector or equivalent	TargetLynx software or equivalent
Waters Acquity C18 BEH UPLC column	1.7 μ m 2.1 $\times$ 50 mm (186002350) or equivalent
Analytical balance	Capable of measuring 0.1 mg
Vortex machine	
Pipettes	1000 μ L, 200 μ L, and 100 μ L

2.4 Results and discussion

A sensitive, rapid, and accurate LC-MS method was developed for LNG. (Figure 2)

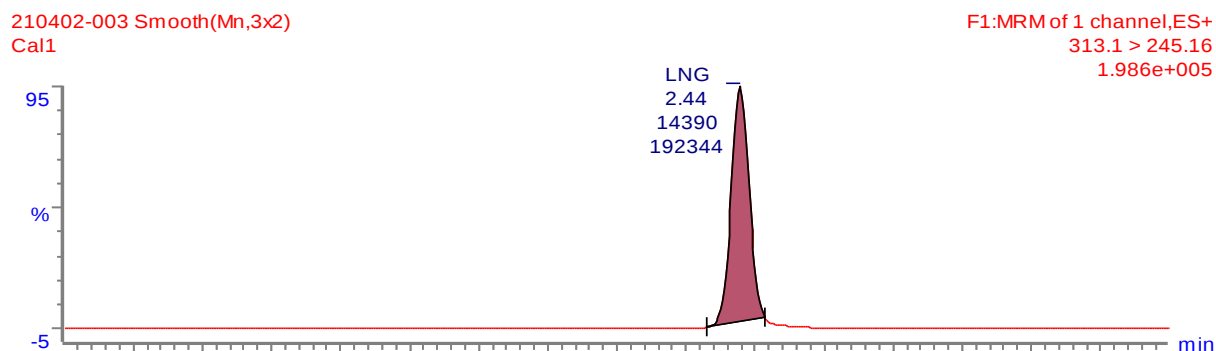


Figure 2 Chromatography of LNG at 20 ng/mL in the USF media

Calibration curve and linearity

Calibration curve is the relationship between known concentrations and experimental response values. It is used to determine the unknown concentration of a sample. The calibration curve should be prepared in the same matrix as the sample in the intended study. Linearity represents the ability of the method to measure test results that are proportional to the concentration of the analyte within a given range. The criteria of linearity included coefficient of determination (R^2) > 0.995, and % Deviation < 15% (< 20% for lowest concentration standard). In this work, at least five standard samples between 20 and 500 ng/mL were used. Linear regression analysis of ng injected vs. 1/X-weighted area response was used for data analysis. Linearity tested on three different days showed R^2 greater than 0.995 (Table 8).

Table 8 Linearity

The linearity was determined by calculating the correlation coefficient (R^2) (n=3).

R^2	Max abs % deviation Lowest std	Max abs % deviation <u>Stds</u>
0.9958	4.0	9.5
0.9973	5.4	6.7
0.9991	3.8	4.7

Compound name: LNG

Correlation coefficient: $r = 0.996708$, $r^2 = 0.993428$

Calibration curve: $899.876 * x + 7527.19$

Response type: External Std, Area

Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

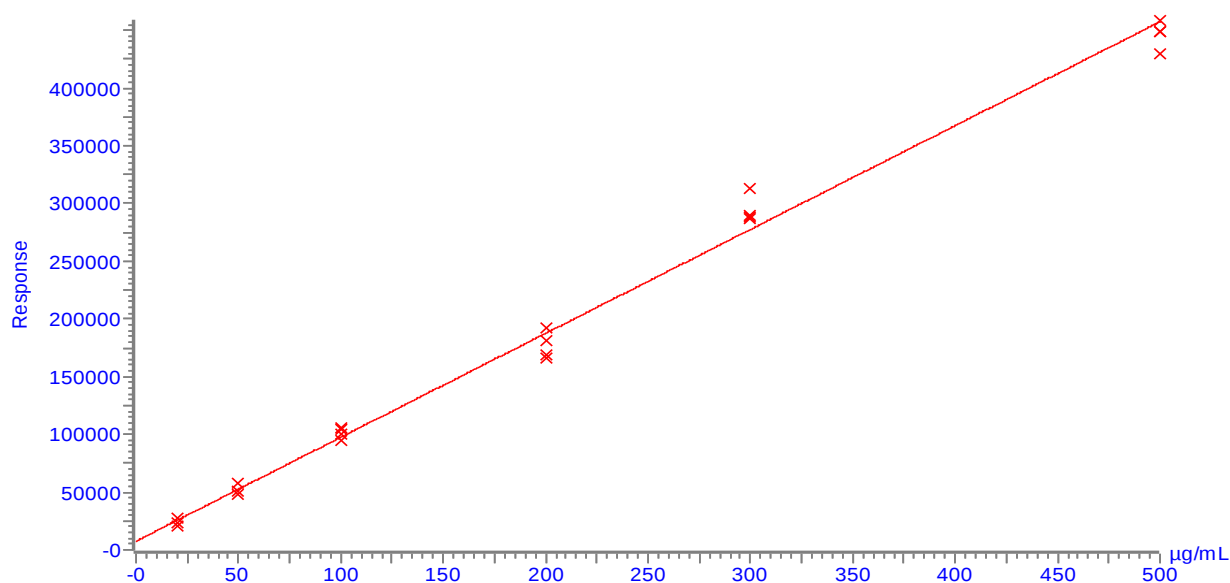


Figure 3 Calibration curve of LNG for LC MS analysis

Accuracy

Accuracy of an analytical method is the degree of closeness between the theoretical value of analytes in the samples and the values by the method using the linear regression equation of the calibration curve. The accuracy of three different levels on three different days was within the range of 90% to 115% (Table 9). The accuracy of LNG was calculated using the equation below.

$$\text{Calculation: \% Accuracy} = \frac{[\text{LNG}]_{\text{Calc}}}{[\text{LNG}]_{\text{Nom}}} \times 100\%$$

Table 9 Accuracy

Intra-day accuracy was performed on 3 different days with the same sample preparation method and instrument method. %Accuracy for different levels of control should fall between 90%- 115%. Values are represented as mean± SD with n=3.

	Nominal ng/mL	% Accuracy		
		Day1	Day2	Day3
Low Concentration Control	40	103.83	98.74	112.26
Mid Concentration Control	150	98.74	96.94	108.62
High Concentration Control	400	106.28	108.62	94.55

Precision

The precision of the method is the closeness of a series of measurements of an analytes when the same analytical procedure is applied repeatedly to multiple aliquots. Criteria of precision was RSD of peak areas < 15% (Table 10).

Table 10 Precision

Intra-day precision was performed on 3 different days with the same sample preparation method and instrument method. The %RSD for standards should be less than 15% (n=3)

	Nominal ng/mL	% RSD		
		Day1	Day2	Day3
Low Concentration Control	40	9.37	1.482	0.634
Mid Concentration Control	150	13.77	0.542	0.822

High Concentration Control	400	8.37	0.142	1.312
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Specificity

Specificity is the ability to accurately and precisely measure the analyte of interest in the presence of other components which may be expected to be present. Specificity evaluation was performed by repeated injections of three different levels of QC samples: low quality control (LQC), middle quality control (MQC), and high quality control (HQC) and USF into the chromatographic system. These results demonstrate that there was no interference from the matrix, thus confirming the specificity of the method (Table 11).

