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A Bioequivalence Study of Pyridoxine Hydrochloride and Doxylamine Succinate 20 mg/20 mg, Modified Release Tablets, Fixed Dose Combination in Healthy Female Adults

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Abstract

Objectives: This is a randomized, single-dose, two-period, two-sequence, and crossover study to evaluate the bioequivalence (BE) profiles of two fixed-dose formulations (FDC) of Pyridoxine 20 mg/Doxylamine 20 mg (P/D) modified release oral tablets in healthy female adults under fasting conditions. **Materials and Methods:** The plasma concentrations of P/D were measured using a validated liquid chromatography mass spectrometry method. Enrolled for the study were 44 female volunteers. Only 41 completed the study. The two formulations of P/D were considered bioequivalent if a 90% Confidence Interval (CI) for ln-transformed ratios of C_{max}, AUC_{0-t} and AUC_{0-inf} fell within the acceptance range of 80.00 - 125.00%. Tolerability and safety were assessed throughout the study. **Results:** The pharmacokinetic (PK) parameters were similar between the Test formulation Pyridoxine 20 mg/Doxylamine 20 mg (T) and Reference formulation (R) Bonjesta® (Pyridoxine 20 mg /Doxylamine 20 mg), (90% CI for all PK parameters were within 80.00 - 125.00%). The 90% CI of the test/reference ratios of log-transformed PK parameter point estimates were to C_{max}: 96.84% CIs (82.80 - 113.26), AUC_{0-t}: 88.94% CIs (80.87 - 97.81) and AUC_{0-inf}: 89.53% CIs (81.72 - 98.09) for Pyridoxine and C_{max}: 89.45% CIs (84.54 - 94.64), AUC_{0-t}: 91.04% CIs (86.63 - 95.68) and AUC_{0-inf}: 90.81% CIs (86.30 - 95.56) for Doxylamine. **Conclusion:** Our results demonstrated BE between two FDC of P/D 20 mg/20 mg modified release formulations: Bonjesta® 20 mg/20 mg (Exeltis Health Care S.L) and Pyridoxine/Doxylamine 20 mg/20 mg (Laboratorios Leti, S.A.V) in healthy female adults under fasting conditions. P/D is a FDC considered the first line treatment of nau-

sea and vomiting during pregnancy (NVP).

Keywords

Bioequivalence, Pyridoxine, Doxylamine, Pregnancy

1. Introduction

A combination of Pyridoxine Hydrochloride and Doxylamine Succinate (P/D) is considered the first line for the symptomatic treatment of nausea and vomiting during pregnancy (NVP). NVP affects up to 85% of pregnant women and is one of the most common indications for hospital admission among pregnant women [1]. NVP is defined as the symptom of nausea and/or vomiting during pregnancy when the onset is prior to 16 weeks of gestation and where there is no other cause [1] [2]. A slow-release combination of P/D is licensed in some countries such as the UK, Australia, Canada and the United States for treatment of NVP as first-line treatment [1]-[4]. A delayed-release formulation containing P/D 10 mg/10 mg has been available in Canada since 1979 and since 2013 in the USA [5]. The combination P/D modified release was approved by the Food and Drug Administration (FDA) in November 2016 for the treatment of NVP when conservative management failed, and the modified formulation of P/D 20 mg/20 mg was introduced to the American market in April 2018 [6].

In a controlled clinical trial conducted in healthy, premenopausal women under fasting conditions, a fixed-dose combination tablet of P/D 20 mg/20 mg tablets was bioequivalent to two combination tablets of P/D 10 mg/10 mg, based on the exposure (AUC) and peak concentration (C_{max}) of Doxylamine and baseline corrected pyridoxal 5'-phosphate [6].

The effect of P/D 20 mg/20 mg modified release is based on two separate compounds. Pyridoxine Hydrochloride is a vitamin B6 analog. The chemical name for Pyridoxine Hydrochloride is 3,4-pyridinedimethanol, 5-hydroxy-6-methyl-, hydrochloride. The empirical formula is C 8H 11NO 3•HCl and the molecular mass is 205.64 [6] [7]. The structural formula is (**Figure 1**):

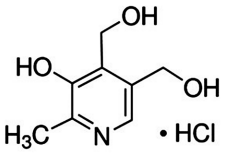

Figure 1. Pyridoxine Hydrochloride chemical structure.

Pyridoxine Hydrochloride is the usual form of vitamin B6 included in pharmaceutical products. Vitamin B6 is a collective name for pyridoxine, pyridoxal, and pyridoxamine, which are related natural compounds with similar biological properties [8]. Pyridoxine is readily absorbed from the GI tract, mainly in the jejunum.

The drug is primarily metabolized in the liver to its four active metabolites pyridoxal, pyridoxal-5-phosphate (PLP), this is the main metabolite with antiemetic activity, others metabolites as pyridoxamine, and pyridoxamine-5-phosphate also participate in biological effects [9]. Following phosphorylation, its main metabolite, PLP, is released into the circulation and is highly protein bound. PLP is a cofactor in over 160 enzyme activities involved in a number of metabolic processes of amino acids, nucleic acids, unsaturated fatty acids, carbohydrates, glycogen, neurotransmitters, and porphyrin. The major metabolite 4-pyridoxic acid is inactive, and is excreted by the kidney [6] [7].

