Women’s health has been a global concern for many decades\textsuperscript{195,196}. The focus of women’s health researchers and health policy planners has also shifted towards PMF, since recent trends suggest an increase in their number and life expectancy\textsuperscript{197}. A total of 130 million Indian women are expected to live beyond menopause by 2015. Under current demographic trends, menopausal and postmenopausal health has emerged as an important public health concern in India owing to improved economic conditions, rapid lifestyle changes, and increased longevity\textsuperscript{198}. Generally, women have more complex and stressful aging process as men do, as a consequence of hormonal changes that occur during menopausal transition\textsuperscript{199}. The onset of this physiological development not only marks the end of women’ reproductive function but makes them more vulnerable to a new set of health problems including cardiovascular diseases, osteoporosis and so on\textsuperscript{199}.

As per World Health Organization menopause is defined as the permanent cessation of menstruation due to loss of ovarian follicular activity\textsuperscript{200}. This definition uses both, a symptom that can be identified by a woman (the end of menstruation) and a sign that can be measured (loss of follicular activities resulting in changes in levels of hormones). The drawback of this definition are; first, follicular activity can continue even in the absence of menstruation, for example, in case of hysterectomy, ovaries may remain functional, second, follicular activity can end, but menstruation can continue through the use of cyclic hormonal therapy and third, how women experience menopause, which may vary within and between the social groups. Most women perceive menopause to be a marker for the end of childbearing.

Researchers and health care providers have generally now agreed to define menopause as the last menstrual period followed by at least twelve months of amenorrhea (no menstrual bleeding). The advantage of this definition is that it identifies a single, measurable variable within this period of transition. The definition also enables to compute median and mean age at menopause for inter- and intra-population comparisons\textsuperscript{201}. Although last menstrual period is a clinically useful marker of an event, the average woman’s sense of the process of the menopausal transition is better described by the term ‘perimenopause’, a phase before menopause actually takes place, when ovarian hormone production is declining and functionally causing most of symptoms. Various staging system have been proposed to
differentiate premenopausal (regular cycling) from perimenopausal (irregular cycling) and postmenopausal (no cycle from last twelve months)\textsuperscript{202}.

As noted, the perimenopause is frequently accompanied by symptoms of varying intensity which are believed to reflect marked fluctuations in levels of estrogen and progesterone or its outright deficiency\textsuperscript{203}. The tissues which are most affected by reduced estrogen level are the ovaries, uterus, vagina, breast, and urinary tract. Tissues such as the hypothalamus, skin, cardiovascular tissues, and bones may also be affected\textsuperscript{204}.

**Hormone Replacement Therapy (HRT):**

HRT, sometimes called estrogen replacement therapy or ERT, refers to hormonal supplements such as estrogen alone or estrogen with another hormone called progesterone (progestin in its synthetic form prescribed to women). HRT replaces hormones that a woman’s body should be making or used to make.

Estrogen and progesterone normally regulate a woman’s menstrual cycle and reproductive health. Estrogen is also important for bone health. Generally, health care providers prescribe HRT for two groups of women:

- Women going through menopause and post-menopause; the natural levels of these hormones drop during menopause. This drop can lead to symptoms such as hot flashes, night sweats, vaginal dryness, and sleep disturbances. HRT may help to reduce some of these symptoms
- Women with certain health conditions; in some cases, women’s bodies don’t make normal levels of the hormones because of a medical problems, such as premature ovarian failure. For these women, HRT replaces the hormones that their bodies should be making.

The NIH conducted the Women's Health Initiative (WHI) trial to learn about the risks and benefits of continuous estrogen + progestin HRT for post-menopausal female.

In one arm of this trial, researchers found that healthy post-menopausal female who took the therapy were at increased risk of invasive breast cancer, coronary heart disease, stroke, and blood clots. There were also benefits of estrogen plus progestin, including fewer cases of hip fractures and colon cancer.

Because the harm of HRT for healthy post-menopausal female in this trial was greater than the benefit, the researchers stopped the trial.
Based on these finding FDA noted that even though HRT effectively lessened some menopause symptoms in healthy post-menopausal female, it carried serious risks. Women should discuss the potential benefits and risks of HRT with their health care provider. The FDA recommends HRT for post-menopausal female be at the lowest doses for the shortest amount of time to reach treatment goals.