Table 11 Specificity

The detection of LQC, MQC and HQC is not affected by the matrix (USF).

Sample ID	Exp ng/ml	Calc ng/mL	%Dev	RT	S/N	Width (min)	Start Time	N	Tf
MATRIX									
LQC	40	40.95	2.4	2.73	1259	0.073	2.63	22173.92	0.76
LQC	40	39.97	-0.1	2.73	1618	0.073	2.63	22133.66	0.76
LQC	40	40.55	1.4	2.73	1497	0.073	2.63	22613.75	0.71
LQC	40	39.72	-0.7	2.73	925	0.073	2.63	22295.36	0.75
LQC	40	40.77	1.9	2.72	1102	0.074	2.63	21832.77	0.79
LQC	40	42.91	7.3	2.72	1236	0.073	2.63	22051.84	0.79
LQC	40	41.42	3.6	2.72	1784	0.074	2.63	21655.95	0.80
LQC	40	42.42	6.1	2.73	1283	0.073	2.63	22163.84	0.71
LQC	40	43.60	9.0	2.73	1413	0.073	2.63	22133.66	0.76
LQC	40	43.01	7.5	2.73	1286	0.073	2.63	22234.52	0.72
MATRIX									
MQC	150	147.44	-1.7	2.73	1273	0.074	2.63	21874.61	0.72
MQC	150	145.10	-3.3	2.73	1368	0.074	2.63	21600.69	0.77
MQC	150	145.24	-3.0	2.73	1310	0.074	2.63	21639.51	0.77
MQC	150	146.88	-2.1	2.72	1596	0.074	2.63	21433.12	0.80
MQC	150	142.56	-5.0	2.72	1460	0.074	2.63	21587.76	0.80
MQC	150	146.00	-2.7	2.73	1344	0.074	2.63	21697.93	0.72
MQC	150	145.92	-2.7	2.73	1253	0.074	2.63	21658.95	0.72
MQC	150	152.34	1.6	2.72	1275	0.074	2.63	21587.76	0.80
MQC	150	154.19	3.0	2.72	1407	0.074	2.63	21529.58	0.80

MQC	150	155.26	3.5	2.72	1250	0.074	2.63	21452.36	0.80
MATRIX						0.000			
HQC	400	421.9	5.5	2.72	1308	0.075	2.62	20932.55	0.77
HQC	400	417.31	4.3	2.72	1304	0.075	2.63	21025.64	0.82
HQC	400	409.43	2.4	2.73	1179	0.075	2.63	21124.19	0.73
HQC	400	410.98	2.7	2.72	1253	0.075	2.62	21175.89	0.76
HQC	400	419.00	4.7	2.72	1288	0.075	2.62	20923.27	0.77
HQC	400	428.16	7.0	2.72	1262	0.075	2.62	20849.29	0.77
HQC	400	437.16	9.3	2.72	1258	0.075	2.63	20932.55	0.80
HQC	400	424.90	6.2	2.72	1220	0.075	2.62	20979.02	0.77
HQC	400	442.90	10.7	2.72	1220	0.075	2.62	20941.83	0.77
HQC	400	439.50	9.9	2.72	1171	0.075	2.62	20979.02	0.77
MATRIX									
Average				2.724333				21573.83	0.77
SD				0.00504					
RSD				0.185002					

Limit of detection and limit of quantification

Limit of detection (LOD) is the lowest analyte concentration that can be reliably detected but not necessarily quantified. The limit of quantification (LOQ) is the lowest analyte concentration that can be determined with acceptable precision and accuracy. Signal-to-noise ratio is used to compare the level of a desired signal to the level of background noise. The noise is measured between two lines bracketing the baseline in the chromatogram and the signal is measured from the middle of the baseline to the top of peak.

The criteria used for the LOD and LOQ are listed below:

LOD = 3.3 x signal-to-noise ratio.

LOQ = 10 x signal-to-noise ratio.

In this study, the LOD and LOQ were found to be 0.417 ng/ml, and 1.25 ng/ml, respectively (Table 12).

Table 12 LOD and LOQ

Sample	Conc (ng/ml)	Ratio	S/N
LOD 1.25ng/ml	1.25	0.077	10
LOD 1.25ng/ml	1.25	0.066	7
LOD 1.25ng/ml	1.25	0.079	9
LOD 1.25ng/ml	1.25	0.073	8
LOD 1.25ng/ml	1.25	0.1	13
LOD 1.25ng/ml	1.25	0.072	14
Average			10.2
LOD 2.5ng/ml	2.5	0.111	11
LOD 2.5ng/ml	2.5	0.104	15
LOD 2.5ng/ml	2.5	0.104	8
LOD 2.5ng/ml	2.5	0.107	16
LOD 2.5ng/ml	2.5	0.123	13
LOD 2.5ng/ml	2.5	0.113	14
Average			12.83

Table 13 Summary of all parameters in LC-MS validation

Parameter	Criteria		Result
Specificity	R_s	≥ 1.8	No other peaks detected
	N	> 3500	21573.83
	T_f	$0.7 \leq T \leq 1.5$	0.77
	t_R	RSD	0.185%
Linearity	R^2	≥ 0.995	≥ 0.996
	% dev	$\leq 15\%$	$\leq 9.5\%$
	Low std,% dev	$\leq 20\%$	$\leq 5.4\%$
Accuracy	% recovery	90 – 115%	94.6 - 112.3 %
Precision	RSD of peak areas	$< 15\%$	$\leq 8.22\%$

2.5 Conclusions

All the parameters in the LC-MS validation study met the criteria. The limit of detection was 0.417 ng/mL, and the limit of quantification was 0.25 ng/mL. Good linearity was observed for the analyte over the linear range. Eventually, this LC-MS method was applied to the analysis of LNG in the dissolution study, thus allowing the rapid and precise determination of the LNG in the dissolution study.

2.6 Acknowledgements

I want to thank Mr. Philip Graebing for all the help in LC-MS study. Whenever I have problems with instrument or sample analysis, he provided me a lot of suggestions to solve the problem. I am also very thankful to Ms. Lin wang for helping me design the experiments for the development of LC-MS method.

3.0 In vitro release profile of IUDs

3.1 Rationale

The release mechanism of IUDs has not been thoroughly studied. Only 40-60% of the drug was released after the labeled usage time¹⁸⁸. Previous accelerated in vitro release studies investigated the full release profile of IUD, but higher temperature, organic solvent, and surfactants were utilized. Both new and discarded IUDs were used in this study. New IUDs were used to study the initial release profile and mechanism of LNG. Discarded IUDs after a certain usage time were used to see the remaining drug release profile and mechanism. One big advantage of using the discarded IUDs is that the release profile can be studied without running a time-consuming dissolution test. Even some accelerated dissolution studies need more than one year to get the whole release profile.

