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The Affect of Capmul, Labrafil and Transcutol on Progesterone 100 Mg Soft Capsules Bioavaialbility in Indian Healthy Adult Postmenopausal Female Subjects Under Fasting Conditions

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Abstract

The aim of this study was to evaluate the affect of solubilizers of capmul, labrafil and transcutol on progesterone 100 mg soft capsules of two different Test batches (Test-1 and Test-2) in comparison with that of Prometrium® (Progesterone USP) capsules 100 mg of Reference Product of Abbott Laboratories, USA in healthy adult, human, post-menopausal female volunteers. This study was an open label, balanced, randomized, three treatment, six sequence, three period, cross-over, single-dose comparative oral bioavailability study of Progesterone USP capsules 100 mg of two different Test batches (Test-1 and Test-2) conducted in 18 healthy adult, human, post-menopausal female volunteers under fasting conditions. Subjects received progesterone 100 mg of either test (Test-1 and Test-2) or reference formulation with a washout period of 7 days. After study drug administration, serial blood samples were collected over a period of 24 hours post dose. The plasma concentrations of progesterone were determined by a validated method using LC/MS/MS. Pharmacokinetic parameters C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, Kel and $T_{1/2}$were determined for both test (Test-1 and Test-2) and reference formulations. The formulations were to be considered bioequivalent if the geometric least square mean ratio of test and reference of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for baseline adjusted data, C_{max} and AUC_{0-t} for baseline unadjusted data were within the predetermined bioequivalence range of 80.00% to 125.00%. A total of 18 subjects were enrolled. No significant differences were found based on analysis of variance. The 90% confidence intervals (CI) for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$of progesterone baseline adjusted data were 617.99-1488.02%, 270.11-683.70%, and 228.82-523.71% respectively. The 90% confidence intervals (CI) for C_{max} and $AUC_{0-t}$of progesterone baseline unadjusted data were 497.80-1180.16% and 156.81-407.82% respectively. Both the test formulations (Test-1 and Test-2) in this study were fails to show the bioequivalence with that of reference formulation for progesterone and were found to have significantly suprabioavailale. The intra subject variability (%) for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$of progesterone baseline adjusted data were found to be 87.49%, 94.16% and 74.66% respectively. The intra subject variability (%) for C_{max} and AUC_{0-t} of progesterone baseline unadjusted data were found to be 85.47% and 97.93% respectively. There was a significant intra subject variability was observed for both the test formulations (Test-1 and Test-2) for progesterone under fasting conditions.

Keywords: Intra-subject variability; Highly variable drugs; Progesterone; Bioequivalence

Introduction

Bioequivalence (BE) studies are an integral component of the ANDA (Abbreviated New Drug Application) approval and marketing of generic drug products. BE studies are generally designed to determine if there is a significant difference in the rate and extent to which the active drug ingredient, or active moiety, becomes available at the site of drug action. According to the criteria developed by the U.S. (United States) Food and Drug Administration (FDA) and generally applied by other regulatory agencies, two pharmaceutically equivalent products are judged bioequivalent if the 90% confidence interval of the geometric mean ratio (GMR) of AUC and C_{max} fall within 80.00-125.00% [1].

The physic-chemical properties includes, progesterone is synthesized from a starting material from a plant source and is chemically identical to progesterone of human ovarian origin. Progesterone has a molecular weight of 314.47 and a molecular formula of $C_{21}H_{30}O_2$. Progesterone (pregn-4-ene-3, 20-dione) is a white or creamy white, odorless, crystalline powder practically insoluble in water, soluble in alcohol, acetone and dioxane and sparingly soluble in vegetable oils, stable in air, melting between 126°C and 131°C [2].

Progesterone is a steroid hormone indicated in the treatment of causing a menstrual period in premenopausal women with absent menstrual periods (secondary amenorrhea) and preventing abnormal overgrowth of the lining of the uterus (endometrial hyperplasia) in postmenopausal women taking estrogen hormone therapy. It plays an important role in the preparation and maintenance of pregnancy [2]. Under its influence the numerous minute glands line the uterine cavity are transformed into secreting glands. This alteration is a part of the change which is essential to provide for the implantation of a fertilized ovum and for the continuing development of the placenta [3]. The main functions ofprogesterone, it acts as a precursor of other sex hormones, including estrogen and testosterone, is necessary for the survival of the embryo and fetus throughout gestation protects against fibrocystic breasts, is a natural diuretic, helps use fat for energy, functions as a natural antidepressant, helps thyroid hormone action, normalizes blood clotting, restores sex drive, helps normalize blood sugar levels, normalizes zinc and copper levels, restores proper cell

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oxygen levels, has a thermogenic (temperature raising) effect, protects against endometrial cancer, helps protect against breast cancer, builds bone and is protective against osteoporosis and is a precursor of cortisone synthesis by adrenal cortex [4,5].

The pharmacokinetic properties of progesterone

Absorption: After oral administration of progesterone as a micronized soft-gelatin capsule formulation, maximum serum concentrations were attained within 3 hours. The absolute bioavailability of micronized progesterone is not known. Table 1 summarizes the mean pharmacokinetic parameters in postmenopausal women after five oral daily doses of Prometrium (Progesterone) Capsules 100 mg as a micronized soft-gelatin capsule formulation [2]. Serum progesterone concentrations appeared linear and dose proportional following multiple dose administration of Prometrium (progesterone) Capsules 100 mg over the dose range 100 mg/day to 300 mg/day in postmenopausal women. Although doses greater than 300 mg/day were not studied in females, serum concentrations from a study in male volunteers appeared linear and dose proportional between 100 mg/day and 400 mg/day. The pharmacokinetic parameters in male volunteers were generally consistent with those seen in postmenopausal women [2].

Distribution: Progesterone is approximately 96 percent to 99 percent bound to serum proteins, primarily to serum albumin (50 to 54 percent) and transcortin (43 to 48 percent) [2].

Excretion: The glucuronide and sulfate conjugates of pregnanediol and pregnanolone are excreted in the bile and urine. Progesterone metabolites are eliminated mainly by the kidneys. Progesterone metabolites which are excreted in the bile may undergo enterohepatic recycling or may be excreted in the feces [2].

Food effect: Concomitant administration of progesterone capsules with food increased the bioavailability of progesterone capsules relative to a fasting state when administered to postmenopausal women at a dose of 200 mg [2].

Study Design

This study was an open label, balanced, randomized, three treatment, six sequence, three period, cross-over, single-dose comparative oral bioavailability study of Progesterone USP capsules 100 mg of two different Test batches (Test-1 and Test-2) conducted in 18 healthy adult, human, post menopausal female volunteers under fasting conditions.

The primary objective of the study was to evaluate the affect of solubilizers of capmul, labrafil and transcutol on progesterone 100 mg soft capsules of two different Test batches (Test-1 and Test-2) in comparison with that of Prometrium®(Progesterone USP) capsules 100 mg of Reference Product of Abbott Laboratories, USA, when given in equal doses of single oral dose in 18 healthy, human, post menopausal female subjects under fasting conditions. The secondary objective of the study was to monitor the adverse events and to ensure the safety of the subjects.

Parameter	Mean ± SD		
	100 mg	200 mg	300 mg
C_{max} (ng/mL)	17.3 ± 21.9	38.1 ± 37.8	60.6 ± 72.5
T_{max} (hr)	1.5 ± 0.8	2.3 ± 1.4	1.7 ± 0.6
$AUC_{(0-10)}$ (ng*hr/mL)	43.3 ± 30.8	101.2 ± 66.0	175.7 ± 170.3

Table 1: Pharmacokinetic Parameters of Prometrium (progesterone) Capsules.

All the subjects provided written informed consent to participate in the study prior to enrolment and were free to withdraw at any time during the study. The study was conducted in compliance with the ICH GCP, ICMR guidelines, and declaration of Helsinki at the research facility.

Material and Methods

The investigational products were supplied by the Aurobindo Pharma Ltd., India for the conduct of this bioequivalence study.

Reference product (R)

Prometrium® (Progesterone USP) capsules 100 mg; each capsule contains 100 mg of progesterone micronized, USP, Mfg by: Catalent Pharma solutions, Marketed by: Abbott Laboratories (in North Chicago IL USA).

Lot No.: 500947.

Exp. Date: 05/2014

Test Product-1 (T1)

Progesterone USP Capsules 100 mg; each capsule containing 100 mg of progesterone Micronized, USP, Manufactured By Aurobindo Pharma Limited, India.

LOT No: 147000010A

Manufacturing Date: 10/20/2011

Test Product-2 (T2)

Progesterone USP Capsules 100 mg; each capsule containing 100 mg of progesterone Micronized, USP, Manufactured By Aurobindo Pharma Limited, India.

LOT No: 147000011A

Manufacturing Date: 10/21/2011

Screening

Volunteers aged from 40-55 years with a body mass index (BMI) in the range of 18-29.9 Kg/m^2 were selected according to the inclusion and exclusion criteria. They wereassessed to be healthy according to medical, systemic and physical examination including vital signs, and normal laboratory test results [haematology, biochemistry, urine analysis], Follicle stimulating hormone (FSH), Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT), Estradiol, Papanicolaou smear, Mammogram, Ultra sound pelvis, 12-lead ECG , Chest X-Ray (PA view) and Screening for infectious diseases including negative HIV 1 and 2, Hepatitis B, Hepatitis C, RPR tests.

Drugs of abuse (Benzodiazepines, Opioids, Amphetamines, Cannabinoids, Cocaines and Barbiturates) in urine, Urine Pregnancy test and alcohol breath analysis test were performed during the study check-in of each period and who tested negative were checked-in.

Drug Administration

Following an over night fasting of at least 10 hours, a single oral dose of Progesterone USP capsules 100 mg, Test-1 (T1) or Test-2 (T2) or Reference (R) product was administered in sitting posture as per randomization schedule with 240 mL of drinking water at room temperature under fasting conditions.