6.1 Demographic assessment:

6.1.1.1. Demographic data for anthropometric analysis:

Results from large trials provide and evidence that hormone replacement therapy (HRT) can blunt the increase in body weight and prevent the shift to a more central, android fat distribution observed in normal women throughout the early postmenopausal period\(^\text{165}\). Recently, a large cross-sectional study, showed a trend for an increase in BMI during the menopausal transition. In addition, another study reported that postmenopausal female treated with combined HRT had lower BMI than untreated control subjects\(^\text{165}\). In a long term, prospective, double blind, placebo-controlled study, the PEPI trial, an increase in body weight during the menopause has been described. After 36 months, the body weight increase was significantly higher in untreated postmenopausal female (12.1 kg) than in women treated with unopposed estrogens (10.7 kg)\(^\text{166-167}\). On the other hand, in patients treated with different estrogen-progestin combinations, the weight gain was lower than but not significantly different from that in the placebo group\(^\text{168}\).

In our study it was observed that there is no significant difference in height, weight and BMI between Group I, Group II, Group III, Group IV, Group V and Group VI of the age 40 to 45, 46 to 50, 51 to 55, 56 to 60, 61 to 65 and 65 to 70 years respectively. Despite having lower prevalence of obesity as defined by BMI, Asian Indian tends to have greater waist circumference and waist to hip ratios, this having a greater degree of central obesity. Again, Asian Indians have more total abdominal and visceral fat for any given BMI and have increased insulin resistance\(^\text{205}\).

These results will help to conduct clinical trials in India with HRT on PMF as there are lot of evidence that the HRT may show different results based on height, weight and thus BMI.
6.1.1.2. Symptoms of post menopausal females:

For centuries, disturbances of mood and behaviour have been associated with reproductive endocrine system change in women. Much of the current understanding of these disorders is based on myths, unwarranted assumptions and conclusions derived from poorly constructed studies. The understanding of relationships among aging, the menopause and behavioural change is inadequate. Many studies have relied on small samples of self-selected women seeking treatment for symptoms. As a result, the actual prevalence of minor psychological and somatic symptoms directly related to lowered levels of ovarian estrogen remains speculative at best.\(^{196}\)

In literature it is reported that the mean age at menopause varies substantially even between the Asian women. For example in Thai women it is 49.30 years, in Malaysian women it is 50.70 years, in Turkish women it is 51.00 years and in Pakistani women it is 50.00 years but almost similar among United Arab Emirates women.\(^{173}\)

According to WHO (1996), a variety of symptoms occur either alone or together are frequently reported as being a part of menopausal symptoms. These include urinary problems, depression, nervous tension, palpitations, headaches, insomnia, and lack of energy, fluid retention, backache and difficulty in concentration. However, it is now recognized that most of these symptoms are not specific to the menopause. Many are related to ageing process or occur because of stresses in mid-life years. Hot flushes and night sweats are the symptoms most consistently associated with menopause, although their prevalence varies in different cultures.\(^{174,175}\)

Long-term consequences of changes in ovarian hormonal levels include morbidities associated with aging such as cardiovascular diseases, osteoporosis, problems related to memorization, urinary incontinence, skin aging and so on. PMF are generally disproportionally affected by osteoporosis, fracture rates among PMFs are approximately twice as high as men. The cause of osteoporosis is very complex but it is clear that hormonal changes after menopause increase the rate of bone resorption, leading to greater risk of osteoporosis. Brain is also a target for estrogen and other gonadal steroids. Subsets of neurons possess intranuclear receptors for estrogen. Moreover, it is found that problems such as Alzheimer’s disease develops earlier in women than in men. This may be related to estrogen loss that occurs with menopause. The incidence of coronary heart disease (CHD) is
extremely rare in premenopausal women, even in high-risk population, and much lower in perimenopausal women than in men of similar age. However, the incidence rapidly increases in women after menopause and loss of ovarian function. Some of the studies show that women who experienced early menopause have increased risk of heart diseases. A cohort study of postmenopausal female, age 50-65 years at enrollment and followed up to 10 years, showed that the risk of cardiovascular mortality was higher for women with early menopause than those with late menopause\textsuperscript{211}.

Urogenital problems are experienced by one-third of women from age of fifty years and onward\textsuperscript{212}. Urinary inconsistence is one of the most significant urogenital disorders. Female’s lower urinary tract is a target organ for the action of the sex steroid hormones estrogen and progesterone since estrogen receptors are found in the urethra and lower urinary tract\textsuperscript{213}. Skin aging has also been reported to be affected by the reduction of female hormones after menopause\textsuperscript{214}. Carbohydrate metabolism and adipose tissue distribution are also regulated by female sex hormones. The metabolic change leads to obesity\textsuperscript{215,216}.

In the present study, we have found various menopausal symptoms among 500 PMFs, (figure 14 and table 5) the prevalence of menopausal symptoms were irregular periods, joint pain, loss of libido, fatigue, sleep disorders, night sweat, hot flushes, weight gain, incontinence, depression, headache / migraine, hair loss and anxiety in 18, 12, 10, 10, 10, 8, 8, 6, 6, 5, 3, 2 and 3 percent (%) of patients respectively. All these observed symptoms are similar to those reported in literatures.