In vitro dissolution testing has been listed as an official test in the United States pharmacopeia (USP) since the early 1960s⁷². It is used for many purposes in the pharmaceutical industry, such as development of new products, quality control to evaluate batch-to-batch consistency, and determination of bioequivalence⁷⁷.

The USP guidelines for selecting test conditions include the selection of dissolution medium, volume of medium (generally no less than three times the volume required for a saturated condition to maintain sink condition), specification for amount dissolved, and selection of apparatus. Dissolution studies are usually performed at 37°C to simulate the physiological temperature in the human body. Currently, there are no standardized dissolution methods that employ biorelevant conditions for female reproductive tract products such as IUDs.

The USP has been accepted by a variety of apparatuses, including seven different types. USP apparatus 1 is the basket method, which is often used for capsules, beads and delayed-release/enteric coated dosage forms, floating dosage forms, surfactants in media. One of the disadvantages is that the basket method will cause a “dead zone” under the basket. USP 2 is the Paddle method, which is the first choice of dissolution. It is often used for tablets, capsules, beads, delayed-release/enteric coated dosage form. It has several advantages, such as easy to use, robust and possible to change the pH. One disadvantage is that USP 2 may cause the coning problem, which is a layer of non-dissolving excipients on top of the rest formulation. This will restrict the drug dissolution process. USP 3 is the reciprocating cylinder, which is useful for tablets and beads. The advantage of this method is its ability to expose the product to a series of solutions with different pHs and immersion rates. USP 4 is a flow-through cell, which consists of a reservoir and a pump for the dissolution medium. It is useful for low-solubility drugs, microparticles, and implants. One advantage of this method is that media pH can be easily changed so that a pH profile can be established. It is used by the pharmaceutical industry for a variety of pharmaceutical forms, including tablets, capsules, and powders. USP 5 is paddle over the disk. The vessel and shaft are the same as the paddle apparatus, but it has a sample holder. The sample holder can hold the product, and its release surface is parallel with the paddle blade. It is usually used for transdermal patches. USP 6 is a rotating cylinder. The dosage unit is placed on the cylinder at the beginning of each test to the exterior of the cylinder. USP 7 is a reciprocating disk. The assembly consists of a set of volumetrically calibrated solutions made of glass, a motor, and drive assembly to reciprocate the system vertically. It's usually used for controlled or extended-release drug products.

Dissolution testing is one of the most important quality control tests. Dissolution is the rate-determining step for hydrophobic, poorly aqueous soluble drugs⁸⁰. It may be influenced by a

variety of factors such as pH, buffer capacity, ionic strength and solubilization effects due to the presence of surfactants. There are five main factors that affect the dissolution rate of the drug: the physicochemical properties of the drug, drug product formulation factors, processing factors, factors relating to the dissolution apparatus, and test parameters.

In order to build a biorelevant dissolution test, several key parameters need to be considered, such as the composition of the dissolution medium, temperature of the dissolution medium, volume of the medium, and timepoint to take the samples. The composition of the dissolution media should be similar to the uterine fluid. The frequency of sampling is also a major concern since the dissolution test should be maintained under sink conditions. Achievement of sink conditions is critical to establish a suitable dissolution method. If the sink condition is not achieved, the dissolution rate cannot be measured consistently. Temperature, pH and volume of uterine fluid also need to be considered before design of experiment. An understanding of uterine fluid composition and uterine environment are important before designing the experiment.

Uterine fluid is the liquid that connects the embryo and uterus and provides a transport and support medium for sperm and unimplanted embryos before and during embryo implantation. Since the chemical and biochemical compositions constantly change based on the hormonal variations, a simulated fluid that quantitatively and qualitatively consisted of the commonly available compounds in the human uterine fluid was developed^{202,203}. The uterine simulant fluid used in this study was firstly developed in 1996. It was used for in vitro study on copper IUD corrosions²⁰⁴. Corrosion of copper has also been studied in the presence of different proteins, such as serum albumin, γ -globulin, and hemoglobin. The amino acid concentration is different with age, diet habits, and gynecological pathologies. Protein concentrations are ranging from 0 g/L to 8 g/L in humans²⁰⁵. The presence of protein accelerated the anodic dissolution process of copper and

enhanced its corrosion. The time dependence of the copper corrosion rates in the simulated fluid at various concentrations has been observed²⁰². Uterine fluid pH also varies from person to person. In a clinical study, pH was determined by the colorimetric method. The pH value of uterine fluid varies from one individual to another; the pH ranges from 6.0-7.9^{206,207}.

The volume of uterine fluid varies from person to person and also changes during the different menstrual phases. A study collected uterine fluid from 12 women at midcycle and mid-luteal phases separately. volume collected in the midcycle is 83 μ L-180 μ L; in follicular phase the volume is 105 $\pm$ 92 μ L; during the luteal phase, volume is 40 $\pm$ 32 μ L; and mid-luteal phase is less 5-35 μ L, which maintains at a constant level.²⁰⁴ Another study indicated that during the menstrual cycle, the uterine simulant fluid would decrease from 125 μ L to 25 μ L²⁰⁵. Notably, the human uterine potassium concentration will increase in the luteal phase of the menstrual cycle. Moreover, the uterine fluid changes in the presence of IUD, including an increase in the total protein amount due to the waste product of lysis cells and becoming less favorable for growth and survival of the preimplantation blastocyst²⁰⁸.

The fluid flow pattern in uterus is important to understand embryo transport. Fluid movements within the uterus are primarily due to myometrial contractions (myometrium is the muscular layer of the uterine wall). During menstruation, one contraction happens every 2-3 mins. During the rest of the cycle, contractions occur every 20-40 seconds. Interestingly, hormone levels also play a role in the rate and intensity of myometrial contractions. Estrogen can accelerate the rate of contractions; in contrast, progesterone will decelerate the rate of contractions²⁰⁹. However, there is no method to quantify the movement pace of the uterine and correlate that with the rpm in the dissolution test. In order to prevent turbulence and provide a reproducible laminar flow, agitation should be maintained at a relatively low rate. The speed is usually 100 rpm for the basket

method. For the paddle method, 50-75 rpm is suggested. Since there is no study that quantifies the movement speed in uterus, the lowest rpm of 50 was used in this study.

Volume of dissolution vessels also need to be calculated before conducting the experiment. The volume should be adjusted as necessary to allow detectable drug concentration, as well as to maintain sink conditions (the ability of the dissolution media to dissolve at least 3 times the amount of drug that is in your dosage form)

If samples were taken every two days with the whole medium changed, the maximum dissolved LNG is around 40µg in two days, based on the theoretical release rate of new Mirena®. The media needed is: $40\mu\text{g}/(1.4\mu\text{g}/\text{mL}) = 28.57\text{mL}$

In order to maintain sink conditions, the medium volume should be capable of dissolving three times of the dissolved drug. Consequently, the volume should be larger than $28.57 \times 3 = 85.71\text{mL}$. Based on the calculation above, 100mL vessel was used in this study.

USP apparatus 2 is easy to use and also a robust dissolution method. The dissolution media was a reported USF. It was equipped with 100mL dissolution vessels to perform the invitro release testing for the LNG IUDs.