Blood Sampling Schedule

A total of 21 blood samples (4 mL each) in each period were

collected in a pre-labeled vacutainer tubes containing K_2 EDTA. The blood samples were withdrawn pre-dose at -24.00, -1.00, -0.50, 0.00 and at 0.33, 0.67, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 6.00, 8.00, 10.00, 12.00, 18.00, and 24.00 hours post dose in each period. The collected samples were centrifuged and separated plasma samples were transferred into pre labeled polypropylene tubes as single aliquot and were stored in a deep freezer maintained at -80°C or colder until Bio-analysis.

Washout period

A period of seven (7) days was given between the three periods.

Study conduct

A total of 18 subjects were enrolled in the study. All the 18 subjects were dosed and all 17 subjects completed the study.

Analytical method

Progesterone was analyzed using validated LC-MS/MS method (MDS SCIEX API 4000 with triple quadruple). The method conditions are as mentioned below [6].

Run time: 3.4 minutes

Polarity: +ve mode.

Column: Zodaic Sil 120-3-C18, AQ 3.0µ 4.6 × 100.

Mobile Phase: 2mM Ammonium Formate (pH 6.2): Methanol: Acetonitrile (ACN) in the ratio of 10:20:70.

Flow rate: 1.0 mL.

Injection volume: 10 µL.

Retention time for progesterone: 2.55.

Internal standard (Progesterone d9) retention time: 2.50.

	Q1	Q3
Progesterone	315.5	97.3
Progesterone d9	324.5	100.3

The MRM (Multiple Reaction Mode) used in the detection of progesterone and its deuterated internal standard (Progesterone d9) was 315.5/97.3 and 324.5/100.3 respectively.

50 mL of internal standard was taken (500 ng/mL) and mixed with 0.4mL of plasma and then 0.4 mL 2% OPA solution was added and vertexed for 10 minutes. Extracted the solution with Solid Phase Extraction using HCB Barry/ICC and conditioned with 1mL methanol and 1mL of milliQ water. The plasma sample was loaded and washed with 1 mL of 0.2% ammonia solution. The obtained solution was washed with 1mL of 10% methanol and then the cartridges dried for 2 minutes. The solution was eluted with 1mL of Acetonitrile (ACN) and evaporated for 10 minutes and finally reconstituted with 0.4 mL mobile phase and injected the sample on LC/MS/MS System. The calibration curve range used is 0.100 ng/mL to 82.525 ng/mL.

Pharmacokinetic and Statistical Analysis

Calculation of pharmacokinetic parameters was done for progesterone baseline adjusted data and progesterone baseline unadjusted data using drug concentration time data by non-compartmental method using Win Nonlin professional software version 5.0.1 (pharsight corporation, USA). Statistical analysis of the pharmacokinetic parameters of the three formulations was carried out using PROC GLM of SAS® release 9.1.3 (SAS Institute Inc., USA) to assess the comparative oral bioavailability of progesterone baseline adjusted data and progesterone baseline unadjusted data. Descriptive statistics, ANOVA, 90% confidence intervals, intra-subject variability was computed and reported for primary pharmacokinetic parameters and descriptive statistics was computed and reported for secondary pharmacokinetic parameters of progesterone baseline adjusted data and progesterone baseline unadjusted data.

Pharmacokinetic parameters of the formulations, based on the following primary and secondary pharmacokinetic parameters were assessed:

For Progesterone baseline adjusted data the following pharmacokinetic parameters were estimated.

C_{max}: Maximum measured plasma concentration over the time span specified.

AUC_{0-t}: The area under the plasma concentration versus time curve, from time 0 to the last measurable concentration, as calculated by the linear trapezoidal method.

$AUC_{0-\infty}$: The area under the plasma concentration versus time curve from time 0 to time infinity.

T_{max}: Time of the maximum measured plasma concentration. If the maximum value occurs at more than one time point, T_{max} is defined as the first time point with this value.

K_{el}: Apparent first order elimination rate constant calculated from a semi-log plot of plasma concentration versus time point. The parameter was calculated by linear square regression analysis using the last 3 (or more) non-zero plasma concentrations.

$T_{1/2}$: The elimination or terminal half-life was calculated as 0.693 / K_{el}.

For Progesterone baseline unadjusteddata the following pharmacokinetic parameteres were estimated.

C_{max}: Maximum measured plasma concentration over the time span specified.

AUC_{0-t}: The area under the plasma concentration versus time curve, from time 0 to the last measurable concentration, as calculated by the linear trapezoidal method.

T_{max}: Time of the maximum measured plasma concentration. If the maximum value occurs at more than one time point, T_{max} is defined as the first time point with this value.

These parameters were derived individually for each analysed subject from the concentration vs time data of Progesterone baseline adjusted and Progesterone baseline unadjusted in plasma. Values below the lower limit of quantification were set to zero. The pharmacokinetic parameters were calculated by non-compartmental model using Win Nonlin Professional Software Version-5.0.1 (Pharsight Corporation, USA).

Results and Discussion

The descriptive statistics, ANOVA, 90% confidence intervals, intra-subject variabilty were computed for the pharmacokinetic parameters of progesterone baseline adjusted data were as mentioned below (Table 2-8).

The linear and semi-log plasma concentration versus time profiles

S.No	Ingredients		Test Formulations		Function of excipients
	Medicament	**Reference**	**Test 1**	**Test 2**	
1	Progesterone	100mg	100mg	100mg	Active
2	Peanut oil NF	√	150 mg	150 mg	vehicle
3	Lecithin NF	√	5 mg	5 mg	emulsifier
4	Transcutol	-	-	35mg	Solubilizer
5	Capmul	-	15mg	-	Solubilizer
6	Labrafil		20mg	-	Solubilizer
	Fill weight	**290mg**	**290mg**	**290mg**	
	Softgel composition				
7	Gelatin NF	√	√	√	
8	Glycerin USP	√	√	√	
9	Titanium dioxide USP	√	√	√	
10	D&C Yellow No. 10	√	√	√	
11	FD&C Yellow No. 6.	√	√	√	

Transcutol® – Diethyleneglycol monoethyl ether
Capmul® – Propyleneglycol monocaprylate
Labrafil® – Lineoylmacrogol glycerides
All the above 3 materials are from Gatefosse vendor.

Table 2: Formulation details of two different Test Batches.

Parameter (Unit)	Mean ± SD (Un-transformed data) Progesterone baseline unadjusted		
	Test-1 (T1)	**Test-2 (T2)**	**Reference (R)**
C_{max}(ng/mL)	8.30 ± 8.10	50.18 ± 53.89	5.79 ± 6.24
$AUC_{0\to t}$ (hr. ng/mL)	19.23 ± 25.26	58.42 ± 61.60	41.26 ± 78.73
T_{max} (hr)	1.50 (0.67-4.00)	0.67 (0.33-1.50)	2.00 (1.00-5.00)

Table 3: Mean ± SD(Un-transformed data)Progesterone baseline unadjusted.

Parameter (Unit)	Mean ± SD (Un-transformed data) Progesterone baseline adjusted		
	Test-1 (T1)	**Test-2 (T2)**	**Reference (R)**
C_{max}(ng/mL)	8.29 ± 8.10	50.18 ± 53.89	4.37 ± 5.23
$AUC_{0\to t}$ (hr. ng/mL)	18.98 ± 25.35	58.42 ± 61.60	15.08 ± 21.94
$AUC_{0\to\infty}$ (hr. ng/mL)	19.82 ± 26.11	60.84 ± 63.16	16.92 ± 23.94
T_{max} (hr)	1.50 (0.67-4.00)	0.67 (0.33-1.50)	2.00 (1.00-5.00)
K_{el} (hr^{-1})	0.27 ± 0.16	0.17 ± 0.16	0.33 ± 0.21
$t_{½}$ (hr)	3.55 ± 1.91	6.06 ± 2.89	3.15 ± 2.00

Table 4: Mean ± SD (Un-transformed data) Progesterone baseline adjusted.

of progesterone (ng/mL) under fasting conditions for baseline unadjusted data of reference product were provided in Figures 1 to 2 and for baseline adjusted data were provided in Figure 7 to 8.

The linear and semi-log plasma concentration versus time profiles of progesterone (ng/mL) under fasting conditions for baseline unadjusted data of test product-1 were provided in Figure 3 to 4 and for baseline adjusted data were provided in Figure 9 to 10.

The linear and semi-log plasma concentration versus time profiles of progesterone (ng/mL) under fasting conditions for baseline unadjusted data of test product-2 were provided in Figure 5 to 6 and for baseline adjusted data were provided in Figure 11 to 12.

Mean and semi-log mean plasma concentration versus time profile of baseline unadjusted progesterone (ng/mL) under fasting conditions were provided in Figure 13 to 14 and for baseline adjusted progesterone (ng/mL) under fasting conditions were provided in Figure 15 to 16.

Based on the profiles it was observed that the test product-2 showed significant improvement in bioavailability both in terms of C_{max} and AUC_{0-t} in comparison with that of reference product, whereas test product-2 showed somewhat lower in comparison of bioavailability. However, progesterone exhibits a high variability in its absorption pattern and it was found be erratic.

The log-transformed pharmacokinetic parameters, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of baseline adjusted data for progesterone in test formulation 1 and test formulation 2 were subjected to analysis of variance (ANOVA) with the main effects of sequence, treatment, and period at 5% level

Parameter	Ln transformed Data – Baseline adjusted data				
	Geometric Mean		(T1/R) Ratio (%)	90% Confidence Interval	Intra Subject CV (%)
	Test-1 (T1)	Reference (R)			
C_{max}	6.20	2.73	227.51	146.61-353.03	87.49
$AUC_{0\to t}$	12.35	7.64	161.53	101.53-256.99	94.16
$AUC_{0\to\infty}$	13.14	9.78	134.42	88.86-203.36	74.66
ANOVA p-value for Lntransformed Data					
Parameter	**C_{max}**	**$AUC_{0\to t}$**	**$AUC_{0\to\infty}$**		
Sequence	0.3177	0.5453	0.5344		
Period	0.2663	0.3906	0.7294		
Treatment	<.0001*	<.0001*	<.0001*		

Table 5: Ln transformed Data – Baseline adjusted data and ANOVA p-value for Lntransformed Data.