6.1.2. Demographic data of laboratory parameters:

Prior to menopause, the risk of CHD in women is 2.5 to 4.5 times lower than that of age-matched men\textsuperscript{217}. This finding correlates to lower levels of LDL-C and higher levels of HDL-C in PMF compared to males of similar age. However, within 10 years of menopause, the risk of CHD in women increases to a level similar to that in men\textsuperscript{218}. The mechanism for this precipitous rise in cardiac risk is likely multifactorial, though alterations in lipid metabolism in the face of estrogen deficiency may play a significant role. Specifically, reductions in HDL-C levels and elevations in total cholesterol, triglycerides, fibrinogen, and lipoprotein (a) levels are observed following menopause\textsuperscript{219-224}.

While age may contribute to altered lipid metabolism, menopause itself has been shown to have a stronger correlation. In a longitudinal study of 541 initially premenopausal
women aged 42 to 50 years, greater changes in blood lipid levels were seen in those women who underwent menopause than in those who did not. In fact, those who maintained their premenopausal status during the study period showed no change in HDL-C levels. These findings support the general consensus that menopause, whether surgical or natural, is associated with a less desirable lipid profile\textsuperscript{221}.

Menopausal women suffer from some of the health problems related to hormone imbalance which in turn leads to elevated levels of lipids (total cholesterol, LDL-C levels) and lower HDL-C levels. Before menopause, the LDL-C levels are lower and rise after menopause. The cause for this is significant reduction in circulating concentrations of estradiol and estrone\textsuperscript{178}.

Lipid profiles are affected by metabolic conditions, and alterations in lipid metabolism have been implicated in atherosclerosis and coronary heart disease. Various study on lipid profile in postmenopausal female indicate that menopause alters the lipid profile in PMFs. It is reported that total cholesterol, LDL-C and TC/HDL-C (atherogenic index) ratio and Apo-B\textsuperscript{179-182} level are significantly higher and HDL-C level is lower in postmenopausal female and women with age greater than 45 years when compared to premenopausal women and women between the age ranges of 25-45\textsuperscript{179}.

According to summary reports from Japan, US and UK, serum lipid profiles regarding CHD risk were better in women than in men until 40 years of age. After 50 years of age, however, serum total cholesterol and LDL-cholesterol levels became higher in women than in men. Serum triglycerides were increased and HDL-C was lowered with age in women, but they remained better than in men throughout life. The 3rd and 4th National Surveys of Circulatory Disorders in Japan reported similar findings: serum total cholesterol and triglycerides were lower in women than in men aged 40 years or below, but increased more markedly in older women than in men, and total cholesterol was higher in women aged 50 years or above and TG in women was almost equal to those in men after 60 years of age. On the other hand, serum HDL-C was higher in women than in men in all age groups, but in women it decreased gradually with age\textsuperscript{225,226}.

No attention was paid to the influence of menopause on serum hepatic enzymes in previous studies. It is noted in one of the study that, increase in serum hepatic enzymes may be a sign of fat accumulation in the abdominal cavity and liver, a characteristic of fat
distribution. Body fat distribution in premenopausal women is very different from that in men but it was reported that fat distribution becomes more android after menopause. Therefore, the increase in serum ALT in postmenopausal female may reflect the changes in body fat distribution after menopause\textsuperscript{227,228}.

6.2. Method development and validation process for hormone analysis.


In the present study LC-MS-MS assay for simultaneous measurement of estrogens was carried out. The inclusion of stable internal standards in each sample allows accuracy and reproducibility. It appears to be largely free of interference or cross-reactivity, a problem that continues to affect even estrogen immunoassays that incorporate sample extraction. Unlike high- and ultrahigh-sensitivity manual immunoassays, which achieve comparable or better sensitivities but require substantial manual labor and prolonged incubation times, this LC-MS-MS assay is fast, with little hands-on time despite the need for sample extraction and derivatization. Moreover, many of the front-end sample-processing steps can be automated by use of robotic workstations, making this assay suitable for high-throughput and short turnaround time applications that require high-sensitivity estrogen measurements.

Most commercial estrogen immunoassays are unsuitable for clinical applications that require a low detection limit, e.g., estradiol measurements in men or children or monitoring of gonadotropin-analog-mediated pituitary-gonadal axis down-regulation before in vitro fertilization and embryo transfer\textsuperscript{229}. The predominant estradiol assays used in the modern clinical laboratory are direct automated immunoassays which lack sensitivity and are subject to interference by cross-reacting substances\textsuperscript{230}. Many of these assays claim detection limits for estradiol of as low as 40 pmol/L\textsuperscript{231}.