3.2 Methods

3.2.1 LNG solubility in Uterine simulated fluid

The LNG solubility was tested in USF at 4 °C, room temperature (RT), and 37°C. 60 ml of USF was stored at 4 °C, RT, and 37 °C for two hours prior the experiment. 18 scintillation vials (20 mL) were prepared and marked. Two vials were prepared for each condition. Samples were taken at 4 hours, 24 hours, 48 hours until equilibrium of dissolved samples are reached.

Around 3 mg of LNG was added to each vial, then 10 ml of USF at the appropriate temperature

was added to each vial. Each vial was shaken vigorously and put on the appropriate shaker (set at 4 °C, RT, or 37°C). At each time point, a 5 ml aliquot was drawn and filtered through a 0.2 µm or 13 mm Nylon filter in which the first 3 ml was discarded. Each filtered sample was diluted ten times and analyzed using the validated LC-MS method.

3.2.2 Filter screening and validation study

As with any dissolution method, it is important to select the right filter prior to starting the tests. This is to make sure the drug is not sticking to the filter and removes insoluble excipients which can interfere with the analysis.

A 5 ml syringe was filled with 200 ng/ml solution of LNG in USF. Bubbles were removed, and the liquid was adjusted to the 5 ml mark on the syringe. After connecting the filter, the content was transferred through the filter into five centrifuge tubes, 1 ml each. The first ml was transferred into the first centrifuge tube, and the second ml transferred into the second tube and so on until nothing was left in the syringe. Filters screened in the study was shown in Table 14.

Table 14 Filters screened in the study

Brand	Filter
Fisherbrand®	PTFE 0.2µm, 25mm
	PTFE 0.45µm, 25mm
	PTFE 0.22µm, 13mm
	PTFE 0.22µm, 13mm
Millex®	Nylon 0.2µm, 13mm
Thermo scientific®	PES 0.45µm, 13mm
	CA 0.45µm, 13mm

The filtered samples were analyzed using the validated quantitative LC-MS method as described above. The initial filtration screening experiments were performed in duplicate for each filter. The recovery rate was calculated by LNG concentration after the filtration divided by the original LNG concentration. Then the study was repeated in triplicate to confirm the most promising filter.

3.2.3 LNG absorption on VMR[®] tube

A 200 ng/ml stock solution of LNG in USF was prepared. A 0.6 ml of the stock solution was transferred to 8 separate centrifuge tubes to represent four-time points in duplicate (T0, D1, D2, D5). All tubes were stored at 4 °C until the time for analysis. All samples were analyzed using the validated LC-MS method.

3.2.4 Dissolution studies for IUDs

USP Dissolution Apparatus 2/Paddle ($37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$) is used. The bottom of the paddle was positioned $25 \pm 2\text{mm}$ above the inner bottom of the vessel. A Swiss-Style sinker, 316SS, 15.5 mm x 5 mm by Distek, was used to keep the device from floating. The rotational speed was set at 50 RPM. Uterine Fluid Simulant (USF) was developed to simulate the fluid produced in the uterus. The composition of USF is listed in Table 15.

The USF was freshly prepared within 24 hours of the experiment to avoid microorganisms grow in the medium. A 2 ml aliquot of the media was withdrawn after 90 minutes of dissolution and replenished with USF to test for dose dumping. Subsequent samples were taken every two days, and then the whole media was replaced to maintain sink condition and avoid bacteria growth. Samples were then filtered directly after sampling using Nylon filter, 0.22 μm , 13 mm, in which

the first 2 ml were discarded. The filtered dissolution samples were analyzed using the validated quantitative LC-MS method.

Table 15 Composition of 100mL uterine simulated fluid

Composition	Amount (g)
Sodium chloride	4.970
Potassium chloride	0.224
Calcium chloride	0.167
Sodium hydrogen-carbonate	0.250
Sodium dihydrogen phosphate dihydrate	0.072
Glucose	0.500
Millipore water	To 1 L
pH	7.4 $\pm$ 1

3.3 Materials and equipment

All the materials used in the dissolution study are listed in Table 16.

Bayer Pharmaceuticals kindly provided all the marketed IUDs used in these studies. Three discarded IUDs was also used in this study provided by Dr. Achilles in Magee Women's hospital. One Mirena[®] IUD was discarded from the patient after the label claim of 5 years usage time. Another Mirena[®] IUD was discarded from the patient after one year of use. A Skyla[®] was also discarded from the patient after one year of use.

Table 16 Materials used in the dissolution study

Material	Manufacturer	Lot Number	Expiration date
Mirena®	Bayer	TU01645	10/23
Skyla®	Bayer	TU03456	10/23
Kyleena®	Bayer	TU76546	10/23
Sodium chloride	Spectrum	2FJ0164	10/21
Potassium chloride	Fisher	180380	3/23
Calcium chloride dihydrate	Spectrum	2FD0321	12/23
Sodium hydrogen-carbonate	Sigma	SLBR9495V	8/23
Sodium dihydrogen phosphate dihydrate	Sigma	BCB24802	5/21
Sinker	Distek	316SS	

3.4 Results and discussion

LNG solubility in the USF was tested for designing the dissolution study. The most optimal filter was chosen for sample extraction after the dissolution test. In vitro release profile of both new IUDs and discarded IUDs were demonstrated below:

3.4.1 LNG solubility in Uterine simulated fluid (USF)

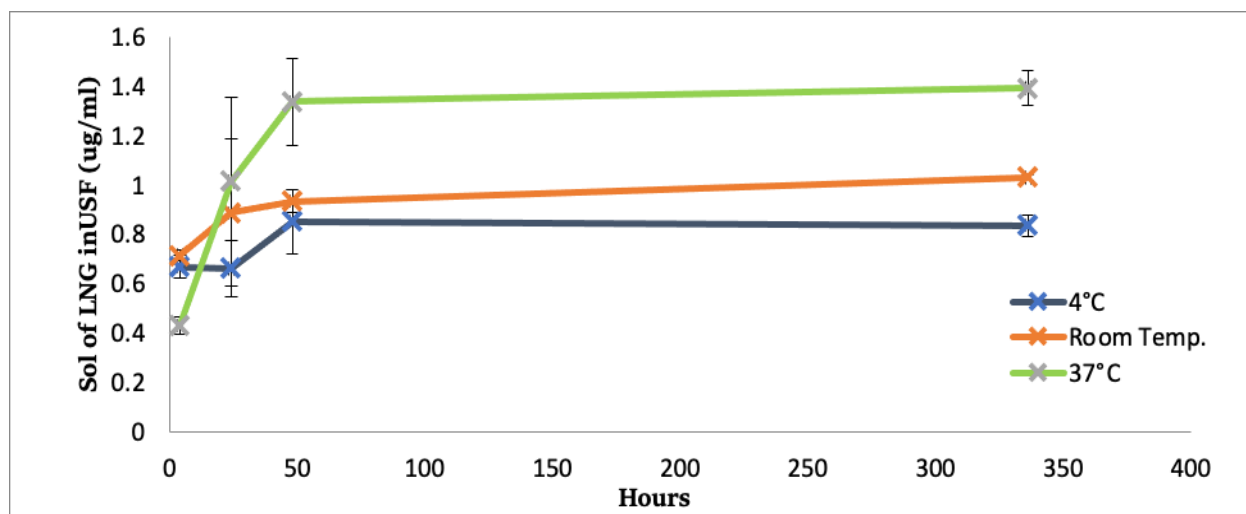


Figure 4 Solubility of LNG in USF

Equilibrium solubility curves of LNG in USF under different temperature was shown in Figure 4. The dissolved LNG amount was measured when reached to equilibrium after 14 days of experiment. The average solubility of LNG was found to be 0.84 ± 0.04 $\mu\text{g/ml}$ at 4°C , 1.03 ± 0.01 $\mu\text{g/ml}$ at Room Temperature (RT), and 1.40 ± 0.07 $\mu\text{g/ml}$ at 37°C , respectively at 14 days. This solubility data was further used to design the sink conditions for the in vitro release experiments.