Parameter	Ln transformed Data – Baseline adjusted data				
	Geometric Mean		(T2/R) Ratio (%)	90% Confidence Interval	Intra Subject CV (%)
	Test-2 (T2)	Reference (R)			
C_{max}	26.14	2.73	958.95	617.99-1488.02	87.49
$AUC_{0\to t}$	32.85	7.64	429.74	270.11-683.70	94.16
$AUC_{0\to\infty}$	33.85	9.78	346.17	228.82-523.71	74.66

Table 6: Ln transformed Data – Baseline adjusted data.

Parameter	Lntransformed Data – Baseline unadjusted data				
	Geometric Mean		(T1/R) Ratio (%)	90% Confidence Interval	Intra Subject CV (%)
	Test-1 (T1)	Reference (R)			
C_{max}	6.24	3.33	187.34	121.67-288.45	85.47
$AUC_{0\to t}$	12.78	12.30	103.94	64.45-167.62	97.93
ANOVA p-value for Lntransformed Data					
Parameter	**C_{max}**	**$AUC_{0\to t}$**			
Sequence	0.2756	0.3989			
Period	0.6331	0.6679			
Treatment	<.0001*	0.0048*			

Table 7: Ln transformed Data – Baseline unadjusted data and ANOVA p-value for Lntransformed Data.

Parameter	Lntransformed Data – Baseline unadjusted data				
	Geometric Mean		(T2/R) Ratio (%)	90% Confidence Interval	Intra Subject CV (%)
	Test-2 (T2)	Reference (R)			
C_{max}	25.54	3.33	766.47	497.80-1180.16	85.47
$AUC_{0\to t}$	31.10	12.30	252.88	156.81-407.82	97.93

Table 8: Lntransformed Data – Baseline unadjusted data.

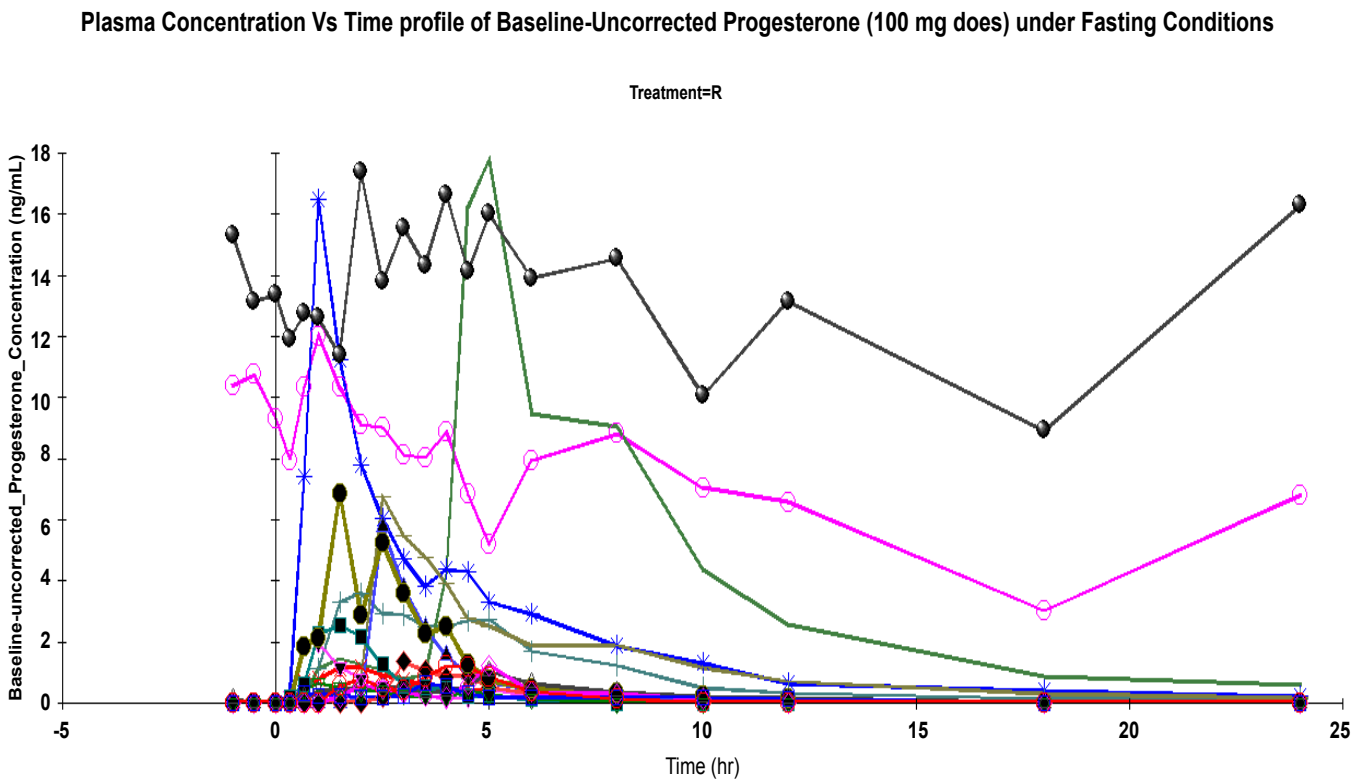

Figure 1: Plasma Concentration versus Time Profile of Baseline Unadjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects in linear scale for reference product.

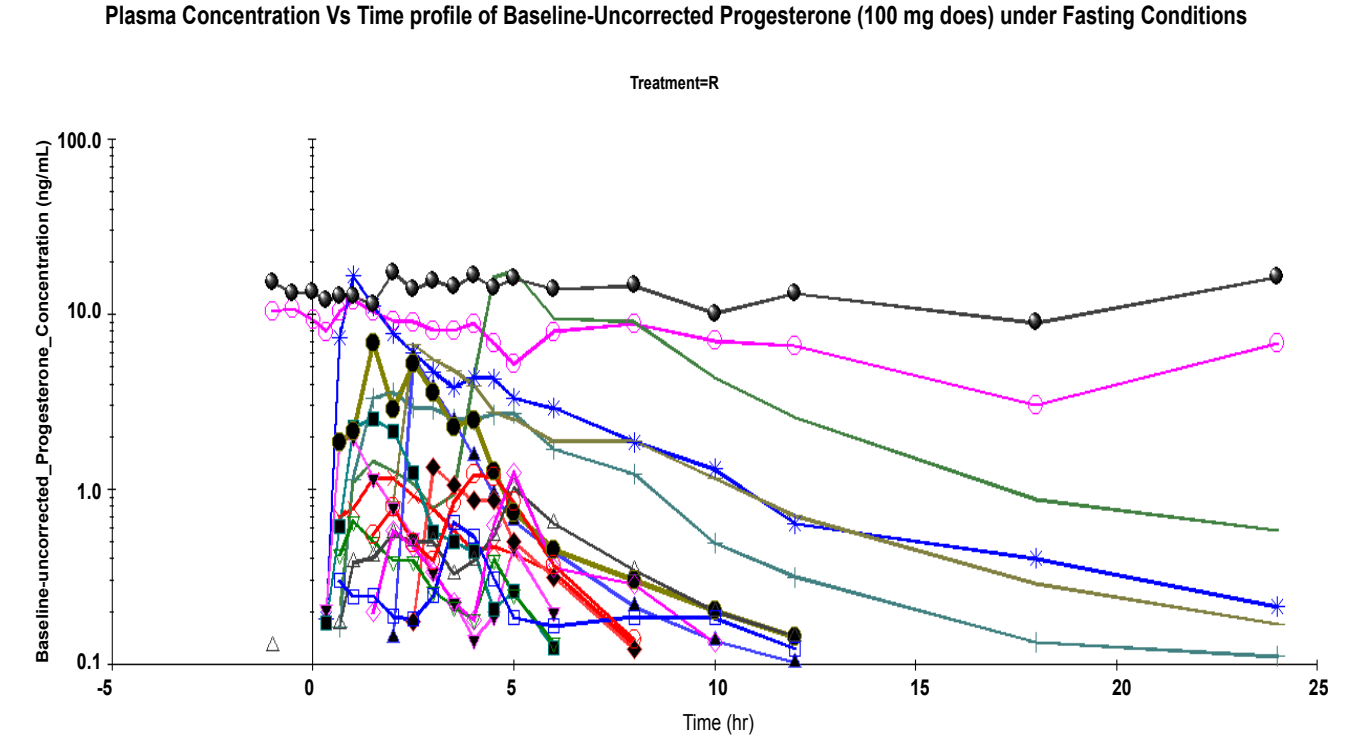

Figure 2: Semi-log Plasma Concentration versus Time Profile of Baseline Unadjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects for reference product.