Because of the above mentioned limitation of immunoassays, chromatographic methods have been considered as an alternative methods, but these methods also have various limitations. Conventional HLPC-based methods have been reported, but they perform poorly in terms of turnaround time and sensitivity\textsuperscript{232}. GC-MS methods for the quantification of estradiol in human serum samples represent the recognized gold-standard method, but their value in a clinical situation is restricted\textsuperscript{233,234}. The complexity of the serum matrix requires purification and concentration of estrogens before derivatization for GC-MS.
analysis. The common methods for purification are solvent–solvent and solid-phase extractions. Some other approaches use affinity columns, purification with Sephadex LH-20, strong ionic exchangers, weak ionic exchanger and reversed-phase HPLC, or various combinations of these methods. In addition to the extraction and derivatization process, reported run times for GC-MS based estrogen assays may be as long as 45 min/sample and are rarely <15 min/sample. The reported GC-MS methods for estradiol not only lack high-throughput capabilities but also have insufficient sensitivity to challenge modern immunoassays for routine clinical use\textsuperscript{235}.

The additional information that is provided by the high-sensitivity estrone measurement might be useful in certain clinical situations. For example, in obese postmenopausal female, the fraction of androstenedione converted to estrone increases, yielding enough estrogen to produce endometrial proliferation and bleeding. In men, estrone concentrations may be of value in the investigation of gynecomastia or the detection of estrogen-producing tumors\textsuperscript{236}.

The current method show acceptable precision (table 33a and 33b) and adequate sensitivity (table 32a and 32b) for the quantification of estradiol and estrone in human plasma samples obtained for pharmacokinetic, bioavailability or bioequivalence studies. The method described is simple, rapid, sensitive, specific and fully validated as per FDA guidelines. The cost effectiveness, simplicity and speed of liquid/liquid extraction and simultaneous quantification of estrone and estadiol make it an attractive procedure in the bioanalysis of estrogens. This method allows the quantitation of estrogen in the 10-1080 pg/mL range.

6.2.2. Method development and validation process for estimation of Progesterone in human plasma by LC-MS-MS method.

During the early stage of method development, both electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI) interfaces coupled to MS-MS were investigated for sensitivity. Because in recent years ESI has become the most widely applied interface for liquid introduction into the mass spectrometer. Also, the advent of the electrospray ionization interface has greatly stimulated the use of APCI technique. ESI and APCI have enhanced sensitivity, improved selectivity, robustness and high sample throughput in mass spectrophotometric methodology. Therefore, high performance liquid
chromatography with mass spectrometry (LC-MS-MS), especially LC-MS-MS, is currently recognized as a powerful tool to characterize complex samples and to analyse biological samples. There was significant gain in sensitivity by the use of APCI was found, which indicates that APCI is a better for less polar and non-polar compounds. The APCI interface was therefore selected.

The LC-MS-MS conditions for the determination of progesterone in human plasma were investigated. The parameters such as temperature, orifice potential, flow of the nebulizer and auxiliary gas were optimized. The full-scan (Q1 Mode) mass spectra of progesterone and internal standard in the MS positive mode indicated that the most abundant ions were the protonated molecular ions. These positive molecular ions were therefore used as the precursor ions in the LC-MS-MS experiment.

LC was coupled to MS for the pre-separation of progesterone and internal standard from the sample matrix. Two typical mobile phases used in HPLC, methanol–and ammonium acetate (95/5% v/v), were investigated. It was found that the intensity by using methanol as mobile phase was slightly higher than that obtained by using ammonium acetate as mobile phase alone. Although the efficiency of ionization for MS detection could be improved if 100% methanol was used as mobile phase. Progesterone and internal standard could be well separated from the matrix by using a mobile phase with combination of methanol and ammonium acetate and hence was used herein.

Hence the method developed for estimation of progesterone from human plasma by LC-MS-MS method using multiple reaction monitoring. The current method has shown acceptable precision (table 49) and adequate sensitivity (table 48) for the quantification of progesterone in human plasma samples obtained for pharmacokinetic, bioavailability or bioequivalence studies. Furthermore, it was utilized for the analysis of subject samples. The method described is simple, rapid, sensitive, specific and fully validated as per FDA guidelines. The cost effectiveness, simplicity and speed of liquid/liquid extraction and quantification of progesterone make it an attractive procedure in the bioanalysis. The validated method allows the quantitation of progesterone in the 10-1500 ng/mL range.
6.3. Comparison of hormone levels between premenopausal and postmenopausal females

Three estrogens (estradiol, estrone and estriol), are naturally produced in the female body. In premenopausal women, estradiol produced by the ovary, is the estrogen in largest quantity. Estradiol is the most potent because it has the highest affinity for estrogen receptors. In premenopausal women, circulating estradiol levels fluctuate from 40 to 200–400 pg/mL across the menstrual cycle\(^{237}\) and in the present study the value was found to be 119.22±9.07 pg/mL. After menopause, estradiol levels drop to less than 20 pg/mL as per literature\(^{237}\) and in the present study it was observed as 67.54±7.96 pg/mL.