The chemical name for Doxylamine Succinate is ethanamine, N, N-dimethyl-2-[1-phenyl-1-(2-pyridinyl)ethoxy]-,butanedioate (1:1). The empirical formula is C 17H 22N 2O•C 4H 6O 4and the molecular mass is 388.46 [6]. The structural formula is (**Figure 2**):

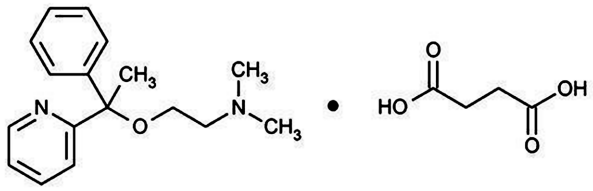

Figure 2. Doxylamine succinate chemical structure.

Doxylamine Succinate is a first-generation antihistamine derivate, a member of the ethanolamine class. It strongly antagonizes histamine's effects on histamine 1 (H1) receptor sites; as a result, it possesses sedative effects. As with other members of this group of drugs, Doxylamine possesses substantial antimuscarinic activity with a low incidence of gastrointestinal adverse effects [10]. As with any other H1 blocker, Doxylamine may exhibit anticholinergic effects if taken in large doses. Doxylamine is well absorbed from the GI tract, with peak plasma concentrations achieved within 2 - 4.5 h, and the therapeutic effects usually persist for 4 - 6 h. Doxylamine is biotransformed in the liver by N-dealkylation to its principal metabolites N-desmethyl and N, N-didesmethyl doxylamine, which are excreted by the kidney [6] [7].

The pharmacokinetics (PK) of P/D 20 mg/20 mg, modified release tablets, have been studied in healthy non-pregnant adult women in a single-dose study (one tablet) and multiple-dose study (two tablets per day on days 1 - 11). PK parameters of P/D 20 mg/20 mg reported in clinical trials and summary of the product, Bonjesta® are: C_{max} (90.4 ± 13 - 98.7 ± 18) ng/mL, $AUC_{0\text{-}t}$ (203.7 ± 51.7) h*ng/mL and $AUC_{0\text{-}inf}$ (233.6 ± 55.9) h*ng/mL [11] [12] for Pyridoxine and C_{max} (90.4 ± 13 - 98.7 ± 18) ng/mL, $AUC_{0\text{-}t}$ (1367 ± 356) h*ng/mL and $AUC_{0\text{-}inf}$ (1425 ± 405) h*ng/mL for Doxylamine, in a single oral dose and fasting condition. The median peak plasma concentration of Pyridoxine was reached within 0.5 hours and Doxylamine within 4.5 hours, post a single dose in fasted condition [6] [7].

After multiple doses, the concentration of Doxylamine was increased and C_{max} value was 1.8 times and the AUC value of absorption, increased by 2 times. Multiple-dose administration reduced the time to peak concentration from a mean of

20.0 hours (range, 2.00 - 23.0) to 3.50 hours (range, 1.00 - 20.0). The mean accumulation index was 1.99, suggesting that Doxylamine accumulates after multiple doses [6]-[10]. Although no accumulation of Pyridoxine was observed, the mean accumulation index of the main active metabolite, pyridoxal-5'-phosphate, after multiple doses was 2.61. Multiple dose administration slightly reduced the time to peak concentration: from a mean of 21.0 hours (range, 15.0 - 23.9) to 15.0 hours (range, 2.00 - 24.0). Administration of a high-fat and high-calorie meal slowed down the absorption of Doxylamine, Pyridoxine and pyridoxine metabolites. Food significantly reduced the bioavailability of Pyridoxine. The Cmax value decreased by approximately 67% and the AUC value by approximately 37% compared to the fasting state. Besides, the C_{max} and AUC values and the main active metabolite, pyridoxal-5'-phosphate, were not affected by food. The principal metabolites of Doxylamine, N-desmethyl-doxylamine and N, N-didesmethyldoxylamine, are excreted by the kidney. The terminal elimination half-life of Doxylamine and Pyridoxine are 11.9 hours and 0.4 hours, respectively [6]-[15].

In the efficacy studies the combination of 10 mg/10 mg of P/D, was evaluated with Pyridoxine and Doxylamine separately, and comparable (90% CI 80 - 120) after administration of the same daily dose. Therefore these results of efficacy of 10mg/10mg tablets support the use of 20 mg/20 mg of P/D [6]-[15]. The efficacy and safety of the combination (P/D) were evaluated in clinical trials randomized, placebo controlled, involving adult women aged 18 years or older with the average gestational age at the time of study [1] [6] [7]. The maximum daily dose was 40mg/40mg of P/D, divided doses per day [1] [6] [13]. The primary efficacy endpoint was the change from baseline in the Pregnancy-Unique Quantification Emesis (PUQE) questionnaire score on day 15. The mean baseline PUQE score was 9.0 in the P/D group and 8.8 in the placebo group. On day 15, the PUQE score of the P/D group was reduced (nausea and vomiting symptoms were relieved) by an average of 0.9 points from baseline compared to placebo (95% CI 0.2 - 1.2; p-value 0.006) [1] [6] [13].

A Cochrane review, systematic reviews and meta-analyses have reported on the safety and efficacy of many antiemetics for use in NVP, with no increased risk of teratogenesis or other adverse pregnancy outcomes [1] [13]. These drugs include: antihistamines (H1 receptor antagonists) such as P/D. Also that review reported safety and efficacy data for first line antiemetics such as P/D and concluded that this treatment should be prescribed initially when required for NVP and HG (Evidence Grade A-UK guidelines) [1].