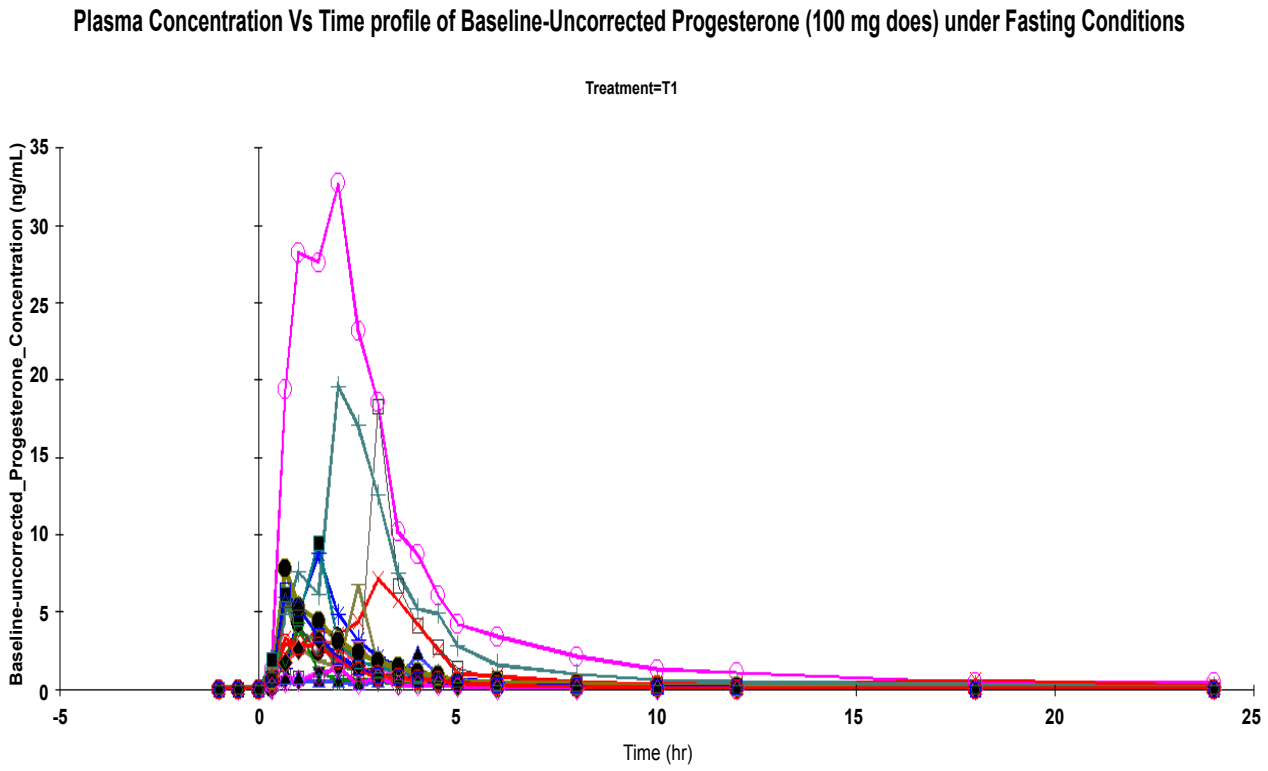

Figure 3: Plasma Concentration versus Time Profile of Baseline Unadjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects for test product-1.

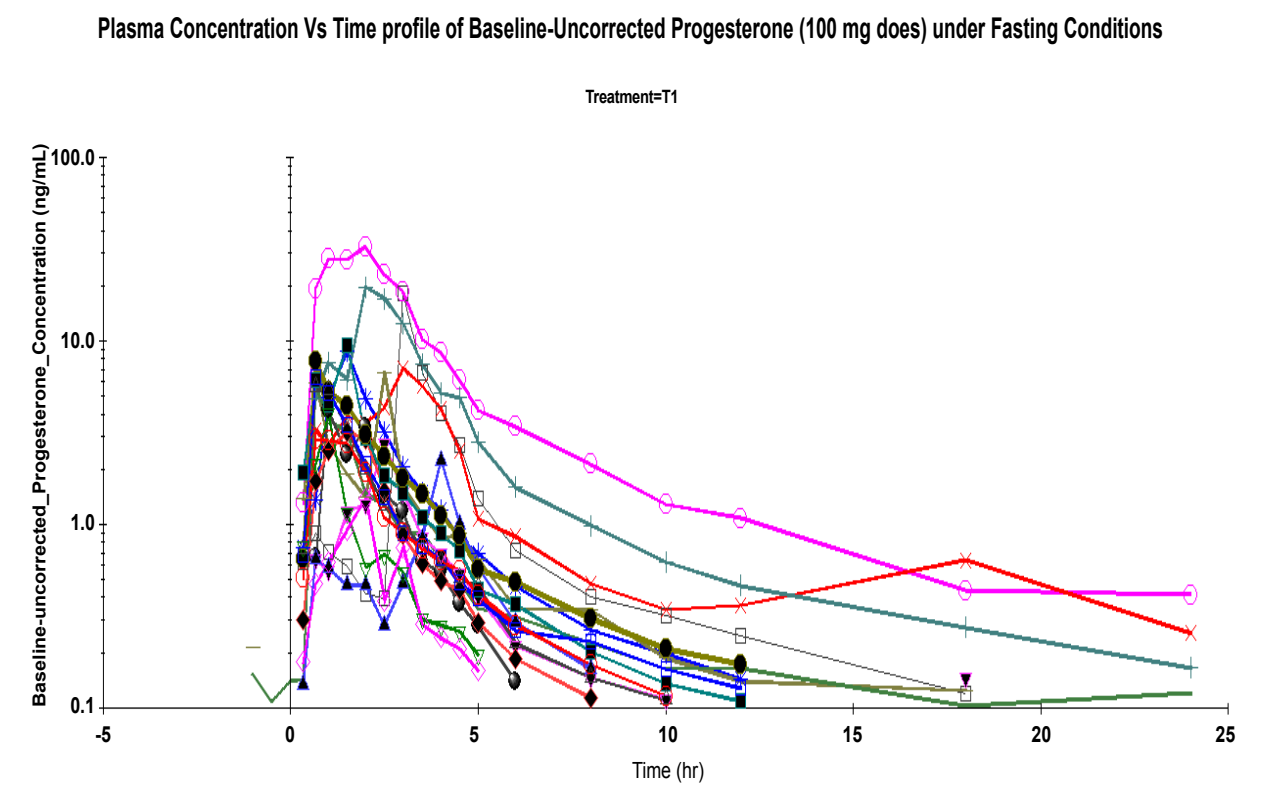

Figure 4: Semi-log Plasma Concentration versus Time Profile of Baseline Unadjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects for test product-1.

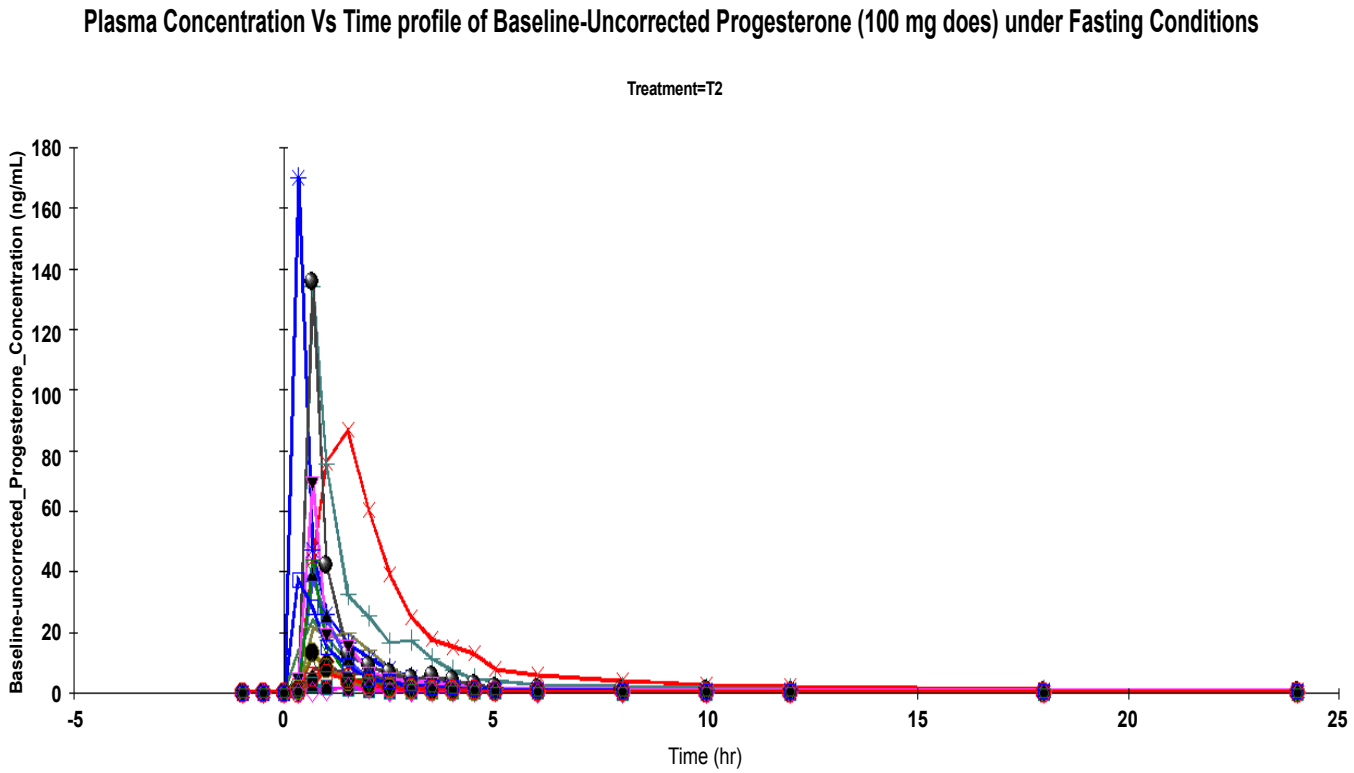

Figure 5: Plasma Concentration versus Time Profile of Baseline Unadjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects for test product-2.

