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Pharmacokinetics and Bioequivalence of Dienogest in Healthy Bangladeshi Female Volunteers: An Open-Label, Single-Dose, Randomized, Two-Way Crossover Study

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Abstract

Background: Dienogest is a potential treatment for pelvic pain associated with endometriosis, a condition of significant concern in gynaecology. The current study was conducted as a crossover-randomized bioequivalence assessment of two oral Dienogest 2 mg formulations, aiming to provide valuable insights for healthcare professionals and researchers in this field. **Objective:** The primary aim of this research was to evaluate and compare the pharmacokinetic characteristics of Dienogest 2 mg tablets. Dinogest (Dienogest 2 mg) tablets, manufactured by Nuvista Pharma Limited in Bangladesh, and Visanne (Dienogest 2 mg) tablets, manufactured by Bayer Pharma in Germany, were the test and reference formulations, respectively. **Materials and Method:** The study was an open-label, balanced, randomized, two treatments, two sequences, two periods, two-way crossover, laboratory blind, single oral dose bioequivalence study conducted in healthy adult females under fasting conditions. The study was carried out on 13 healthy, non-pregnant female subjects, and all the subjects completed both study periods with a 15-day washout in between. Randomization was used to assign the test and reference formulations to the subjects. Following each oral administration, a series of blood samples were obtained at different time intervals from pre-dose to 72 hours post-dose and analyzed for Dienogest concentrations using a validated bio-analytical method. A standard non-compartmental model was used to analyze the pharmacokinetic parameters. The primary pharmacokinetic parameters were peak plasma drug concentration (C_{max}), the area under the

plasma concentration-time curve from time zero to time t (AUC_{0-t}), and AUC from t = 0 to infinity ($AUC_{0-\infty}$). The other PK parameters included time to reach C_{max} (T_{max}), terminal elimination rate constant (K_{el}), and half-life ($t_{1/2}$). **Result:** The ratios and 90% CI for the geometric mean test/reference were 95.53% (86.70% - 105.26%) for C_{max}, 101.75% (95.42% - 108.49%) for AUC_{0-t}, and 101.54% (95.59%% - 107.87%) for $AUC_{0-\infty}$. The formulations were bioequivalent since the 90% CIs for the geometric mean test/reference ratios were 80% to 125%, according to the predetermined range of US Food and Drug Administration (FDA) requirements. **Conclusion:** This single-dose investigation shows that the Dienogest test and reference formulations exhibited a rate and degree of absorption that met the regulatory requirements for bioequivalence.

Keywords

Dienogest, Bioequivalence Study, Endometriosis, Novus CRSL

1. Introduction

Endometriosis is a chronic, neuro-inflammatory condition defined as the development of endometrial glands and stroma-like lesions outside of the uterus [1] [2]. Endometriosis frequently exhibits symptoms (dysmenorrhea, deep dyspareunia, chronic pelvic pain, etc.) that overlap with other gastrointestinal and gynecologic conditions, making diagnosis more difficult [3]. Consequently, for many of these women, the diagnosis of endometriosis can be difficult and lengthy, and often, the delay results in a reduced quality of life [4]. Treatment consists of the surgical removal of lesions and hormonal medication [5]. Endometriosis is estimated to affect 6% - 10% of women of reproductive age [6]. Treatment of endometriosis consists of either medical or surgical management [7]. The surgical management of endometriosis is effective but has several controversial features [8]. Endometriosis must be regarded as a chronic pain disorder with a high recurrence rate, even after surgical removal [9].

Since endometriosis is essentially a hormonal disease, hormonal drug therapy is currently considered an essential and effective therapy [10]. Endometriosis has been treated with various hormones and medicines [11]. Specific medical therapies that are approved for the treatment of endometriosis include gonadotropin-releasing hormone (GnRH) agonists, danazol, the Combined Oral Contraceptive Pill (COCP), and certain progestins [12].