The purpose of our study was to assess and compare the PK profiles and safety of Bonjesta® (Exeltis, Exeltis Health Care S.L, España) as reference (R) formulation to Pyridoxine/Doxylamine (Laboratorios Leti, S.A.V., República Bolivariana de Venezuela) as test (T) formulation of Pyridoxine Hydrochloride/Doxylamine Succinate 20 mg/20 mg, in healthy female adults, non lactating, non pregnant, under fasting condition, randomized crossover study.

This study was conducted by a Clinical Research Operator (CRO), at Synergen Bio Private Limited, India. Pharmadesk Solutions Pvt. Ltd., was in charge of monitoring the study and was present during the conduct of the study.

2. Materials and Methods

2.1. Ethical Approval

The study was conducted ethically in accordance with the principles of the ICMR guidelines (2017) [16], New Drugs and Clinical Trials Rules 2019 India [17] and adhere to the ethical principles of the Declaration of Helsinki [18] the International Conference on Harmonization Good Clinical Practice Guidelines [19]. The study protocol (N˚123-23) was approved by Kusum Independent Ethics Committee, on March 22, 2024 (Version: 01, Dated March 01, 2024) and certified by CDSCO/DGHS to Synergen Bio Private Limited.

2.2. Study Design

This was a randomized, single oral dose, two-treatment, two-period, two-sequence, and crossover bioequivalence (BE) study under fasting conditions comparing two P/D 20 mg/20 mg formulations. Pyridoxine/Doxylamine 20 mg/20 mg modified release tablets, were provided as the Test formulation (T) by Laboratorios Leti S.A.V., República Bolivariana de Venezuela, batch BP-2101-009F, date of expiry 03/2025, and the Reference formulation (R) Bonjesta® 20 mg/20 mg modified release tablets, by Exeltis, Exeltis Healthcare, España, batch N˚30041-4, date of expiry 01/2026.

2.3. Subjects

All volunteers underwent a screening procedure. Forty-four (44) female healthy subjects, non-smoking, non-lactating, non-pregnant, aged between 18 and 45 years, who met the inclusion and exclusion criteria were enrolled. These female subjects were selected on the basis of laboratory evaluations during screening, demography, height, weight and BMI), clinical examination along with vital signs, questioning on medical history, recording of electrocardiogram (12-lead ECG) and laboratory investigations of blood as well as urine within 21 days prior to dosing. However, a satisfactory chest X-Ray (P/A view) taken less than 180 days prior to the first dosing of period 01 would be accepted. A urine screen for drugs of abuse and an alcohol breath test was undertaken at the time of check-in in each period. Urine pregnancy test was done at the time of screening and hCG pregnancy test was done during check-in of each period. Study specific test urine porphobilinogen was done at the time of screening.

2.4. Drug Administration

The subjects were randomized, to one of the two sequences (T R) or (R T). The randomization schedule was generated using Statistical Analysis Software (SAS® version 9.4). One single dose was administered in each period. Subjects who received T formulation in period I were administered R formulation in period II and *vice versa*. The pre-screening period was 21 days. The total duration of the clinical phase of the study was 26 days (08 Aug 2024 - 10 Sept 2024) with a washout period of 21 days, considering the terminal half-life for P/D.

The subjects were admitted to the facility overnight before study. In each pe-

riod, after an overnight fasting of 10 hours, each subject received a single oral dose (P/D 20 mg/20 mg) of either one T or R, as per randomization schedule with 240 mL ± 2 mL of water at ambient temperature in sitting position for 02 h after dosing. A total of 20 × 5 ml of venous blood samples were collected through cannula from each subject during each period, withdrawn at pre-dose (00-00 h) and 00.17, 00.25, 00.50, 00.75, 01.00, 01.50, 01.50, 02.00, 02.50, 03.00, 03.50, 04.00, 04.25, 04.50, 04.75, 05.00, 05.50, 06.00, 07.00, 08.00, 10.00, 12.00 16.00 and 24.00 h. While 48.00 and 72.00 hours, post dose, blood samples were collected by direct venepuncture on ambulatory basis. The subjects received standardized meals (2500 Kcal) on the day of check-in and approximately 4.00, 8.00 and 12.00 hours post dose in each period and drinking water was provided *ad libitum.*

2.5. Analytical Procedure

Venous blood samples were collected in pre-labelled K_2EDTA (ethylenediaminetetraacetic acid) vacutainers and were centrifuged at 3800 ± 20 rpm for 10 min at 10 ± 2˚C within 45 minutes of sample collection. Plasma was separated, labelled and stored at −70˚C ± 15˚C before analysis. Plasma concentrations of Doxylamine and Pyridoxine were determinated by using a validated LC-MS/MS method in the Bioanalytical Research Department of Synergen Bio Pvt. Ltd, Pune, India. Analytical instruments were employed including a Shimadzu Quaternary Pump, an autosampler with sample (LC-40D XR) and shimadzu column oven for temperature control, with detection via the MS/MS Triple Quad 4500, the analysis was performed using a Termo Hypurity TM C18 100 mm * 4.6 mm, 5 ɯm. Pyridoxine in human plasma were developed and validated over a calibration range 0.501 ng/mL to 100.625 ng/mL for using pyridoxine and pyridoxine D3 as an internal standard.