3.4.2 Filter screening and validation study

The recoveries of LNG under different filtration conditions are shown below for the filter screening experiments.

Table 17 Recovery of LNG using PES 0.45 μm , 13mm by Thermo scientific®

mL discarded	Recovery %
0.0	19.0
1.0	41.8
2.0	66.8
3.0	90.6
4.0	94.3

Table 18 Recovery of LNG using Nylon 0.2 μm , 13mm by Millex®

mL discarded	%Recovery
0.0	29.5
1.0	67.1
2.0	99.2
3.0	93.2
4.0	91.6

Table 19 Recovery of LNG using CA 0.45µm, 13mm by Thermo scientific®

mL discarded	% Recovery
0.0	22.9
1.0	67.7
2.0	79.2
3.0	88.8
4.0	76.6

Table 20 Recovery of LNG using Nylon 0.45 µm, 13mm by Fisherbrand®

mL discarded	%Recovery
0.0	24.2
1.0	34.5
2.0	40.6
3.0	50.6
4.0	62.6

Table 21 Recovery of LNG using PDVF 0.22µm, 13mm by Fisherbrand®

mL discarded	% Recovery
0.0	87.3
1.0	90.7
2.0	92.5
3.0	90.0
4.0	88.1

For the filters tested, Nylon 0.2 µm, 13mm by Millex® showed the best recoveries. This most promising filter was further tested in triplicate for validation purpose. The recoveries for the validation experiments are shown below:

Table 22 Recovery of LNG using Nylon by Millex®

mL discarded	STD	RSD	%Recovery
1.0	1.7	14.62	36.53
2.0	0.77	3.16	77.32
3.0	1.52	4.97	96.61
4.0	0.53	1.68	99.86
5.0	0.6	1.86	101.83

* concentration used is 30 ng/ml

3.4.3 In vitro release profile of new IUDs

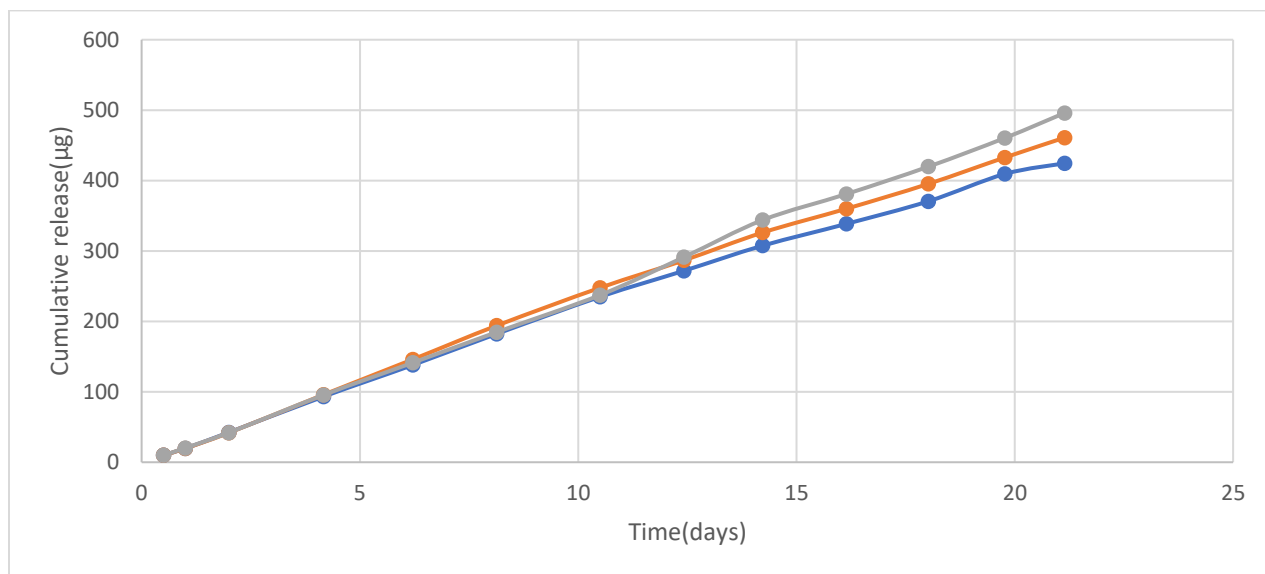


Figure 5 In vitro release profile of Mirena® (n=3)

The in vitro dissolution release rate of new Mirena® was found to be 20.998 µg/day, which can be correlated with the in vivo release rate of 20µg/day.

The release of new Mirena® can be fitted into zero order release model:

Equation is $y=20.998x+7.00184$, $R^2=0.9979$

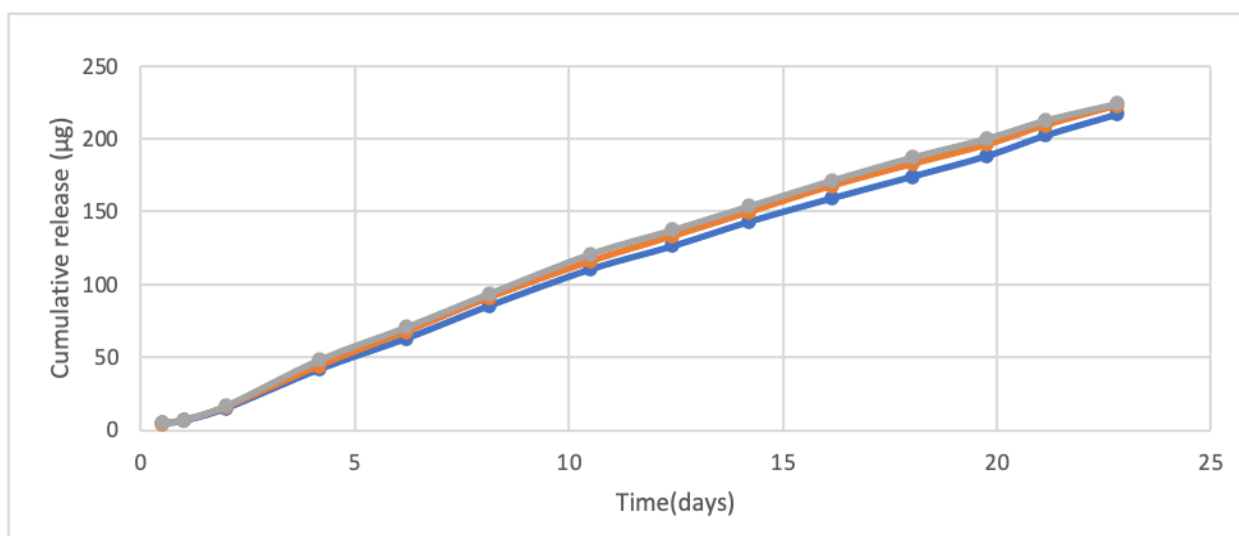


Figure 6 In vitro release of new Skyla® (n=3)

The in vitro dissolution release rate of new Skyla[®] was 10.071 µg/day, which can be correlated with the in vivo release rate of 8µg/day over the first year.