Dienogest is a new generation of progestin that has become one of the most used drugs in all endometriosis phenotypes for long-term treatment [13] [14]. According to the ESHRE (European Society of Human Reproduction and Embryology) guidelines, progestins are primarily used as a first-line, long-term treatment that is highly effective and acts on multiple sites of action [15]. Dienogest is almost completely absorbed and has a high oral bioavailability of more than 90% [16]. As it has a relatively short half-life of 10 hours, there is no risk of accumulation of

the drug in the body even after multiple dosages. Orally administered Dienogest is excreted through urine within 24 hours [17]. Its molecular formula is $C_{20}H_{25}NO_2$, with a molecular weight of 311.4 g/mol [18]. Chemically, Dienogest is described as (17*α*-cyanomethyl-17*β*-hydroxy-estra-4,9-dien-3-one [19]. Its structural formula is displayed in **Figure 1** [20].

Peak serum concentrations of approximately 47 nanograms per mL are reached about 1.5 hours after single ingestion [21].

Bioequivalence studies play a crucial role in evaluating a drug's efficacy by providing scientific evidence of therapeutic equivalence between different formulations. If two drugs are bioequivalent, they are expected to be the same for all intents. This study aims to investigate the bioequivalence of test formulations to reference formulations of Dienogest in healthy Bangladeshi female volunteers under fasting conditions [22]. Demonstrating bioequivalence ensures the safety, efficacy, and affordability of generic medications, ultimately benefiting patients, healthcare providers, and healthcare systems alike.

2. Methods

2.1. Study Centre and Study Period/Duration

The bioequivalence trial was conducted in 2023 at Novus Clinical Research Services Limited, a DGDA-approved Contract Research Organization (CRO) in Dhaka, Bangladesh. The clinical stage of the study was performed from August 29 to September 18, 2023, and the analytical stage from October 17 to October 31, 2023.

2.2. Ethical Consideration

The Bangladesh Medical Research Council (BMRC) of the National Research Ethics Committee (NREC) reviewed and approved the study protocol and all study documentation on January 22, 2023 (Registration No.: 50730102022). The study was also approved by the Directorate General of Drug Administration (DGDA) on April 13, 2023 (Reference No.: DGDA/CTP-04/2016/8688).

Good Clinical Practice, Good Laboratory Practice, Pharmaceutical Administration Law, and the Declaration of Helsinki (and its amendments) were all followed during the experiment.

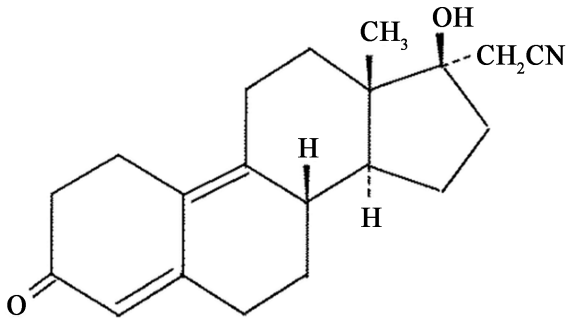

Figure 1. Chemical structure of Dienogest.

2.3. Identity of Investigational Products

Table 1 summarises the investigational products that were used in the research procedure. The doses of Dienogest used in this study were determined based on the recommended dose for endometriosis [23].

2.4. Study Subjects

A total of 27 healthy, adult, registered female volunteers were randomly selected for screening from the registered volunteers of Novus Clinical Research Services Limited. Among them, 13 eligible subjects aged 18 to 45 years with a body mass index between 18.5 and 29.99 kg/m^2 were included in the study. To confirm the eligibility of volunteers, chest radiography, electrocardiography, and laboratory investigations such as CBC, Blood glucose, HbA1C, serum creatinine, SGPT, SGOT, Uric Acid, Urea, Lipid profile, routine urine examination, etc., were carried out before 12 days of the first dosing.

Subjects were excluded from the study if any abnormalities were found in clinical investigations. It was confirmed that the subjects recruited for the study met inclusion and exclusion criteria. Each participant gave written informed consent before the screening, and study-specific informed consent was obtained from each participating subject before check-in.