Doxylamine in human plasma were developed and validated over a calibration range 1.028 ng/mL to 202.050 ng/mL for using Doxylamine D5 as an internal standard. Project samples analysis for Doxylamine was carried out using a calibration range of 201.743 ng/mL to 1034 ng/mL. The incurred sample reanalysis (ISR) was performed on 200 samples for Pyridoxine and 200 samples to Doxylamine. Out of 200.197 and 200.185 samples were found within acceptance criteria for Pyridoxine and Doxylamine respectively. The mass spectrometer was operated in positive electrospray mode. Identifications were based on multiple reactions monitoring transitions; m/z 134.10 - 170.20 for Pyridoxine drug and m/z 137.10 - 173.10 for the IS-Pyridoxine D3, and m/z 167.10 - 271.30 for Doxylamine drug and m/z 172.10 - 276.20 for the IS-Doxylamine D5. The inter-batch calibration standard was 0.36% to 1.75% with accuracy 94.04% to 103.61% for Pyridoxine and 0.12% to 1.09% with accuracy 97.55% to 100.95% for Doxylamine.

2.6. Statistical Analysis

The sample size calculation for the study was based on intra-subject coefficient of variation (CV%) for P/D 20 mg/20 mg, modified release, obtained from published literature (C_{max}: 19% to 21% to Pyridoxine, and 15% to 20% to Doxylamine) [6] [7] [10] [11] with the expected 31% CV and the ratio within 80 and 125% [6] [7]

[10] [11]. Based on a sample size, 44 subjects were sufficient to demonstrate BE between the two P/D formulations (with a power of ≥80% at 5% level of significance). Statistical analysis was conducted on all of the subjects who complete both periods of the study as per protocol, using PROC GLM procedure of SAS® (Software version 9.4, Institute. Inc., CARY, USA).

The primary PK parameters were evaluated and adhered to bioequivalence guidelines, according to EMA guidance CPMP/EWP/QWP/1401/98 Rev.1/corr** [20], based upon measured concentrations of parent compound of P/D [21]: maximum peak concentration (C_{max}), area under curve from time 0 to last measurable concentration ($AUC_{0\text{-}t}$) and area under curve from time 0 to infinity ($AUC_{0\text{-}inf}$). Other PK parameters evaluated were: Time to reach C_{max} (T_{max}), time required for plasma concentration to decrease by 50% ($T_{1/2}$), $AUC\_{}_{\%Extrap}$, constant of elimination (K_{el}) and the number of points (N_{points}) of the terminal log-linear phase used to estimate the terminal rate constant. The natural log transformed (*i.e.*, Ln-transformed) values for PK parameters C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$, was analyzed for statistical difference between T and R formulations with ANOVA by a Generalized Linear Model (GLM) ANOVA using SAS®. Based on these parameters, the 90% confidence intervals (CIs) were constructed for the least square mean differences of log-transformed PK parameters C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ for P/D. The formulations were regarded as bioequivalent when the 90% CIs of the T and R ratio of C_{max} and $AUC_{0\text{-}t}$, ranged from 80% to 125% [20] [21].

2.7. Safety Assessments

The safety of two formulations was evaluated through the assessment of adverse events monitoring throughout the study. Vital signs were measured during baseline screening, and at the conclusion of the study.

3. Results

3.1. Characteristics of the Subjects

A total of 44 healthy female adults were included. They had a mean age of 33.4 years, mean weight of 62.7 kg, mean height of 152.8 cm, and body mass index of 26.8 kg/m^2 (Table 1).

Table 1. Demographic profile of subjects completing the bioequivalence study.

Age	Mean ± SD		33.4 ± 5.28
(Years)	Range		19 - 44
Age group		**F**	**%**
	19 - 40	40	90.90%
	41 - 64	4	8.10%
TOTAL	19 - 64	44	100%
BMI (kg/m²)	Mean ± SD		26.85 ± 2.31
	Range		(21.57 - 29.73)

Continued

Race	Asian	44	100%

Results are displayed as n (%) or mean ± standard deviation (SD).

Efficacy analysis

The pharmacokinetic parameters listed below were derivated indiviadually for each analysed subjects, forty-four (44) subjects data was used for PK analysis and forty one (41) subjects data was used for statistics evaluation. A non-compartmental analysis was applied for the estimation of PK parameters C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, T_{max}, Kel (h − 1), and $T_{1/2}$, of P/D in plasma concentration which are presented in (Table 2 and Table 3 for Pyridoxine, and Table 4 and Table 5 for Doxylamine), analysis of variance analysis from Ln C_{max}, and Ln $AUC_{0\text{-}t}$, The oral dosing of P/D 20mg/20mg for 72 h post-dose is represented on arithmetic and logarithm scales, as shown in Figure 3 and Figure 4 for Pyridoxine, Figure 5 and Figure 6 for Doxylamine.

Pyridoxine results

Table 2. Descriptive statistics of pharmacokinetic parameters of test product (T) and reference product (R) for pyridoxine (N = 41).