The release of new Skyla[®] can be fitted into zero order release model:

Equation is $y=10.071x+4.7218$, $R^2=0.9928$

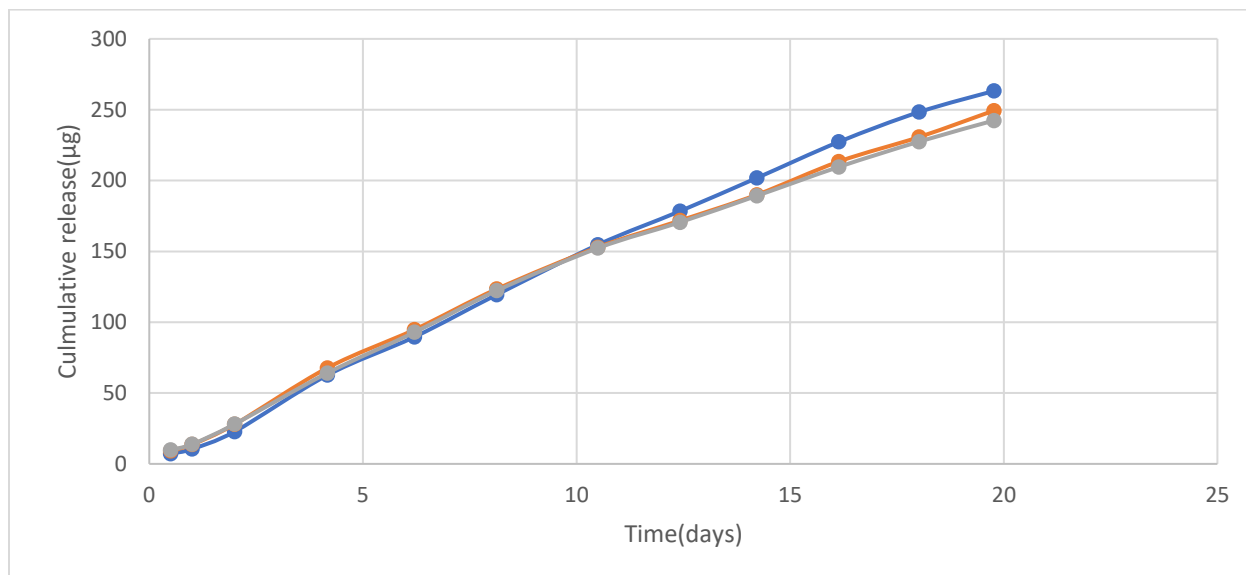


Figure 7 In vitro release of new Kyleena[®] (n=3)

The in vitro dissolution release rate of new Kyleena[®] average release rate was 11.994 µg/day in average, which can be correlated with the in vivo release rate 12.6 µg/day over the first year.

The release of new Kyleena[®] can be fitted into zero order release model:

Equation is $y=11.994x+12.594$, $R^2=0.9916$

3.4.4 In vitro release profile of discarded IUDs

All the discarded LNG IUDs were firstly cleaned under the Ultraviolet (UV) light in order to prevent the growth of bacteria during the dissolution test. Sample extractions were same as the new IUDs and their release profiles are listed below:

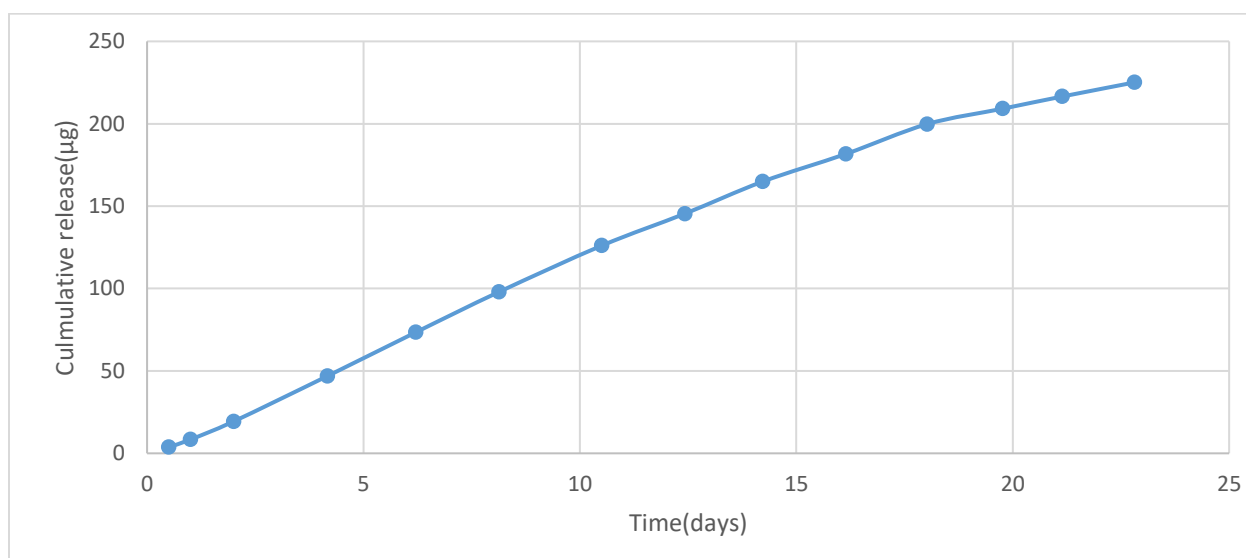


Figure 8 In vitro release profile of discarded Mirena[®] after five years of use

The in vitro dissolution release rate of old Mirena[®] after five years in women's body was found to be 10.435 µg/day, which can be correlated with the in vivo release rate 10µg/day after five years of usage.