2.5. Study Design

This study was performed under fasting conditions using a single-centre, randomized-sequence, single-dose, two-period, two-treatment crossover design. Eligible subjects were randomized to one of the two dosing-order subgroups, T/R and R/T. SAS® (SAS Institute Inc., USA) was used to randomise. The subjects in one sequence group were administered a single tablet of the test formulation with 240 mL of water in the first period, and after a washout period, individuals received a single tablet of the reference formulation in the second phase. The participants in the alternative sequence group received a reference tablet in the first period and a test tablet in the second period. The randomisation code was under controlled access till the completion of the analysis. The analysts were blinded to the sequence of administration of test and reference formulations throughout the analysis procedures.

Table 1. Identification of the experimental product (s).

IMP details	Test product (T)	Reference product (R)
Trade Name	Dinogest	Visanne
Generic Name	Dienogest	Dienogest
Specification	2 mg/tablet	2 mg/tablet
Batch/Lot No.	104223002	WEU6CF
Expiry Date	Dec' 2024	Dec' 2023
Manufacturer	Nuvista Pharma Ltd., Dhaka, Bangladesh	Bayer Pharma, Germany

Subjects were checked in the facility the day before the investigation's medication was administered in each period to ensure an overnight fast of at least 10 hours. There was a 15-day washout period between two consecutive dosing periods of the study, which was considered appropriate as per requirements by the FDA and the EMA [24].

2.6. Standard Meal and Fluid

The standard meal plan was identical for both study periods, and all in-house subjects received it at 04.00 hours following dosing. Except for one hour before and one hour after dosage, subjects were allowed to drink any amount of water they desired [25].

2.7. Blood Sampling

Venous blood samples (5 mL) were collected from each subject approximately 22 times through an indwelling cannula to assay. Dienogest from predose to 72 hours postdose at preset time points (0.00 (pre-dose), 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00, and 72.00 hours) and placed in a K_2EDTA tube. Every plasma sample was centrifuged for 10 minutes at 5˚C ± 3˚C at 3500 rpm. Two duplicate tubes containing evenly divided plasma were frozen at −60˚C, one for testing and the other for backup.

2.8. Safety Assessment

Clinical examination and vital sign measurements were carried out to monitor the subjects' safety at baseline and 1.00, 3.00, 5.00, 7.00, 9.00, 13.00, 26.00, 35.00, 48.00, and 72.00 hours after the dose, as specified in the protocol. However, investigations such as CBC, Blood glucose, HbA1C, serum creatinine, SGPT, SGOT, Uric Acid, Urea, Lipid profile, routine urine examination, etc and physical examinations, including 12-lead ECG and X-rays, were carried out at the time of screening and after the trial. Any adverse effects (AEs) that happened during the trial were tracked. Throughout the study, adverse events were evaluated for their severity, duration, and correlation with the study medication.

2.9. Analytical Method

Dienogest plasma concentrations were determined using a previously validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Protein precipitation was used to pretreat plasma samples. Chromatographic separation was done at 40˚C using a Thermo Scientific Hypersil Gold column (4.6 × 50 mm, 5.0 µm). The plasma linearity ranges from 1.000 ng/mL to 200.000 ng/mL. The intra-assay %CV and accuracy (relative error) for Dienogest were 1.30% to 6.22% and 99.5% to 110.2%, respectively, while the inter-assay %CV and accuracy were 3.34% to 4.87% and 103.6% to 107.4%.

The assay sequence was as follows: calibration standards of 1.000, 2.000, 10.000, 20.000, 40.000, 80.000, 160.000, and 200.000 ng/L, volunteers' plasma

samples, and quality-control samples of 3.000, 25.000, 100.0, and 150.00 ng/L throughout all sequences.

2.10. Pharmacokinetic and Statistical Analyses

Pharmacokinetic parameters were calculated using WinNonlin software, and statistical comparisons of pharmacokinetic parameters were carried out using SAS® statistical software (Version 9.4; SAS Institute Inc., USA). Pharmacokinetic primary parameters like C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ and secondary parameters like T_{max}, $t_{1/2}$, K_{el}, and $AUC_{Extrapolation}$ were determined for all the subjects who had completed both study periods. The two preparations will be bioequivalent if 90% Confidence Intervals (CI) for test/reference ratios of C_{max}, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ fall between the range of 80% and 125% [26] [27].