Form	Variable	Mean	SD	Minimum	Median	Maximum	CV%
R	C_{max} (ng/mL)	62.6940	24.4640	27.7020	61.8710	135.4490	39.0213
	$AUC_{0\text{-}t}$ (ng * hr/mL)	70.5483	22.1550	35.2540	67.7190	114.0970	31.4040
	$AUC_{0\text{-}\infty}$ (ng * hr/mL)	71.0220	22.1234	35.7680	68.3590	114.4570	31.1500
	T_{max} (hr)	2.0024	1.7575	0.5000	1.0000	5.0000	87.7691
	K_{el} (hr^{-1})	3.2060	1.2676	0.4000	3.1180	7.4590	39.5399
	$t_{1/2}$ (hr)	0.2798	0.2537	0.0900	0.2200	1.7300	90.6782
	Residual_area	0.7544	0.7356	0.1500	0.5000	3.9900	97.5108
	$AUC_{\_\%Ratio}$	99.2456	0.7356	96.0100	99.5000	99.8500	0.7412
T	C_{max} (ng/mL)	62.7046	28.0120	14.1220	57.9370	128.2460	44.6731
	$AUC_{0\text{-}t}$ (ng * hr/mL)	63.8400	23.6629	26.9420	52.4220	123.7050	37.0660
	$AUC_{0\text{-}\infty}$ (ng * hr/mL)	64.4915	23.3942	28.5970	52.9660	124.0120	36.2748
	T_{max} (hr)	1.3727	1.4490	0.2500	0.7500	5.0000	105.5564
	K_{el} (hr^{-1})	2.7596	1.2035	0.3190	2.7140	4.9440	43.6110
	$t_{1/2}$ (hr)	0.4063	0.4770	0.1400	0.2600	2.1800	117.3824
	Residual_area	1.3749	2.3329	0.1600	0.5700	13.8300	169.6812
	$AUC_{\_\%Ratio}$	98.6251	2.3329	86.1700	99.4300	99.8400	2.3654

Data presented as mean ± SE. C_{max}: maximum concentration, $AUC_{0\text{-}t}$: area under the plasma concentration—time curve from time 0 to the last measurable concentration; $AUC_{0\text{-}\infty}$: area under the plasma concentration—time curve from time 0 to infinity, T_{max}: time to reach C_{max}, Kel: elimination rate constant, $T_{1/2}$ time required for the drug concentration to fall by 50%. Residual area: expressed as% of total value of AUC AUC% ratio: relation between AUC and drug concentration.

Table 3. Geometric least squares means, ratios, 90% confidence intervals, power and ISCV for pharmacokinetic parameters (C_{max}, AUC_{0-t} and AUC_{0-inf}) of pyridoxine (N = 41).

Parameter	Geometric LSM		Geometric Mean Ratio (90% Confidence Interval) (%)	Intra-subject CV%	Power	BE result
	Treatment T	Treatment R	T vs R			
C_{max} (ng/mL)	56.451	58.291	96.84 (82.80% - 113.26%)	44.01	64.61	YES
AUC_{0-t} (ng * hr/mL)	59.744	67.173	88.94 (80.87% - 97.81%)	25.97	96.96	YES
AUC_{0-inf} (ng * hr/mL)	60.599	67.684	89.53 (81.72% - 98.09%)	24.90	97.87	YES

Data presented as a% mean ln transformed. C_{max}: maximum concentration, AUC_{0-t}: area under the plasma concentration—time curve from time 0 to the last measurable concentration; $AUC_{0-\infty}$: area under the plasma concentration—time curve from time 0 to infinity. GMR: Geometric mean ratios n = 24, PK: Pharmacokinetics, CI: Confidence interval, ln: natural logarithm.

The Test/Reference geometric mean ratios (GMR) and 90% CIs for the logarithm of C_{max}, AUC_{0-t} and AUC_{0-inf}, are presented in (Table 3) for Pyridoxine. The BE results were as follows: Ln C_{max} 96.84% (82.80 - 113.26%), AUC_{0-t}: 88.94% (80.87 - 97.81%) and AUC_{0-inf}: 89.53 (81.72% - 98.09%), these values are within the 90% CIs and acceptance criteria of 80 - 125%. There were no significant differences between the PK parameters of Pyridoxine in the two formulations, Test and Reference ($P > 0.05$). The oral dosing of Pyridoxine 20 mg for 72 h post-dose is represented on arithmetic and logarithm scales, as shown in (Figure 3 and Figure 4).

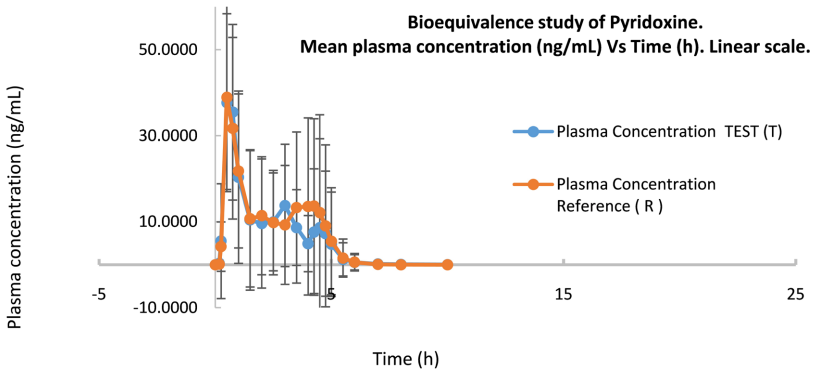

Figure 3. Linear plot of mean plasma concentrations of pyridoxine vs. time for test formulation (T) and reference formulation (R) (N = 41). Mean Pyridoxine 20 mg plasma concentration versus time (h) profile for each formulation is presented in an arithmetic scale, following a single oral dose. Red line indicates the Reference formulation (Exeltis Heath Care S.L) and blue line indicates Test formulation (Laboratorios Leti S.A.V).