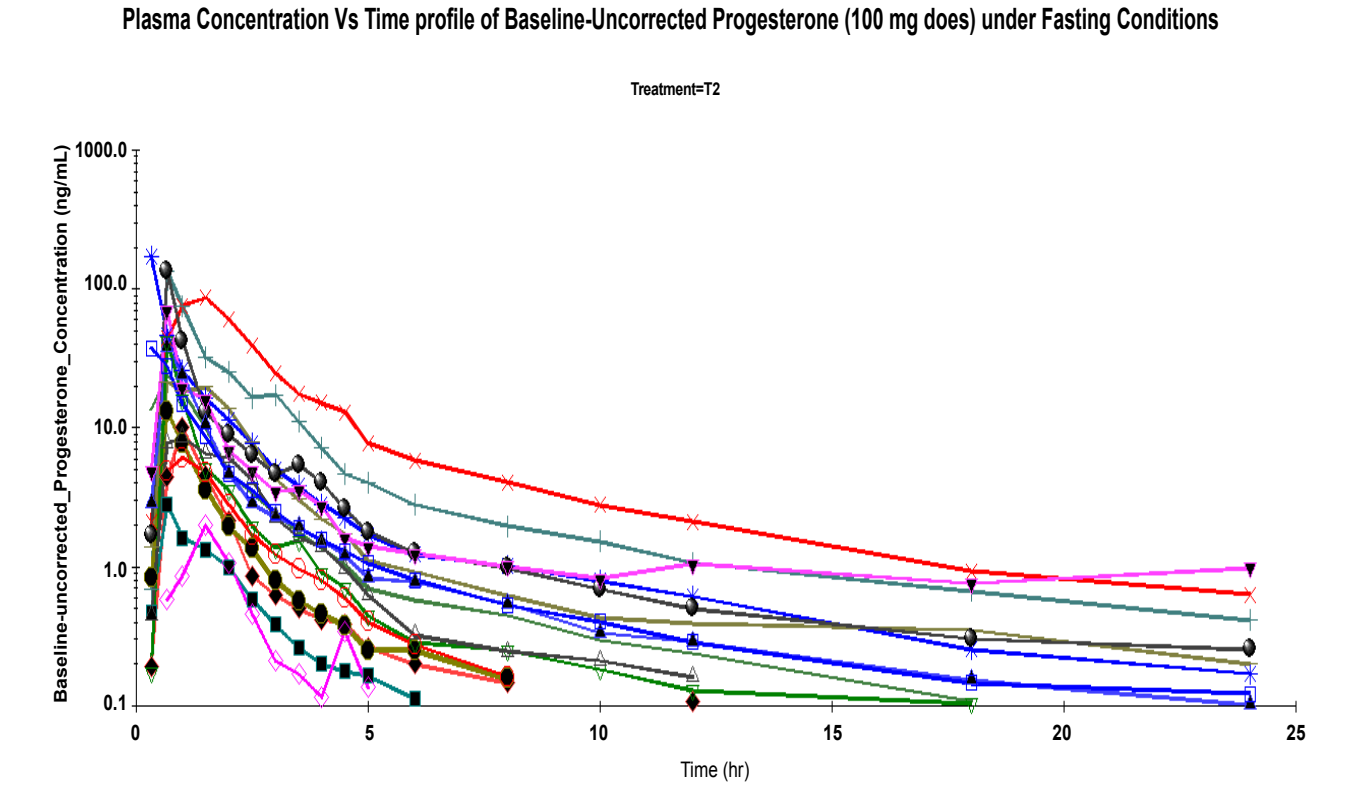

Figure 6: Semi-log Plasma Concentration versus Time Profile of Baseline Unadjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects for test product-2.

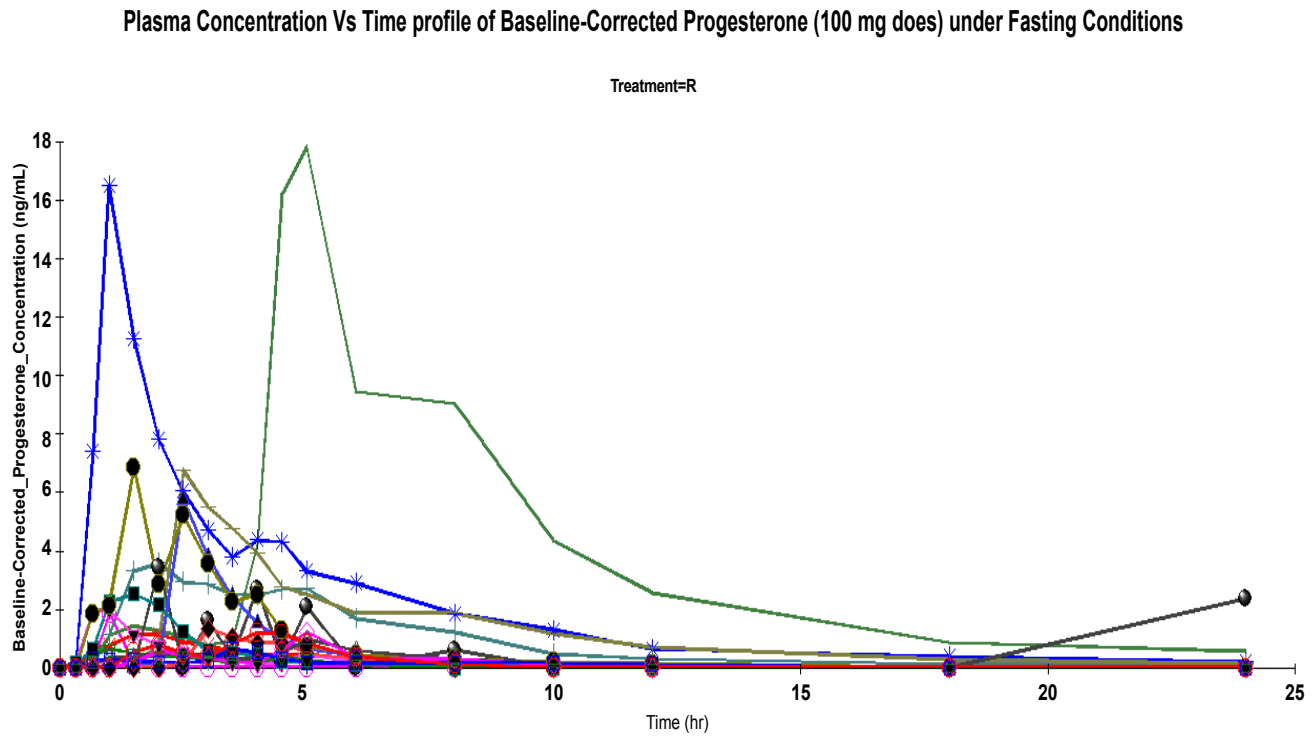

Figure 7: Plasma Concentration versus Time Profile of Baseline Adjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects in linear scale for reference product.

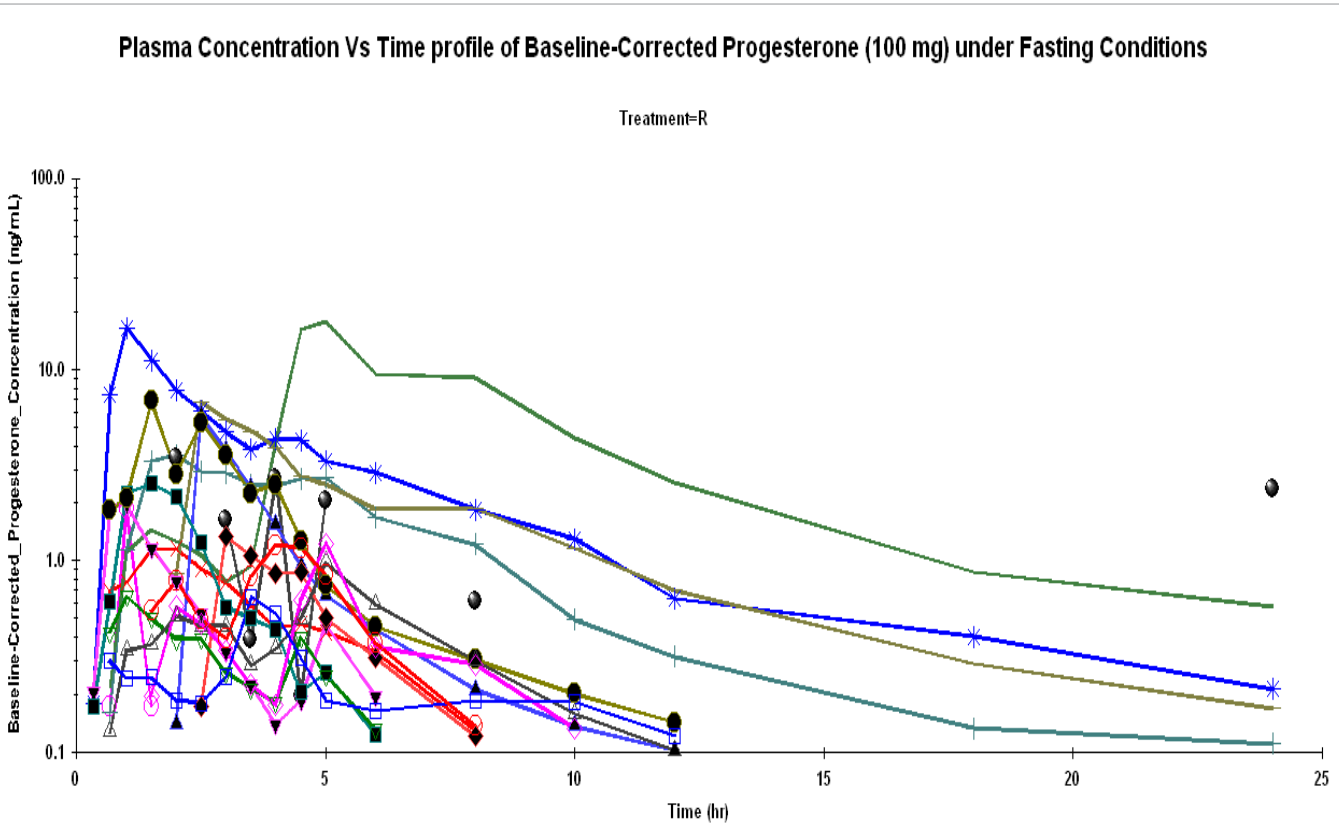

Figure 8: Semi-log Plasma Concentration versus Time Profile of Baseline Adjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects for reference product.