3. Results

3.1. General Characteristics of the Subjects

A total of 13 participants were enrolled from 27 screened volunteers, and all finished the clinical phase of the study. Table 2 displays the demographic information of all enrolled subjects.

3.2. Method Validation

All Dienogest calibration curve standards are within the acceptance limit (1 - 200 ng/mL). The correlation coefficient was higher than 0.999. There were no visible interferences, and the chromatograms produced were entirely distinct from each other. The method validation followed international guidelines of the Food and Drug Administration (FDA) [28] and the European Medicines Agency (EMA) [29].

3.3. Tolerability and Safety Assessment

All Adverse Events (AEs) were closely observed and monitored throughout the study. During the clinical stage, mild forms of AEs were observed (subjects 4, 8, and 9 experienced vomiting, and subject 9 had diarrhoea) and resolved spontaneously under medical supervision.

3.4. Pharmacokinetic Parameters

Figure 2 and Figure 3 show the mean plasma concentration-time curves of the

Table 2. Demographic Characteristics of the Subjects (n-13).

Characteristic	Values
Age, mean (SD), range, years	26 (5.44), 18 - 35
Weight, mean (SD), range, kg	57.04 (12.54), 40.70 - 77.80
Height, mean (SD), range, cm	152.80 (8.29), 143 - 168
BMI, mean (SD), range, kg/m^2	24.30 (4.27), 18.80 - 29.99

BMI = Body mass index; SD = Standard deviation.

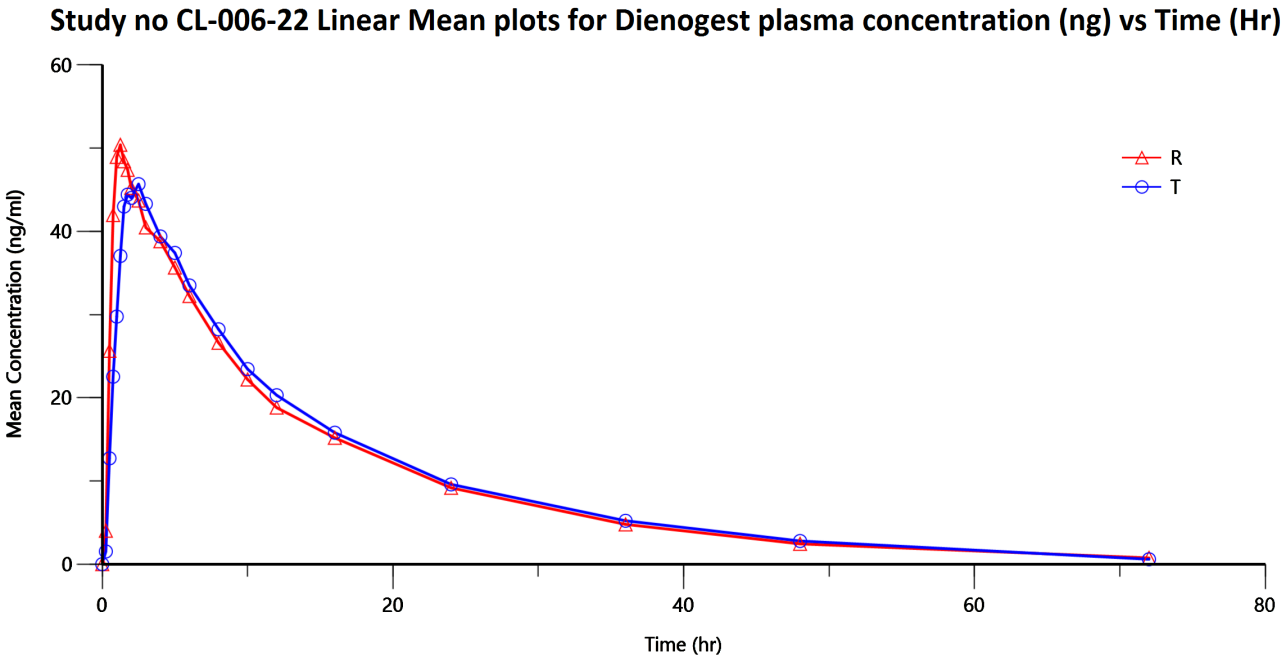

Figure 2. Linear Plot of Mean Plasma Concentration (ng) versus Time (hr).