In menopause the reduced estradiol levels are believed to influence hepatic lipid and lipoprotein metabolism, which in turn increases the heart diseases and atherosclerosis. Estrogen and progesterone are the two primary sex hormones, estrogen rises in the first half of the cycle, peaks at ovulation, then falls in second half as progesterone rises. Sometimes estrogen is too high relative to progesterone. If estrogen levels remain high in proportion to progesterone and women develops infertility, hyperamenorrhea (heavy bleeding), fibroids, breast cancer and stroke. If progesterone levels are high in proportion to estrogen, it may lead to endometrial cancer, cervical and ovarian cancer\(^{182}\).

Estrone a metabolite of estradiol is produced via the conversion of androstenedione in adipose tissue. In PMFs the normal value are reported to be 12.5 to 144.4 pg/mL\(^{240}\) and in the present study it was observed as 79.07±6.87 pg/mL. In post menopause it was observed as 34.82±2.41 pg/mL and reported normal values is 32.09±4.6 pg/mL\(^{239}\). In postmenopausal female, the ovary ceases producing estradiol, but the adrenal gland continues making androstenedione, the immediate precursor to estrone, so the levels of estrone remain unchanged, whereas the plasma levels of estradiol fall significantly\(^{238}\).

The reported value of progesterone in premenopausal female is 0.47 ± 0.03 ng/mL and in PMF is 0.20±0.05 ng/mL\(^{241}\). In the present study the observed values are 8.14±1.04 and 0.17 ± 0.02 ng/mL for premenopausal female and PMF respectively.
6.4. Pharmacokinetic study of hormones in post menopausal females of India

6.4.1. Pharmacokinetic study of estrogen (estradiol and estrone) tablets in post menopausal females:

The present study was carried out with the aim to assess different pharmacokinetic parameters of estrogens (estradiol and estrone).

Many women in their middle to late 40s who are approaching menopause begin to experience bothersome symptoms such as hot flashes and night sweats and seek therapeutic interventions from their clinicians. Hormone therapy, with estrogen or estrogen plus progestogen (in women with a uterus), has been studied extensively and is the most consistently effective treatment for vasomotor symptoms. Because of large-scale prospective clinical trials such as the Women’s Health Initiative, which demonstrated that hormone therapy is not without risks, the FDA and several professional organizations currently recommend prescribing the lowest effective dose for the shortest duration of time consistent with treatment goals for the individual woman\textsuperscript{189,190}.

As age increases, the number of women entering the menopausal years is expanding rapidly. The goals of HRT are to replace estrogen in a manner that alleviates menopausal symptoms (hot flashes, urinary symptoms, and vaginal atrophy are the three symptoms for which women most frequently seek treatment), prevent the development of osteoporosis, decrease the risk of cardiovascular disease, and improve cognitive ability and mood, without increasing the risk of uterine cancer, breast cancer, ovarian cancer, or gallbladder disease\textsuperscript{191}. None of the pharmacologic formulations on the market today are refined enough to meet all of these goals; therefore, the “estrogen dilemma” or risk/benefit controversy over the use of HRT is an ongoing issue of concern for researchers, clinicians, and most especially, postmenopausal female. The clinician prescribing these medications and the women using them must carefully review the mechanism of action, adverse effects, contraindications, and the potential risks before initiating therapy\textsuperscript{191}.

Estrogens are among the most common pharmacologic agents prescribed. This article presents a basic overview of the physiology and pharmacology of estrogen to provide the reader with a foundation for reviewing the literature on risks, benefits, and clinical management. The population of women requesting pharmaceutical treatment for ERT/HRT alone is growing rapidly. Because the benefits and risks associated with these drugs are still
being elucidated, the choices made for individual women must be based on a thorough understanding of what is known to date. Clinicians prescribing these drugs are referred to the articles listed in the appendix for a review of the clinical management and informed consent and are urged to use continued critical examination of the evidence to guide their practice.

As explained in many literatures in the next decade many menopausal women will turn to the medical community for guidance on postmenopausal health. Although estrogen replacement therapy (ERT) and hormone replacement therapy (HRT, estrogens combined with progestins) are commonly prescribed treatments, currently only 10% to 35% of postmenopausal female consistently use ERT or HRT.