The release of discarded Mirena[®] after five years of use can be fitted into zero order release model: Equation is $y=10.435x+5.8205$, $R^2=0.9958$

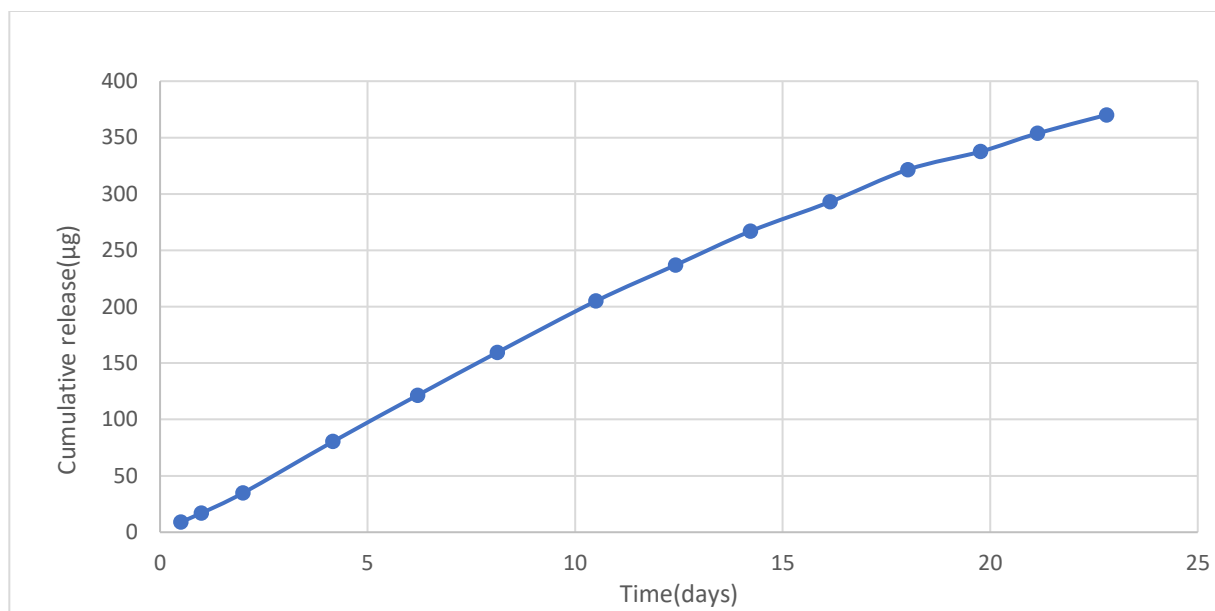


Figure 9 In vitro release profile of discarded Mirena® after one year of use

The in vitro dissolution release rate of old Mirena® after one year in women's body was 16.752 µg/day, which can be correlated with the in vivo release rate 18µg/day after one year usage.

The release of discarded Mirena® after one year of use can be fitted into zero order release model: Equation is $y=16.752x+12.618$, $R^2=0.9895$

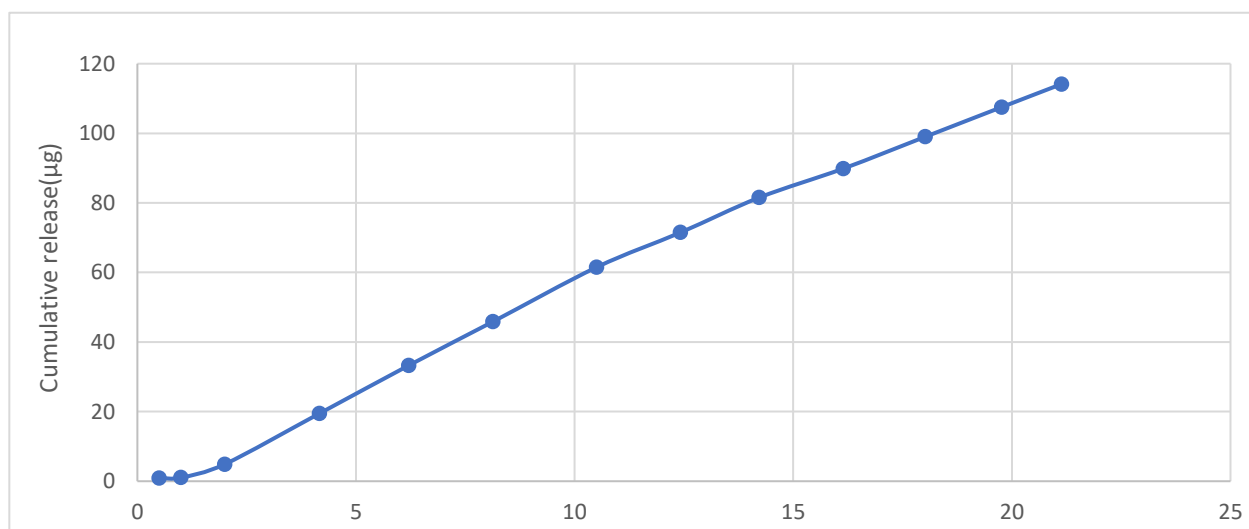


Figure 10 In vitro release profile of discarded Skyla® after one year of use

The in vitro dissolution release rate of old Skyla[®] was 5.6959 µg/day, which can be correlated with the in vivo release rate 5µg/day after one-year usage.

The release of discarded Skyla[®] can be fitted into zero order release model: Equation is $y=5.6959x-2.613$, $R^2=0.9958$

The sensitive and accurate LC-MS method was used for quantification of LNG. No interference of LNG was observed for the USF media. This study demonstrated that our newly developed dissolution method could mimic the in vivo release profiles of LNG IUDs. The release profile of all these IUDs can be fitted into the zero-order release model. The in vitro release parameters are being used by our collaborators to optimize PBPK models for the female reproductive tract.

The release rate of these IUDs correlate with the theoretical release rate in vivo. The work in this thesis has several limitations. Firstly, the dissolution medium used in this study was a reported uterine simulated fluid which is a salt solution. However, the real-world uterine fluid is a complex fluid which contains multiple proteins, such as serum albumin, γ -globulin, and hemoglobin. In a clinical study, urine secretions were aspirated from 56 fertile women. Reverse phase high performance liquid chromatography was used to analyze the concentration of 18 amino acids within the uterine fluid and blood serum. The total human uterine fluid was observed to contain an average amino acid concentration of 3.54 mM, which was not significantly altered by age, BMI and cycle phase or the presence of gynecological pathologies²⁰⁵. Given the high volume (100 mL) of dissolution media used in this study and low concentration of uterine fluid, protein binding was not considered to significantly affect the in vitro drug release rate. It is exciting to find that the in vitro release profiles in the simplified USF media were well correlated with the in vivo release profiles of commercial LNG IUDs. From a practical standpoint, protein in the

dissolution test will be a nutrition source for bacteria and may contaminate the dissolution instrument. A previous study in our lab found dissolution medium containing protein was contaminated overnight. Especially in this study, discarded IUDs taken from the body may have unknown bacteria that may contaminate the dissolution test. Since the simplified dissolution test has already demonstrated the ideal results, the development of a more complex medium is not considered necessary.

Another limitation of this study is the liquid volume used in this study was much larger than the actual uterine fluid volume for maintaining the sink conditions and sampling at multiple timepoints. From a practical standpoint, 100mL vessel is the lowest volume we can use to serve these two purposes.

The last limitation of this study is the lack of investigation of some dissolution parameters such as the RPM. An appropriate RPM is important to obtain reproducible and valid dissolution testing results to assure a properly qualified dissolution test. Due to the lack of literature on the movement speed of uterus, 50 RPM was used in this study.