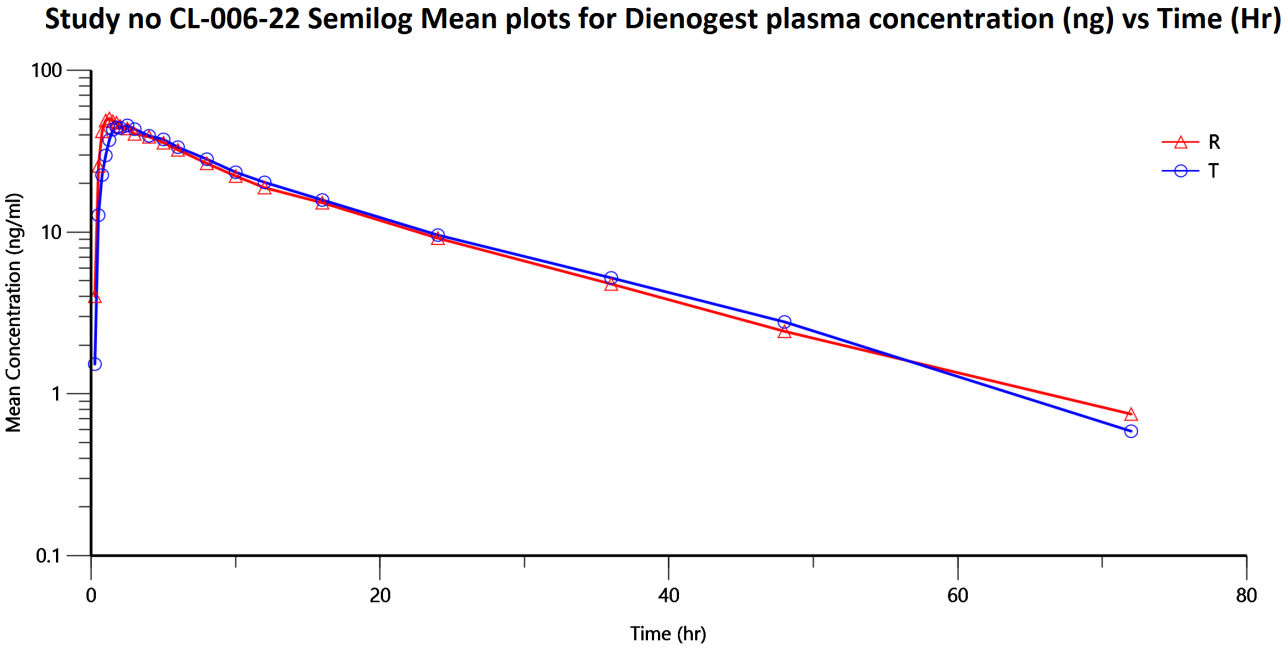

Figure 3. Semilog Plot of Mean Plasma Concentration (ng) versus Time (hr).

two formulations. The superimposable figures suggest that the two formulations have equivalent mean plasma concentration-time curves.

Table 3 reports the pharmacokinetic results, and Table 4 shows the geometric means, geometric mean ratios, and 90% CIs for the pharmacokinetic parameters of the Dienogest 2 mg tablet.

The impact of formulations, sequences, and periods on log-transformed pharmacokinetic variables was evaluated using the analysis of variance (ANOVA) [30]

Table 3. Pharmacokinetic parameters (N = 13).