The exact concentrations of estrogens required to relieve postmenopausal symptoms and to prevent bone loss and heart disease are unknown. Systemic estradiol and estrone concentrations, as well as the ratios of estrone to estradiol achieved, differ with various estrogen products. Clinically effective doses of CEE produce estradiol and estrone levels comparable to those of the normal menstrual cycle. Transdermal or percutaneous estradiol administration results in higher estradiol concentrations than does oral estrogen treatment. Intravaginal products achieve high systemic levels of estradiol similar to those of transdermal products. Estradiol vaginal creams result in higher estradiol concentration, whereas higher estrone concentrations result from CEE vaginal creams. Additionally, higher estradiol and estrone concentrations were found with intravaginal CEE than with oral CEE.

The bioavailability estrogen depends on estrogen formulation and route of administration, other factors such as serum proteins and cigarette smoking. The majority of circulating estrogen is bound to serum proteins, which are important in the transport, distribution, and metabolic clearance rate of estrogens. The metabolic clearance rate for both unconjugated equilin and estrone is approximately 10-fold higher than that of their conjugated counterparts. This may be explained by the differences between conjugated and unconjugated estrogens in binding to albumin. Conjugated estrogens such as the sulfated esters of equilin, estrone, and estradiol bind with high affinity to albumin. In contrast, unconjugated equilin, estrone, 17β-dihydroequilin, and 17β-estradiol bind with high affinity to albumin. Cigarette smoking can increase the hepatic metabolism of oral estrogens. In
few studies it was discovered up to a 50% reduction in estrogen levels in postmenopausal female who smoked compared with those who did not. Consequently, the beneficial effects of ERT and HRT on serum lipoproteins and bone loss were reduced in smokers compared with nonsmokers 258.

In the present study we administered 2 mg of estrogen tablets to PMF which was well tolerated and absorbed, figure 66, 67 for estradiol and 68, 69 for estrone. The Cmax for estradiol was reached at 420.42±37.74 pg/mL at $t^{1/2} 14.42±1.42$ hr (Table 70) with baseline correction and Cmax is 541.58±98.88 pg/mL at $t^{1/2} 31.71±5.79$ hr (Table 71) without baseline correction.

The Cmax for estrone reached at 262.86±11.79 pg/mL at $t^{1/2} 15.53±2.24$ hr (Table 72) with baseline correction and Cmax is 290.19±11.68 pg/mL at $t^{1/2} 31.87±3.42$ hr (Table 73) without baseline correction.

Postmenopausal health in women can be improved with HRT because estrogens relieve symptoms of estrogen deficiency, prevent or delay cardiovascular disease and osteoporosis, and improve quality of life. Progesterone on the other hand protects the endometrium from the proliferative effects of estrogen. Although several estrogen and progestrene products are available for the treatment of estrogen deficiency in postmenopausal female, the efficacy of a particular estrogen and progesterone may be determined by the pharmacokinetics or formulation of each product.

6.4.2. Pharmacokinetic study of Progesterone capsules in post menopausal females.

Oral micronized progesterone has been used successfully to protect the endometrium from the proliferative effects of estrogen. It is reported that administration of 100 mg micronized progesterone for 25 days of the month protected the endometrium from proliferation and resulted in induction of amenorrhea in >90% of women 242. The Postmenopausal Estrogen/Progestin Interventions (PEPI) trial compared micronized progesterone with medroxyprogesterone acetate and found micronized progesterone to have an effect on the endometrium as efficacious as that of medroxyprogesterone acetate while having a less negative impact on high-density lipoprotein cholesterol 243. Recent evidence has caused concern about the cardiovascular consequences of the use of medroxyprogesterone acetate and has raised the question of selecting and optimizing progestin doses and route of administration 244.
Various non-oral routes of administration of progesterone have been used in treating postmenopausal female receiving estrogen replacement. Intramuscular and vaginal progesterone produced similar serum levels of progesterone and effects on the endometrium. It is has been reported that a gonadal women undergoing embryo donation achieved a secretory endometrium with vaginal micronized progesterone. The delivery of progesterone to the uterus was enhanced by the vaginal route compared with a standard intramuscular regimen. All women with benign endometrial hyperplasia treated effectively with a vaginal cream consisting of 100 mg natural micronized progesterone in a polyethylene glycol base used from days 10 to 25 of the cycle. Other investigators have also reported success in achieving luteal serum progesterone concentrations and secretory changes in the endometrium in response to vaginal application of progesterone. Even studies with low serum concentrations of progesterone have shown beneficial effects on the endometrium, suggesting a direct transit to the uterus for a “first uterine pass effect”.