Furthermore, the actual uterus has a biorhythm of oxygen tension that cannot be simulated in vitro. There will be a rise in oxygen tension around the time of ovulation⁹⁷. A study placed a fiber optic oxygen sensor inside the uterus and found that the oxygen tension varies minute from minute²¹⁰.

Since the data generated in this study meet the in vivo results, limitations discussed above are not considered significant to affect the results.

3.5 Conclusions

In this work, a biorelevant dissolution method was developed and validated. USF was used to simulate the fluid produced in the uterus. USP Dissolution Apparatus 2/Paddle ($37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$) was utilized, and sink conditions were maintained in the USP media. Under our optimized conditions, the in vitro release profiles of three marketed new, and three discarded LNG IUDs were all closely correlated with the in vivo release profiles reported in the literature. Combining the release rate of new Mirena[®], discarded Mirena[®] after five years of use and one year use, this dissolution method can not only apply to new IUDs but also employ discarded IUDs.

By studying the release rate of those discarded IUDs, we can confirm whether they can be extended to use longer than the label claim. The previous real-time release testing for the IUDs was done in 0.9% w/v NaCl at 37°C . The duration of this real-time study was 924 days, which is particularly time-consuming. However, by using the discarded IUDs, the whole release profile of IUDs can be studied instead of running the dissolution experiment exceptionally long. This can save time and money for research.

Our biorelevant dissolution method and the resulting PBPK models will be invaluable tools for the improvement of product development, manufacturing, and quality control, especially for products targeting the female reproductive tract such as IUDs.

3.6 Acknowledgements

I am sincerely grateful for all the preliminary dissolution data provided by Dr. Alantary and Mr. Prithivirajan Durairajan for helping me set up the dissolution machine and. I would also like

to thank Dr.Archillies for providing all the discarded IUDs from Magee Women Hospital. These IUDs were taken from women's bodies and provided for this in vitro study.

4.0 Future directions

Dissolution testing plays a crucial role in pharmaceutical product development and product-to-product comparisons. There is no biorelevant dissolution method that can simulate the in vivo release for IUDs. The data generated in this study fills the gap unknown in vitro release data of LNG IUDs. The ongoing dissolution study will be continued for a longer period of time to further validate its ability to predict the in vivo release rate and to examine its prediction potential for the subsequent years of IUD usage.

In 2020, the FDA approved extending the Mirena[®]'s indication to up to 6 years of pregnancy prevention. The release rate of Mirena[®] progressively decreases to 10µg/day after five years of use and 9µg/day after six years of use²¹¹. This dissolution study may be used in the future to study whether the duration of IUD use can be longer. Previous accelerated dissolution studies found the zero-order release model can be fitted in the range of 0-30% release, and the Higuchi model can be fitted into 30% to complete release¹⁸⁸. The release mechanism transition time in vivo is not known for now. Since this dissolution method can simulate the in vivo release rate, the generated in the future may fill this gap.

In addition, other experiments may be done in the future to make the dissolution test more robust. For instance, the effects of protein binding (i.e., adding proteins such as albumin to reach the measured amino acid concentration in the uterus) and pH (several pH values measured at different phases of a menstrual cycle) may be studied to better understand how the in vivo release rate varies within a menstrual cycle, which may help identify during which days of the cycle the LNG concentrations are the lowest. If successful, such information can be used to reduce the

failure rate of contraception by advising the women to add a second contraceptive method during the identified period. Moreover, the biorelevant dissolution method may be used to evaluate the in vivo release profiles of other products that target the uterus. Besides the composition of the dissolution medium, parameters in the dissolution study also can be investigated in the future, such as different agitation speeds (75RPM, 100RPM), the volume of the dissolution vessel.

The data generated in this study are important parameters that will be used to build PBPK models of the female reproductive tract. A PBPK model that can accurately describe the female reproductive tract would potentially provide a mathematical framework for evaluating alternative drug products administered into this space and increase the efficiency of the development of these locally administered complex drug products.

There are no generalized PBPK modeling and simulation platforms that characterize both intravaginal and intrauterine drug delivery. To date, there are only two PBPK models of drug delivery to the female reproductive tract. One is a model of intravaginal delivery of dapivirine, the other is a model of intrauterine delivery of Levonorgestrel for Skyla[®]. Multiple microbicide dosage forms have been included in the modeling of vaginal drug delivery, such as gel and intravaginal ring. The vaginal drug delivery modeling can help with the understanding of microbicide in five ways: 1) time to the beginning of the prophylactic activity, level and duration of activity; 2) comparisons between different dosage regimens; 3) comparisons between modeling results of different dosage forms; 4) interpretation of the formulation's aging during storage; 5) comparisons between human studies and animal studies. The predictions of models can also be applied to in vitro experiments of drug transport and through tissue specimens. The use of modeling can improve the understanding of determinants of vaginal drug distribution and guide experimental design²¹². IUD as a reference drug product through the female reproductive tract has an important

role in developing the PBPK model. This in vitro release data will be incorporated into a generalized PBPK model to predict the release and absorption of API in the female genital tract. It can be used as a surrogate for the in vivo study, thus reduce the cost during the development of pharmaceutical products. It can also be employed in new drug development including candidate selection, formulation development, dose range, and design of clinical studies.

Our biorelevant dissolution method and the resulting PBPK models will be invaluable tools for improvement of future generic IUD development, manufacturing, and quality control. They could be employed in pharmaceutical research and development (R&D) and quality control settings to evaluate IUD performance and help in accelerating the development of more user-acceptable IUDs. This study can also facilitate the process of permitting biowaivers through an in vitro method, therefore reducing the necessity for clinical studies. On account of women's growing interest in using IUDs, more IUDs for greater uptake and higher usage will be developed in the future to meet women's various reproductive needs.

Appendix A: Abbreviations used

ACN: Acetonitrile
API: Active pharmaceutical ingredients
AUC: Area under curve
BV: Bacterial Vaginosis
CDC: Centers for Disease Control and Prevention
EC: Emergency contraception
EE: Ethinyl Estradiol
ENG: Etonogestrel
FSH: Follicle-stimulating hormone
FDA: Food and Drug Administration
GnRH: Gonadotropin-release hormone
HRT: Hormone replacement therapy
HQC: High quality control
HIV-1: Human immunodeficiency virus-1
HSV-2: Herpes simplex virus
IUD: Intrauterine contraceptive device
LC-MS: Liquid chromatography-mass spectrometry
LH: luteinizing hormone
LNG: levonorgestrel
LOD: Limit of detection
LOQ: Limit of quantification
LQC: Low quality control
MQC: Middle quality control
MTP: Multi-purpose platform
N-9: Nonoxynol-9
NGMN: Norelgestromin
PBPK: Physiologically based pharmacokinetic
PDMS: Polydimethylsiloxane

PEVA: Polyethylene vinyl acetate
R&D: research and development
S/N: Signal-to-noise rate
SHBG: Sex hormone-binding globulin
STDs: Sexually transmitted diseases
USF: Uterine simulate fluid
USP: United States Pharmacopeia
UPA: ulipristal acetate
UV: Ultraviolet
VCF[®]: Vaginal contraceptive film

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