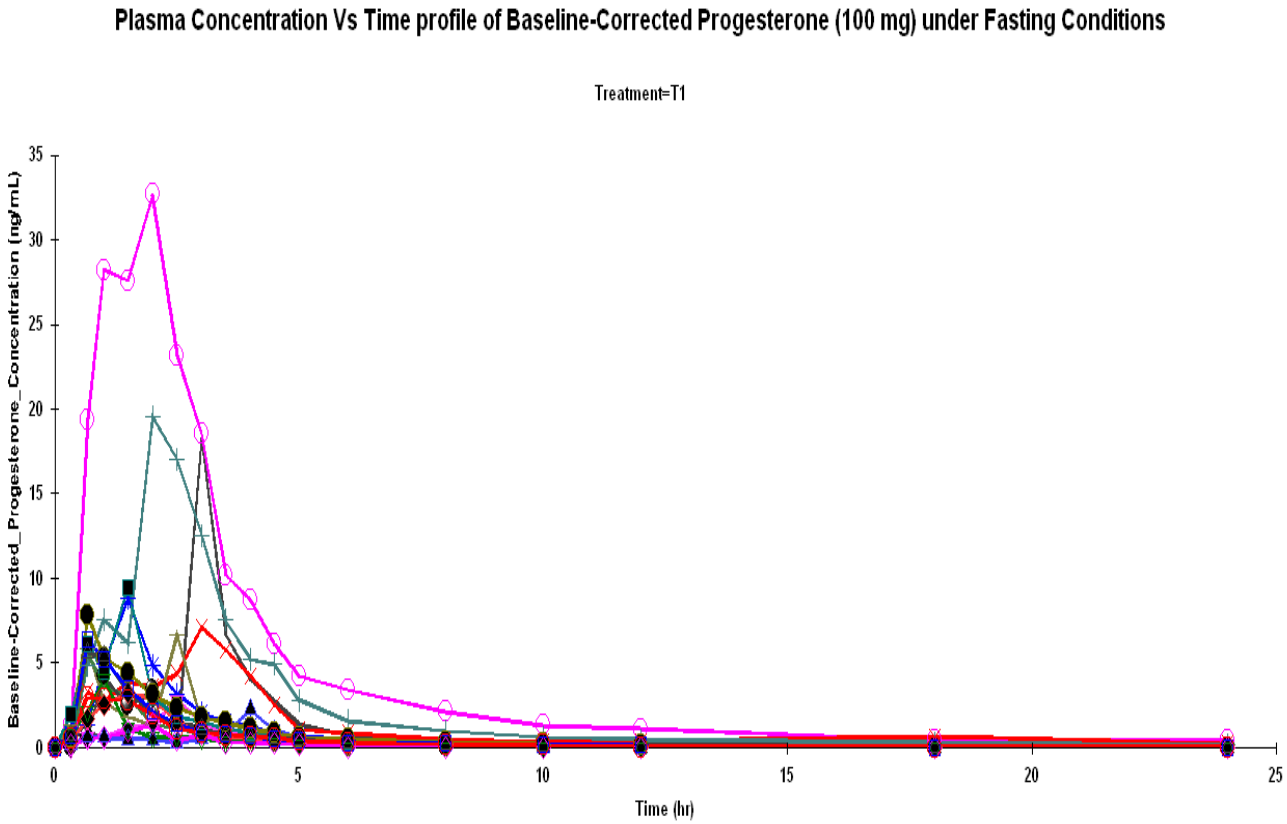

Figure 9: Plasma Concentration versus Time Profile of Baseline Adjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects for test product-1

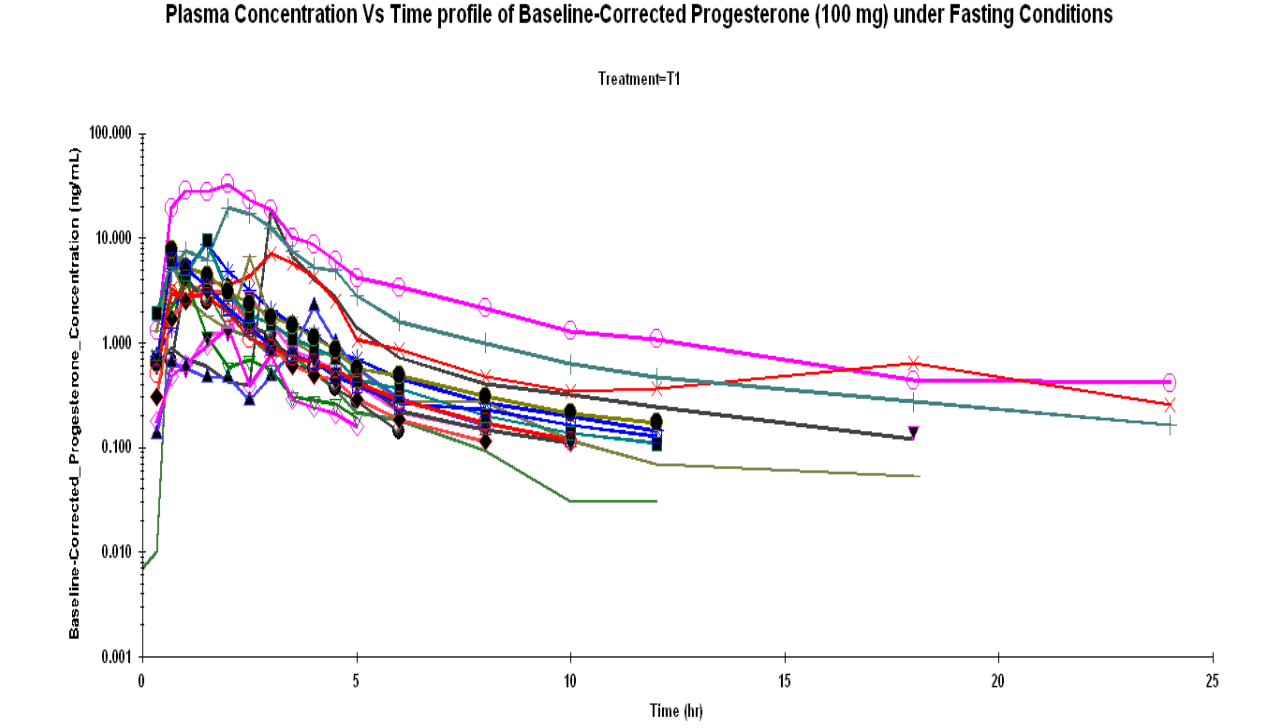

Figure 10: Semi-log Plasma Concentration versus Time Profile of Baseline Adjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects for test product-1.

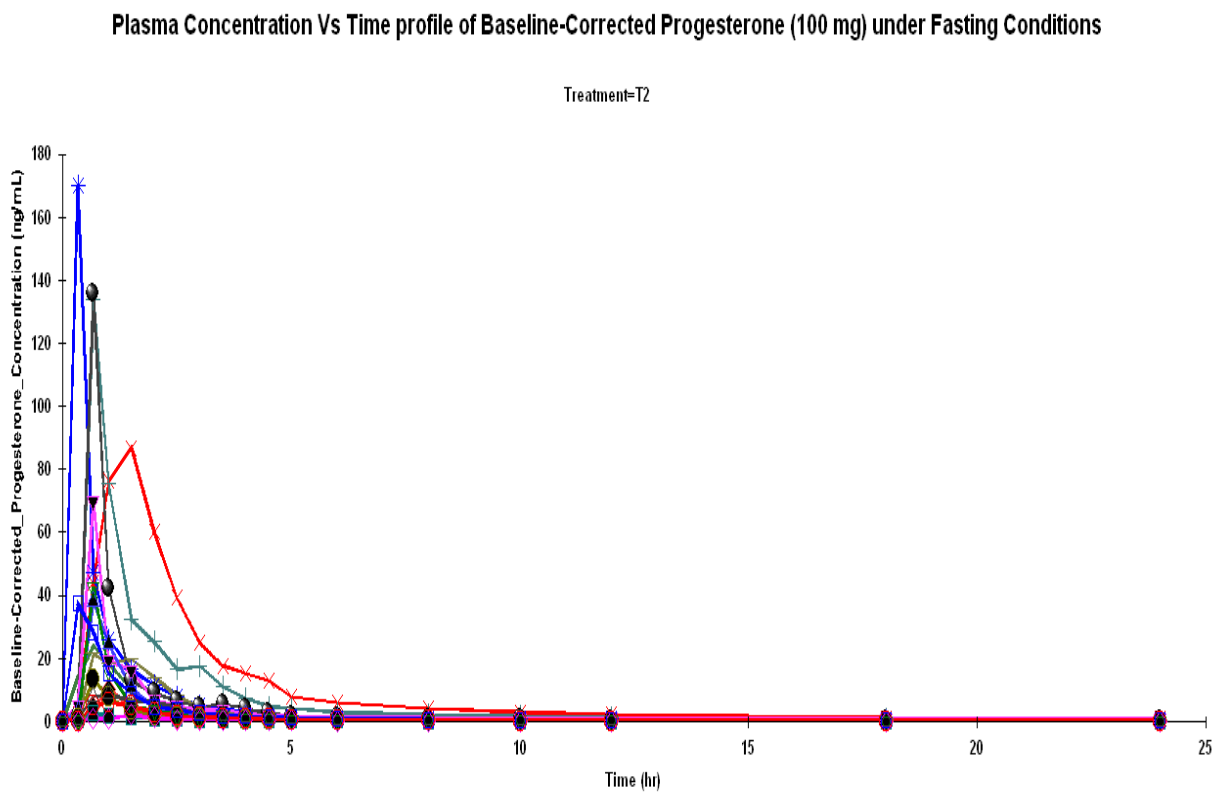

Figure 11: Plasma Concentration versus Time Profile of Baseline Adjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects for test product-2.