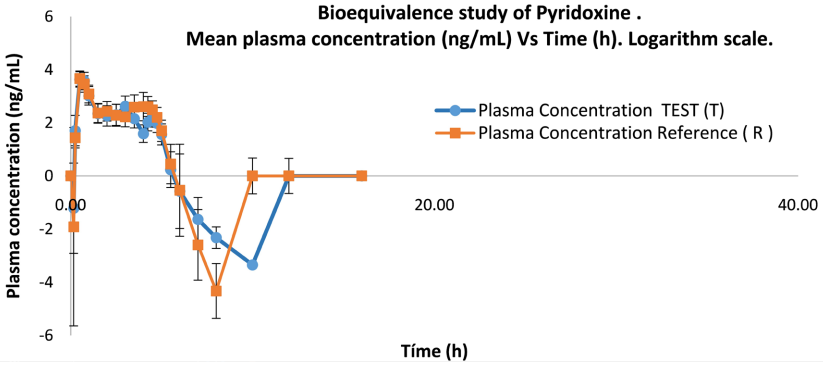

Figure 4. Ln-linear plot of mean plasma concentrations of pyridoxine vs. time for test formulation (T) and reference formulation (R) (N = 41). Mean Pyridoxine 20 mg plasma concentration versus time (h) profile for each formulation is presented in a logarithm scale, following a single oral dose. Red line indicates Reference formulation (Exeltis Heath Care S.L) and blue line indicates Test formulation (Laboratorios Leti S.A.V).

Doxylamine results

The mean plasma pharmacokinetics parameters corresponding to Doxylamine, Test and Reference formulations are presented in Table 4, and the GMR is summarized in Table 5.

Table 4. Descriptive statistics of pharmacokinetic parameters of test product (T) and reference product (R) for doxylamine (N = 41).

Form	Variable	Mean	SD	Minimum	Median	Maximum	CV%
R	C_{max} (ng/mL)	106.5452	19.0541	78.2000	102.2410	159.5210	17.8836
	AUC_{0-t} (ng * hr/mL)	2013.4618	401.2959	1112.4360	2000.3270	3116.2550	19.9306
	AUC_{0-inf} (ng * hr/mL)	2123.5189	436.0986	1136.7680	2126.6120	3236.7420	20.5366
	$T_{max\ (hr)}$	4.5315	0.9050	2.5000	4.5000	7.0000	19.9720
	K_{el} (hr^{-1})	0.0440	0.0061	0.0320	0.0440	0.0570	13.8058
	$t_{1/2}$ (hr)	16.0256	2.2316	12.1900	15.7800	21.4200	13.9253
	Residual_area	5.0141	1.9673	2.1400	4.7500	11.7800	39.2353
	$AUC_{\_\%Ratio}$	94.9859	1.9673	88.2200	95.2500	97.8600	2.0712
T	C_{max} (ng/mL)	96.3638	21.9736	55.9670	96.2020	149.7450	22.8027
	AUC_{0-t} (ng * hr/mL)	1844.0081	422.8515	905.1480	1845.5760	3148.2600	22.9311
	$AUC_{0-\infty}$ (ng * hr/mL)	1938.7458	453.3832	929.9220	1902.9820	3324.7920	23.3854
	T_{max} (hr)	4.8329	0.8914	3.5000	4.5000	8.0000	18.4438
	K_{el} (hr^{-1})	0.0443	0.0056	0.0350	0.0450	0.0550	12.6638
	$t_{1/2}$ (hr)	15.9129	2.0643	12.5300	15.5800	20.0300	12.9723
	Residual_area	4.7759	1.6876	2.3700	4.3000	8.3800	35.3368
T	$AUC_{\_\%Ratio}$	95.2241	1.6876	91.6200	95.7000	97.6300	1.7723

Data presented as mean ± SE. C_{max}: maximum concentration, AUC_{0-t}: area under the plasma concentration—time curve from time 0 to the last measurable concentration; AUC_{0-inf}: area under the plasma concentration—time curve from time 0 to infinity, T_{max}: time to reach C_{max}, Kel: elimination rate constant, $T_{1/2}$ time required for plasma. Residual area: expressed as% of total value of AUC AUC% ratio: relation between AUC and drug concentration

Table 5. Geometric least squares means, ratios, ISCV, powers and 90% confidence intervals for pharmacokinetic parameters (C_{max}, AUC_{0-t} and AUC_{0-inf}) of doxylamine (N = 41).

Parameter	Geometric LSM		Geometric Mean Ratio (90% Confidence Interval) (%)	Intra-subject CV%	Power	BE Result
	Treatment T	Treatment R	T vs R			
C_{max} (ng/mL)	93.978	105.064	89.45 (84.54% - 94.64%)	15.26	100.00	YES
AUC_{0-t} (ng * hr/mL)	1798.547	1975.519	91.04 (86.63% - 95.68%)	13.41	100.00	YES
AUC_{0-inf} (ng * hr/mL)	1889.303	2080.455	90.81 (86.30% - 95.56%)	13.75	100.00	YES

Data presented as a% mean ln transformed. C_{max}: maximum concentration, AUC_{0-t}: área under the plasma concentration—time curve from time 0 to the last measurable concentration; $AUC_{0-\infty}$: area under the plasma concentration—time curve from time 0 to infinity. GMR: Geometric mean ratios n = 24, PK: Pharmacokinetics, CI: Confidence interval, ln: natural logarithm.