Few study reported that 45 to 90 mg vaginal progesterone gel induced normal secretory transformation of the endometrium corresponding with plasma progesterone levels of 3.4 to 3.6 ng/mL. Another route of administration available is nasal spray. 5 to 7 days of treatment with a nasal spray containing 34 mg progesterone resulted in secretory endometrial changes in postmenopausal women treated with estrogen. Thus it seems possible that the absorption of progesterone across mucus membranes, vaginal mucosa, and the skin may result in an increased bioavailable level of the hormone, therefore providing an endometrial effect with lower serum levels of progesterone.

Many women are currently using progesterone creams sold without prescription to manage menopausal symptoms. Some of these women are using estrogen together with these creams and thus without any other progestin. Pharmacokinetics of progesterone is quite complex, and thus the serum levels are not obvious and may be counterintuitive.

It is reported that after oral administration, progesterone is rapidly absorbed, rapidly metabolized from the intestines and during the first hepatic pass, cleared from the circulation. Maximal plasma progesterone concentrations are reached simultaneously with progesterone metabolites within 4 hour. Micronization of natural progesterone improves its absorption and bioavailability. After ingestion of 200 mg of micronized progesterone, mean serum progesterone concentrations are attained in 2-4 hours and remain significantly
elevated for the next 6-7 hours\textsuperscript{192}. Nevertheless even higher doses of oral micronized progesterone (200 – 300 mg/day) have failed to induce uniform secretory endometrial features in menopausal women\textsuperscript{193}.

Progesterone support during the luteal phase and early pregnancy is mandatory in in-vitro fertilization (IVF) cycles downregulated with gonadotrophin-releasing hormone analogues (GnRH-a)\textsuperscript{197}, and in women treated for oocyte donation\textsuperscript{185,186}. At times, such supplementation should be continued for several weeks. In recent years newer formulations have been developed for natural progesterone administration. However, the most reliable formulation to meet the patient’s compliance as well as therapeutic efficacy is still under investigation however as a conventional treatment capsule formulation are in more use due to various advantages\textsuperscript{194}.

But different routes of progesterone administration have been analysed, such as intranasal, sublingual, rectal routes. However oral, intramuscular and vaginal route have been frequently used as they have greater advantages compared to other convectional route\textsuperscript{194}.

In one of the study 50 mg and 100 mg vaginal tablets demonstrated pharmacokinetic patterns of rapid absorption, reaching a $t_{\text{max}}$ within 6 hr, with a $t_{1/2}$ of about 13 hr\textsuperscript{186}.

A wide variation exists in progesterone serum concentrations during the menstrual cycle. It is accepted that concentrations above 3.3 ng/mL indicate that ovulation has occurred\textsuperscript{187}, while mid-luteal phase values of 10 ng/ml and above demonstrate adequate corpus luteum function\textsuperscript{188}.

In the present study the 200 mg progesterone was administered as micronized formulation in the form of capsule which resulted the absorption of progesterone figure 76, table 74 with $C_{\text{max}}$ of 4.87±1.01 ng/mL at $t_{1/2}$ 8.29±2.01 hr with base line correction, and figure 77, table 75 with $C_{\text{max}}$ 4.93±1.01 ng/ml at $t_{1/2}$ 19.10±5.44 hr without base line correction suggestive of good absorbance and acceptable dose for Indian females.

The convenience of orally administered progesterone is however indisputable, its use has been associated with systematic adverse effects, e.g. drowsiness, flushing, and nausea. Sedative and hypnotic effects or fluid retention have also been attributed to progesterone or its metabolites after oral ingestion. Pharmacokinetic properties of orally administered
progesterone are further influences by food uptake or by characteristics of progesterone preparation such as vehicle and particle size.\(^{183}\)

In the present study the pharmacokinetic data for hormones, estrogen (estradiol and estrone) and progesterone are presented in two ways i.e. with baseline correction data and without baseline correction data.\(^{259}\)

The figure 66 (table 70) represent pharmacokinetic profile of estradiol with baseline correction and figure 67 (table 71) represent pharmacokinetic profile of estradiol without baseline correction, figure 68 (table 72) represent pharmacokinetic profile of estrone with baseline correction and figure 69 (table 73) represent pharmacokinetic profile of estrone without baseline correction. Figure 76 (table 74) represent pharmacokinetic profile of progesterone with baseline correction and figure 77 (table 75) represent the pharmacokinetic profile of progesterone without baseline correction.