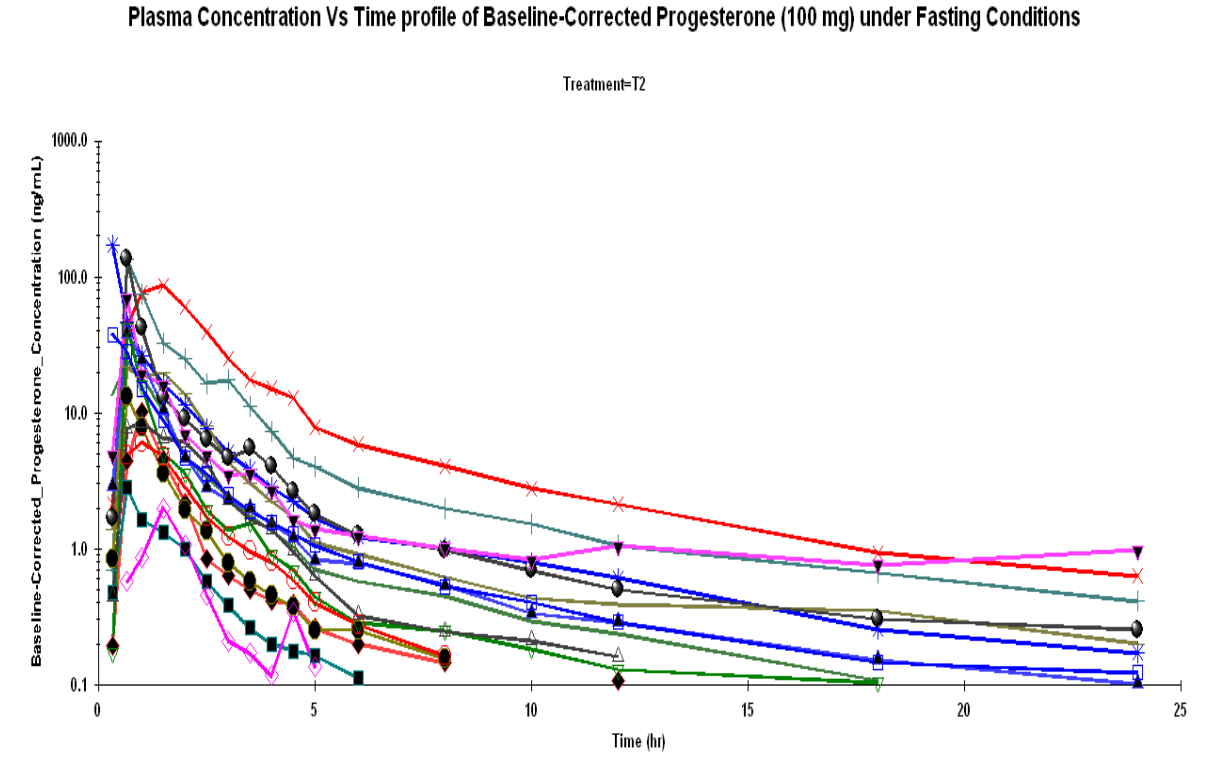

Figure 12: Semi-log Plasma Concentration versus Time Profile of Baseline Adjusted Progesterone (ng/mL) Under Fasting Conditions for all 18 subjects for test product-2.

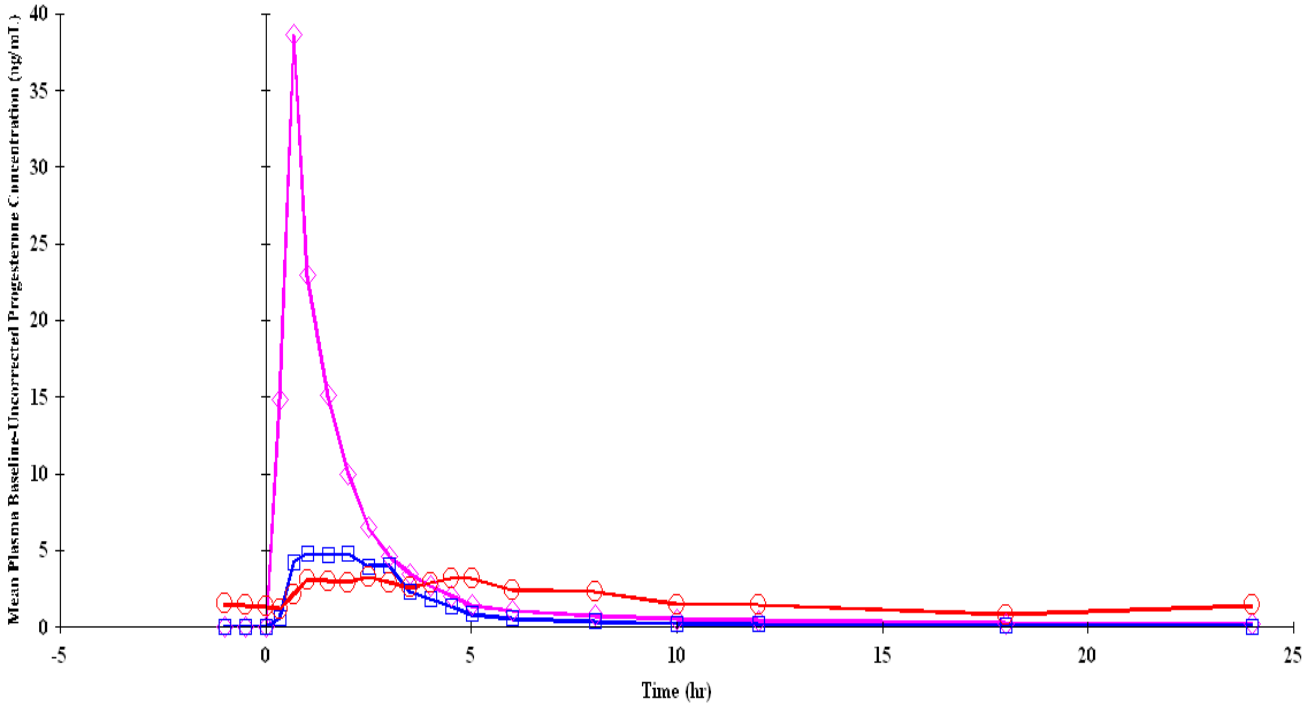

Figure 13: Mean Plasma Concentration versus Time Profile of Baseline Unadjusted Progesterone (ng/mL) Under Fasting Conditions.

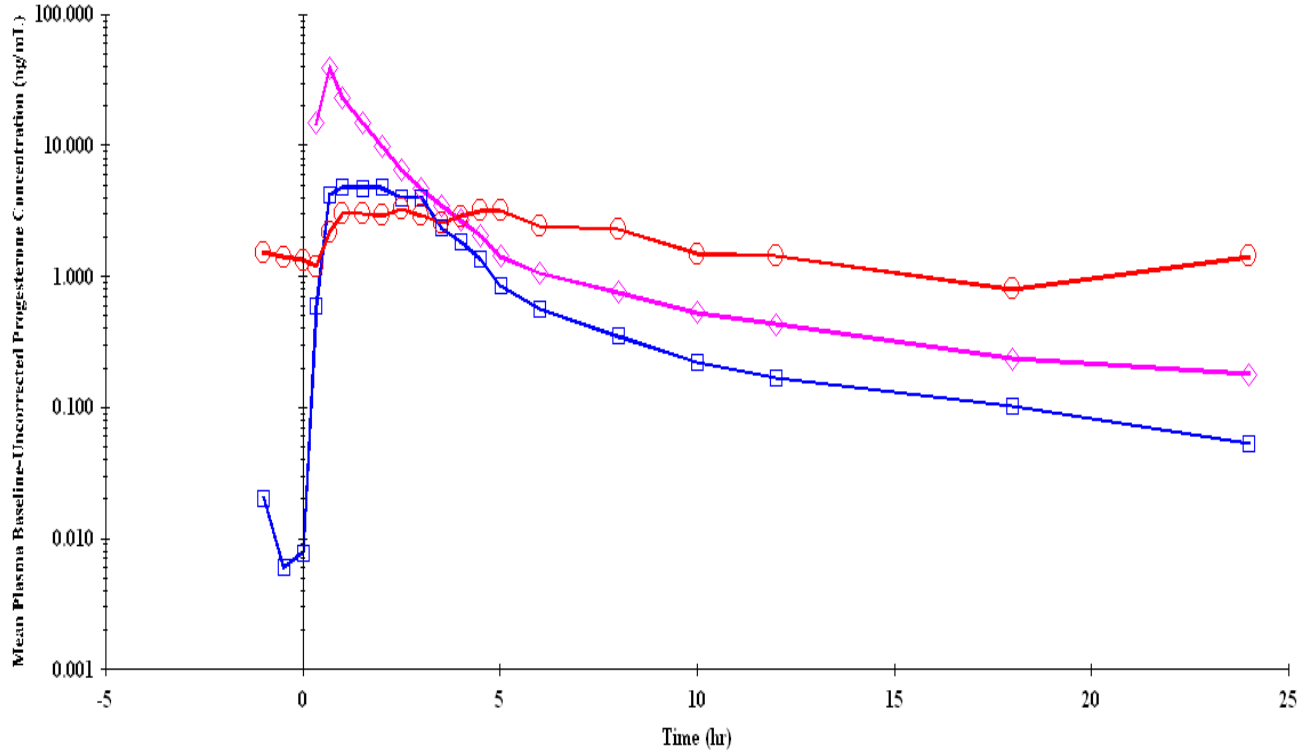

Figure 14: Semi-log Mean Plasma Concentration versus Time Profile of Baseline Unadjusted Progesterone (ng/mL) Under Fasting Conditions.