The BE results were as follows: Ln C_{max}: 89.45 (84.54% - 94.64%) AUC_{0-t}: 91.04 (86.63% - 95.68%) and AUC_{0-inf}: 90.81 (86.30% - 95.56%) these values are within the 90% CIs and acceptance criteria of 80 - 125%. There were no significant differences between the PK parameters of Doxylamine in the two formulations, Test and Reference ($P > 0.05$). The oral dosing of Doxylamine 20 mg for 72 h post-dose is represented on arithmetic and logarithm scales, as shown in (Figure 5 and Figure 6).

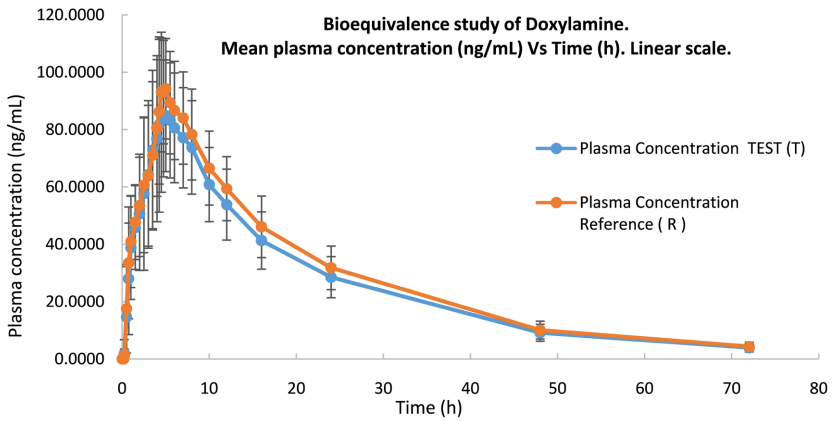

Figure 5. Linear plot of mean plasma concentrations of doxylamine vs. time for test formulation (T) and reference formulation (R) (N = 41). Mean Doxylamine 20 mg concentration versus time (h) profile for each formulation is presented in an arithmetic scale, following a single oral dose. Red line indicates Reference formulation (Exeltis Heath Care S.L), and blue line indicates Test formulation (Laboratorios Leti S.A.V).

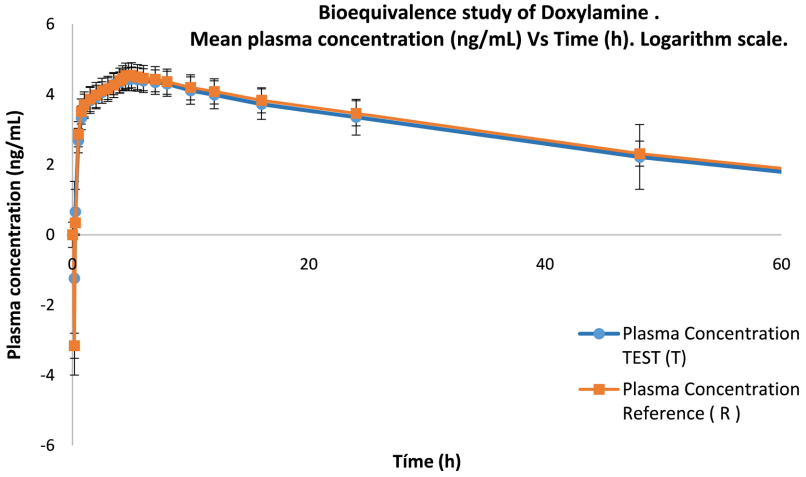

Figure 6. Ln-linear plot of mean plasma concentrations of doxylamine vs. time for test formulation (T) and reference formulation (R) (N = 41). Mean Doxylamine 20 mg concentration versus time (h) profile for each formulation is presented in a logarithm scale, following a single oral dose. Red line indicates Reference formulation (Exeltis Heath Care S.L), and blue line indicates Test formulation (Laboratorios Leti S.A.V).

3.2. Efficacy Conclusions

The 90% confidence intervals of the ratio of geometric least squares means for the Ln-transformed pharmacokinetic parameters C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ for Piridoxyne/Doxylamine are within the acceptance limits of 80.00% - 125.00%.

3.3. Safety and Tolerability

All female adults subjects (44) were dosed in period 01 and 41 subjects were dosed in period 02. Out of 44 subjects, 41 subjects completed the study, 03 subjects did not report to the clinical facility for period 02 check in and were considered as "drop-out" from the study. All subjects who had received at least one dose of investigational product were included in safety evaluation.

A total of five (05) adverse events (Table 6) were observed in the study, involving 05 subjects (*i.e.*, subjects 02, 06, 18, 23 and 29) out of 44 subjects; during post-study clinical laboratory safety evaluation (clinically significant changes in laboratory parameters).

Table 6. Adverse event reported.

Adverse event	Test product (T)	Reference product (R)	Post study (percentage %)
Decreased haemoglobin	-	-	03 (3.53%)
Increased platelet count	-	-	01 (1.18%)
Increased RBC count	-	-	01 (1.18%)
Total	-	-	**05 (5.89%)**

The T and R formulations were comparable in their safety and tolerability. All adverse events were graded as "mild" in intensity. 01 adverse event was "possibly" and 04 adverse events were "unlikely" related to the investigational products. No moderate, severe, serious or life-threatening adverse events were reported during the course of the study. The T and R formulations were comparable in their safety and tolerability.