The mean of the pre-dose estradiol levels should be used for the baseline adjustment of the post-dose levels. Baseline concentrations should be determined for each dosing period, and baseline corrections should be period specific. If a negative plasma concentration value results after baseline correction, this should be set to 0 prior to calculating the baseline-corrected pharmacokinetic parameters.\(^{260}\)

After calculation of the pharmacokinetic parameters in case of without base line correction data, hormone concentrations were observed 80.79 pg/mL (estradiol), 31.81 pg/mL (estrone) and 0.02 ng/mL (progesterone), before administration of the drug. So after baseline correction of pre dose hormone concentration, the Cmax is increased  to 420.42 pg/mL (estradiol-table 70), 262.86 pg/mL (estrone-table 72) and 4.87 ng/mL (progesterone-table 74) respectively while in case of without base line correction it was 541.58 pg/mL (estradiol-table 71), 290.19 pg/mL (estrone-table 73) and 4.93 ng/mL (progesterone-table 75). Similarly concentration profile, half life, Kel, AUC0 – t and AUC\(\infty\) are different than that observed in case of without base line correction. Further with baseline correction and without baseline correction, it was observed that the tmax value remained almost same.

In our study it was found that the values of Cmax are different in tables (70, 71, 72, 73, 74 and 75) and the corresponding graphs (figures 66, 67, 68, 69, 76 and 77), as they were calculated based on different mathematical models. The mean of plasma drug concentrations for all subjects was calculated for each sampling time point and the same
values were used to plot the graph “time versus concentration”. While the value of maximum concentration of drug in plasma of each subject was taken and their mean was calculated as the Cmax which is represented in the tables.

The tmax is the time after administration of a drug when the maximum plasma concentration is reached; when the rate of absorption equals the rate of elimination. Hence the variation in the Cmax also affects the tmax\(^{261}\).

**Scope and Limitation of the study:**
The present study has been planned and executed with certain aim and objectives, with following limitations;

1. The demographic analysis provides a good scope for understanding of various normal laboratory ranges in Indian postmenopausal females. This gives a fare idea to all the investigators and pharmaceutical company within India and rest of the world, who are interested to know these values, either for their routine clinical use or for conduct of clinical trial in India on post menopausal females. As many pharmaceutical companies across the globe like USA, UK, Australia and New Zealand have the laboratory ranges of their country. It becomes very difficult to understand the laboratory profile of Indian post menopausal females due to lack of data, hence it becomes difficult to match with their protocol requirement. This study provides normal ranges of laboratory profiles of Indian post menopausal females so one can refer and design the protocol or clinical study as per Indian requirements.

   At the same time this study has some limitations like;

   a. The study has been conducted in only one part of India i.e. Ahmedabad, Gujarat.

   b. Only the healthy post menopausal females are included in the study

2. LC–MS-MS is becoming the method of choice for hormone quantitation in clinical samples. Biological matrix used to develop and validate a method may be significantly different than the matrix obtained from treated volunteers or patients due to reasons like use of excipients, concomitant medications or their metabolites.

   Extensive work was performed to achieve highly sensitive and rugged methods. The present research work describes the procedures adopted to
develop and validate bioanalytical methods for the research phase pharmacokinetic studies in human studies. The method was applied to measure the concentration of hormones, in plasma thus helping in understanding the pharmacokinetic profile of the administered hormone.

At the same time the method has a limitation of using only one type of mobile phase for both estrogen and progesterone, where a multiple mobile phase would have given a better sensitive method.

3. The comparison of estrogen and progesterone between pre menopausal females and post menopausal females of India has shown the normal values which can be used to compare whether the externally administered hormones have absorbed and available for estimation.

At the same time the study has a limitation of using limited number of volunteers.

4. The pharmacokinetic data provide the details of behavior of hormone in the body after oral administration where when we compared the values of orally administered formulation shown an increase in the plasma concentration time profile with that of normal range obtained as mentioned above.

At the same time the study has a limitation of;
  a. Limited number of volunteers used
  b. The study was conducted on healthy volunteers and not on patients.
  c. Only one formulation type and dose is used.

**Future recommendation:**

*As the life of the PMF are increasing and newer drug formulations are being discovered and tried on the females for hormone replacement therapy, a better understating about usage of these formulation in Indian scenario is required. Though these formulations offer better control of menopause associated symptoms, more studies should be planned. In this view the following future recommendation is advised;*

1. The demographic analysis should be carried out on patients as well along with healthy volunteers.

2. The demographic analysis should be carried on and compared between each group with more number of subjects.
3. The robust, rugged and sensitive method should be developed for estimation of these hormones by using different mobile phase.

4. The comparison of hormones in pre menopausal and post menopausal females should be carried out on patients who actually suffering from hormone disorders.

5. The pharmacokinetic study should be carried out with various formulations and doses in order to get a better formulation which relives the menopausal symptoms with minimal dose and with lesser side effects.

6. The pharmacokinetic study should also be carried out on patients.