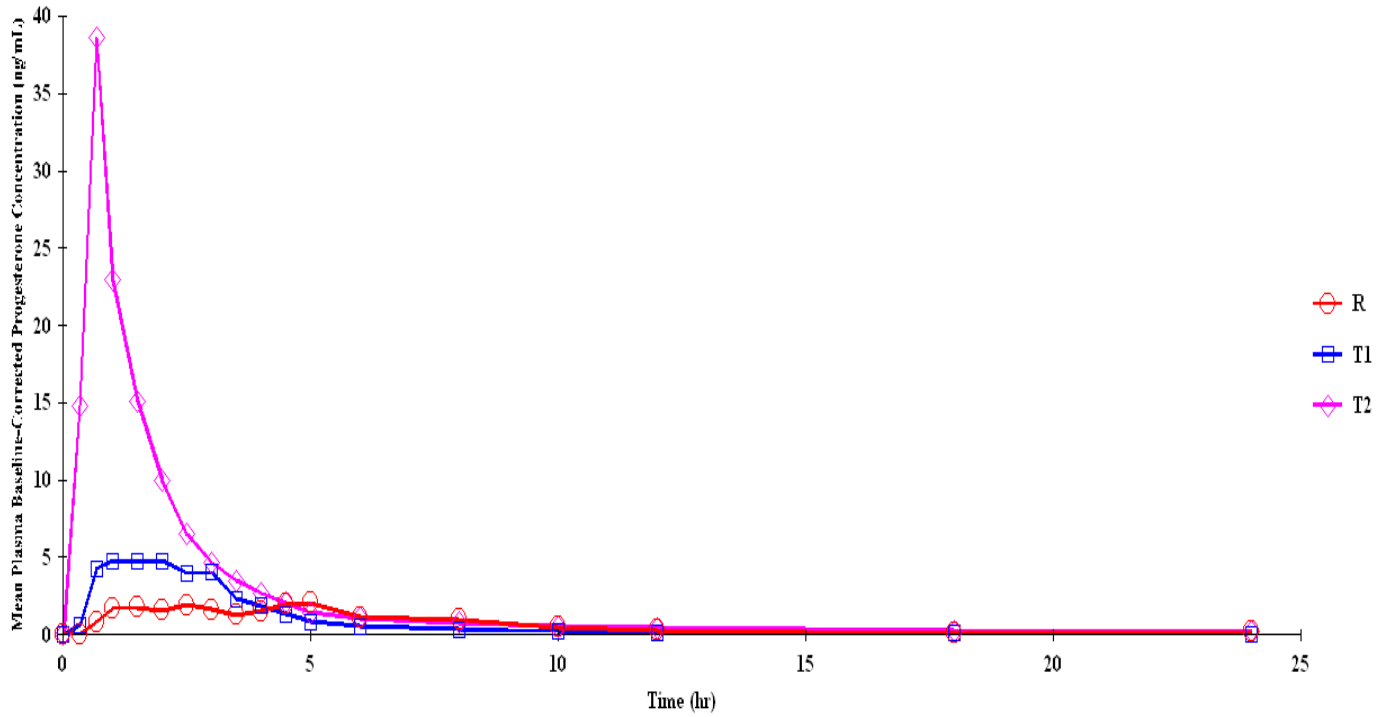

Figure 15: Mean Plasma Concentration versus Time Profile of Baseline Adjusted Progesterone (ng/mL) Under Fasting Conditions.

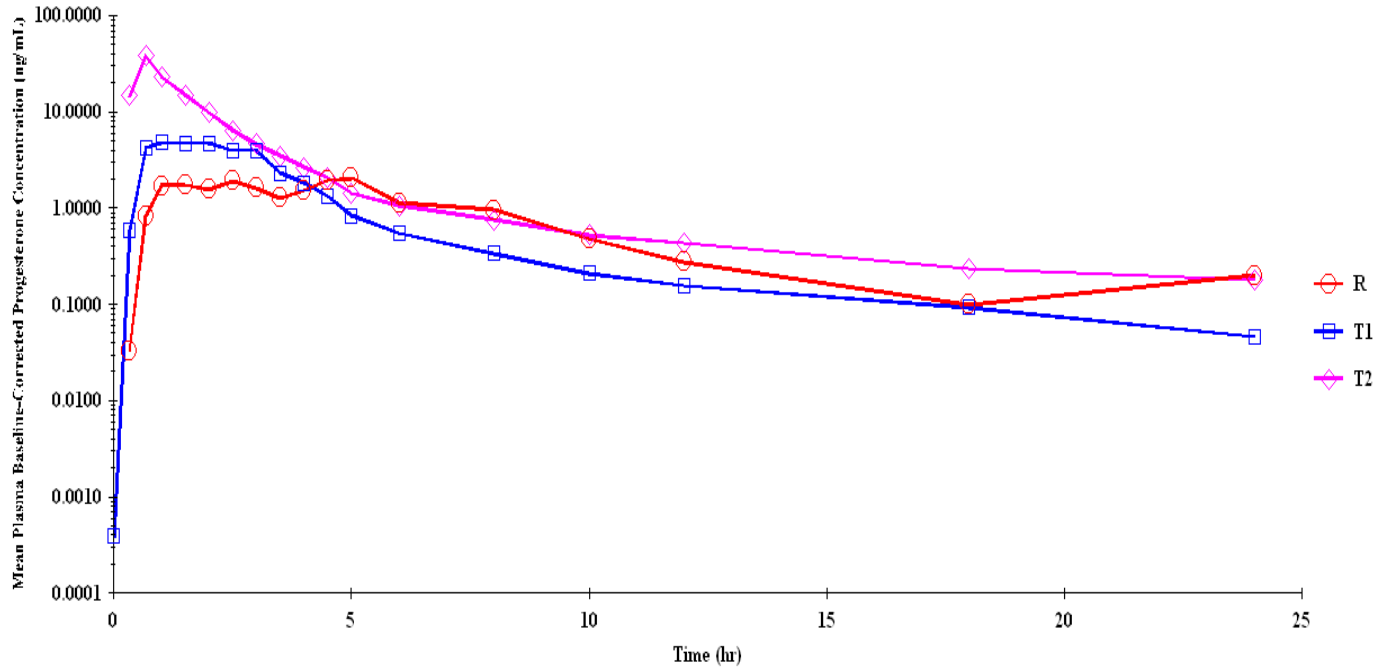

Figure 16: Semi-log Mean Plasma Concentration versus Time Profile of Baseline Adjusted Progesterone (ng/mL) Under Fasting Conditions.

of significance. Only treatment effect was found to be statistically significant effect ($p<0.05$) for both base adjusted and unadjusted data due to increase in the bioavailability of both the test products .

Test Formualtion-1:. The obtained geometric least squares means for test product-1 of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were found to be 6.20, 12.35 and 13.14 respectively. The obtained geometric least squares means for reference product of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were found to be 2.73, 7.64 and 9.78. The Test-1/Reference ratio for C_{max}, AUC_{0-t} and

$AUC_{0-\infty}$ were found to be 227.51%, 161.53% and 134.42% respectively. The 90% confidence intervals for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ using average bioequivalence criterion approach were found to be 146.61-353.03%, 101.53-256.99% and 88.86-203.36% respectively (Table 4).

The log-transformed pharmacokinetic parameters, Cmax and AUC0-t of baseline unadjusted data for progesterone were subjected to analysis of variance (ANOVA) with the main effects of sequence, treatment, and period at 5% level of significance. The obtained intra-subject variabilty for Cmax and AUC0-t were found to be 85.47% and 97.93% respectively. The obtained geometric least squares means for test product-1 of Cmax and AUC0-t were found to be 6.24 and 12.78 respectively. The obtained geometric least squares means for reference product of Cmax and AUC0-t were found to be 3.33 and 12.30. The Test 1/Reference ratio for Cmax and AUC0-t were found to be 187.34% and 103.94% respectively. The 90% confidence intervals for Cmax and AUC0-t using nominal average bioequivalence approach were found to be 121.67-288.45% and 64.45-167.62% respectively (Table 6).

Test Formualtion-2: The obtained intra-subject variabilty for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were found to be 87.49%, 94.16% and 74.66% respectively for baseline adjusted data The obtained geometric least squares means for test product-2 of progesterone baseline adjusted data for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were found to be 26.14, 32.85 and 33.85 respectively. The Test-2/Reference ratio for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were found to be 958.95%, 429.74% and 346.17% respectively. The 90% confidence intervals for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ using average bioequivalence criterion approach were found to be 617.99-1488.02%, 270.11-683.70% and 228.82-523.71% respectively (Table 5).

The Test-2/Reference ratio of progesterone baseline unadjusted data for Cmax and AUC0-t were found to be 766.47% and 252.88% respectively. The 90% confidence intervals for Cmax and AUC0-t using nominal average bioequivalence approach were found to be 497.80-1180.16% and 156.81-407.82% respectively (Table 7).

The obtained 90% confidence intervals were fell outside the acceptance range of 80.00-125.00% and test product-1 and test product-2 were showed supra bioavailability in comparsion with that of reference product. The Cmax and AUC were found be lower for test product-1 in comparison with that literature (Table 1), wehreas for test product-2, the Cmax was increased three fold higher and AUC was increased 1.5 times higher in comparison with that literature. Test product-2 showed significant higher bioavailability in comparison with that of test product-1. The observed high significantly high intra subject variability for progesterone under fasting conditions might be due to following factors including low apparent absolute and relative bioavailability of progesterone less than 10%, lack of aqueous solubility of progesterone, poor absorption when taken orally unless micronized in oil, fluctuation in endogenous levels of progesterone through various factors (e.g. stress, mood changes etc), high intra-subject variability and lack of sensitive bioanalytical method.

Conclusions

Based on the results obtained using average bioequivalence criterian, the test product-1 and test product-2 were showed supra bioavailability in comparsion with that of innovator. There was a significant intra subject variability was observed for C_{max} and AUC for baseline unadjusted and baseline adjusted progesterone under fasting conditions. The test product-2 showed 3 fold increase in C_{max} and approximately 1.5 times increase in AUC for baseline adjusted data indicating the effect of solubilizers Transcutol® (Diethyleneglycol monoethyl ether), Capmul® (Propyleneglycol monocaprylate and Labrafil® (Lineoyl macrogol glycerides). However there is a significant intra-subject variability exists in both the test formulations. Stable baseline values are also not detected indicated that a sensitive LOQ of 5 pg/mL is required for appropriate detection baseline concentrations.

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