4. Discussion

NVP affects up to 85% of pregnant women and is one of the most common indications for hospital admission among pregnant women, with typical stays of between three and four days [22]. This condition can vary in a range of severity from mild nausea to the most severe form in these patients, impacting their quality of life and increasing the cost associated with the use of health resources and health services [22].

Pyridoxine/Doxylamine (P/D) modified release, is a combination considered as first line treatment of NVP, was approved by the US Food and Drug Administration (FDA) and has been available in Canada and other countries [1]-[5]. The formulation of P/D 20mg/20mg oral tablets, has been only licensed for treatment of NVP in the UK [1].

This study assessed the bioequivalence of P/D 20 mg/20 mg modified release tablets, in a healthy adult, non lactating, non pregnant female subjects, under fasting condition. It was a randomized crossover study, two treatments, two periods and one single dose. The 90% CIs for P/D was assessed by determined bioequivalence standards of 80% - 125% for C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ [20] [21]. The study included 44 healthy female subjects, aged between 18 and 45 years. Only 41 completed the study and were included in PK evaluation and statistical analysis, it was a sufficient number of subjects to ensure statistical power to demonstrate the bioequivalence between both formulations.

The PK mean values of Pyridoxine for C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ were 62.6940 ng/mL, 70.5483 h * ng/mL and 71.0220 h * ng/mL to R and 62.7046 ng/mL, 63.8400 h * ng/mL and 64.4915 h * ng/mL to T formulations respectively. The PK mean values of Doxylamine for C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ was 106.5452 ng/mL, 2013.4618 h * ng/mL and 2123.5189 h * ng/mL to R formulation and 96.3638 ng/mL, 1884.0081 h * ng/mL and 1938.7458 h * ng/mL to T formulation, which are included into the range of BE acceptance (limit of 80% - 125%) (**Table 2** and **Table 4**). Other parameters evaluated were $T_{1/2}$ (0.27 ± 0.25 h and 0.49 ± 0.47 h) for Pyridoxine T and R formulations y and $T_{1/2}$ (16.02 ± 2.23 h and 15.91 ± 2.06 for Doxylamine R and T formulation, respectively (**Table 2** and **Table 4**). These PK parameters of P/D reported in this study are comparable to those PK data found in other clinical trials in a single oral dose and fasting condition [6]-[11] [13]-[15].

The 90% CIs for GMRs ranged from Ln C_{max} was 96.84% with CIs (82.80 - 113.26), $AUC_{0\text{-}t}$ was 88.94% with CIs (80.87 - 97.81) and $AUC_{0\text{-}inf}$ was 89.53% with

CIs (81.72 - 98.09) was determined for Pyridoxine and Ln C_{max} was 89.45% with CIs (84.54 - 94.64), $AUC_{0\text{-}t}$ was 91.04% with CIs (86.63 - 95.68) and $AUC_{0\text{-}inf}$ was 90.81% with CIs (86.30 - 95.56) for Doxylamine. These values are within the 90% CIs and acceptance criteria of 80 - 125% and this BE is based on (90% CI) of Pyridoxine and Doxylamine [20].

Following the EMA BE guideline [20] [21] the evaluation of BE was based on measured concentrations of the parent compound [21]. The reason for this is that C_{max} of a parent compound is usually more sensitive to detect differences between formulations in absortion rate than C_{max} of a metabolite [6] [7]. The active metabolites of pyridoxine, pyridoxal 5'-phosphate and pyridoxal no were measured. An adequate washout period was used to avoid the carryover effect [7].

The mean P/D plasma concentrations-time curves of T product vs R product, in arithmetic and logarithm scale were similar (**Figures 3-6**). BE was demonstrated between the T and R formulations, in oral tablets, modified release at a dose of 20 mg/20 mg, in fasting condition. The mean plasma concentration profiles of Pyridoxine demonstrated high variability in both plasma concentration profiles per hour to T and R formulations (**Figure 3** and **Figure 4**).

NVP is a common condition that affects the health of a pregnant woman and her fetus [1]. It can diminish a woman's quality of life and also significantly contributes to health care costs and time lost from work [22]. P/D as pharmacological treatment in the early stages may prevent more serious complications, including hospitalization, and result in a safe and effective available treatment. Evidence suggests that delayed release formulations of P/D are more effective than other antiemetics in the treatment of NVP [1].

Royal College of Obstetricians and Gynaecologists of the UK, recommended in the NVP management guidelines 2025, P/D 20 mg/20 mg as a first-line treatment [1].

Regulatory agencies in Latin America are demanding more attention in the process of conducting bioequivalence studies to make decisions ensuring greater access and adherence to these treatments [23]-[25].

5. Limitations

We did not evaluate the fed condition for supportive evidence of bioequivalence.

6. Conclusion

This study of a single oral dose of Pyridoxine/Doxylamine 20 mg/20 mg modified release tablets, demonstrated bioequivalence of the T formulation to R formulation, in healthy female subjects under fasting conditions. The PK profiles for both P/D products were similar and demonstrated by the 90% CIs of C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ within the accepted BE criteria of 80% - 125%.

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Author's Contributions

EP, AI, AT, XS, and JGC performed the statistical analysis, interpretation, writing, revision, of the manuscript.

Declaration of Patient Consent

The authors certify that they have obtained all appropriate patient consent.

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Use of Artificial Intelligence (AI)-Assisted Technology for Manuscript Preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

Conflicts of Interest

All authors are employees of Laboratorios Leti S.A.V. and may hold share and/or stock options in the company. The authors have no other potential conflicts of interest relevant to this study.

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