Visanne (Dienogest 2 mg) tablets (Reference Product)						
Variable	**Arithmetic Mean**	**SD**	**CV%**	**Min**	**Median**	**Max**
Primary Variable						
C_{max} (ng/mL)	55.0587	12.02436	21.8	39.105	51.127	78.118
AUC_{0-t} (hr*ng/mL)	694.8138	194.88638	28.0	408.241	696.563	1100.844
$AUC_{0-\infty}$ (hr*ng/mL)	727.4534	203.11283	27.9	434.141	729.589	1162.059
T_{max} (hr)	1.6346	0.93883	57.4	0.750	1.250	4.000
AUC_% Extrap_obs (%)	4.5198	1.37358	30.4	1.945	4.527	6.452
$T_{½}$ (hr)	12.8260	3.04074	23.7	8.436	12.320	17.699
Kel (hr^{-1})	0.0570	0.01379	24.2	0.039	0.056	0.082
Dienogest 2 mg Tablets (Test Product)						
Secondary Variable						
C_{max} (ng/mL)	52.9390	13.48170	25.5	35.817	51.465	89.783
AUC_{0-t} (hr*ng/mL)	707.0434	186.81099	26.4	440.166	736.734	1007.867
$AUC_{0-\infty}$ (hr*ng/mL)	738.9579	194.24527	26.3	459.597	768.867	1041.096
T_{max} (hr)	2.0192	0.59039	29.2	1.000	2.000	3.000
AUC_% Extrap_obs (%)	4.3629	1.08353	24.8	2.899	4.179	5.932
T½ (hr)	12.6727	2.63849	20.8	9.594	11.491	17.078
Kel (hr^{-1})	0.0568	0.01095	19.3	0.041	0.060	0.072

C_{max}: maximum plasma concentration of the drug, AUC_{0-t}: area under the plasma concentration-time curve from time zero to the time of the last measurable concentration, $AUC_{0-\infty}$: area under the plasma concentration-time curve from time zero to infinity, T_{max}: time to reach maximum plasma Concentration, $T_{1/2}$: half-life of the drug, K_{el}: elimination rate constant.

Table 4. Summary results.

Parameter	Geometric Least Squares Means (GEOLSM)		T/R Ratio (%)	90% Confidence Interval		Intra Subject CV (%)	Power (%)
	Test Product	Reference Product		Lower Limit (%)	Upper Limit (%)		
C_{max} (ng/mL)	51.368	53.773	95.53	86.70	105.26	13.79	96.01
AUC_{0-t} (hr*ng/mL)	680.392	668.707	101.75	95.42	108.49	9.10	99.90
$AUC_{0-\infty}$ (hr*ng/mL)	711.497	700.698	101.54	95.59	107.87	8.57	99.95

Table 5. P-values for sources of variations obtained from the analysis of variance (ANOVA).

ANOVA p Values			
Parameters	**LC_{max}**	**$LAUC_{0-t}$**	**$LAUC_{0-\infty}$**
Sequence	0.4850	0.6515	0.6743
Period	0.4148	0.7241	0.6127
Formulation	0.7197	0.6374	0.6584

model. No significant period or sequence effects were detected. The ANOVA results are displayed in Table 5.

4. Discussion

For C_{max}, the ratios of least-squares mean (with 90% confidence intervals) were 95.53% (86.70% - 105.26%). For AUC_{0-t} and $AUC_{0-\infty}$, the ratios of least-squares mean (with 90% confidence intervals) were 101.75% (95.42% - 108.49%) and 101.54% (95.59% - 107.87%), respectively.

All of the 90% CI of the pharmacokinetic parameters (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$) were within the bioequivalence acceptable range of 80% to 125%. Moreover, the C_{max} profile of Dienogest 2 mg was almost identical for the test and reference products. The absence of sequence effects in the ANOVA also indicated the absence of a carry-over effect.

5. Limitations

There are some limitations in this current study, as with any other bioequivalence study. The results were obtained from healthy adult individuals of a defined age range who were given a single dose of the formulation in compliance with regulatory criteria. The pharmacokinetics might differ among patients in different age groups. The findings of this study may not be generalized to a specific target population. A non-compartmental model was used to calculate the pharmacokinetic parameters, which are based on certain assumptions about the pharmacokinetic behaviour of the drug, such as uniform distribution and linear kinetics. Any deviations from these presumptions may impact the precision of the model's predictions.

6. Conclusion

The test product Dinogest (Dienogest 2 mg) tablet was unequivocally bioequivalent with the reference product Visanne® (Dienogest 2 mg) tablet in healthy adult participants under fasting conditions, per regulatory requirements. Both formulations were well tolerated.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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