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Pharmacokinetic Evaluation of Ursodeoxycholic Acid, Unconjugated and Conjugated, within Two Oral Formulations in Healthy Male Subjects

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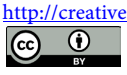

Abstract

Background: This study aimed to evaluate the bioequivalence (BE) of two oral formulations of ursodeoxycholic acid (UDCA), administered as 300 mg tablets (two tablets; 600 mg total dose), and to assess the pharmacokinetics (PK) and bioequivalence (BE) of UDCA in 24 healthy male subjects. **Methods:** A Phase I, randomized, open-label, two-period, two-sequence, two-treatment, single-dose crossover study was conducted under fed conditions. Subjects received a 600-mg oral dose of either the test or reference formulation in each period. Plasma PK parameters (C_{max} and $AUC_{0\text{-}72}$) for unconjugated and total UDCA were quantified using a validated LC-MS/MS method. **Results:** The geometric mean ratios (test/reference) and corresponding 90% confidence intervals (CIs) for unconjugated UDCA were: C_{max}: 103.42% (90.34% - 118.40%), $AUC_{0\text{-}t}$: 105.09% (94.95% - 116.30%). For total UDCA (conjugated + unconjugated), the results were: C_{max}: 100.27% (89.50% - 112.33%), $AUC_{0\text{-}72}$: 96.96% (80.49% - 116.80%). The PK profiles of the test and reference formulations were similar, as demonstrated by the 90% CIs of C_{max} and $AUC_{0\text{-}72}$ parameters falling within the accepted BE range of 80% - 125%. **Conclusions:** Baseline-corrected C_{max} and $AUC_{0\text{-}72}$ for unconjugated and total UDCA met the regulatory criteria for bioequivalence. The test formulation is therefore bioequivalent to the reference formulation. No adverse events were reported.

Keywords

Bioequivalence, Ursodeoxycholic Acid, Pharmacokinetics, Primary Biliary Cholangitis

1. Introduction

Ursodeoxycholic acid (UDCA), also known as ursodiol, is a naturally occurring secondary bile acid present in low concentrations in human bile. Chemically, UDCA (**Figure 1**) (3α,7β-dihydroxy-5β-cholan-24-oic acid; $C_{24}H_{40}O_4$) is a white, crystalline powder that is freely soluble in ethanol and glacial acetic acid, slightly soluble in chloroform, sparingly soluble in ether, and practically insoluble in water. Its molecular weight is 392.56 g/mol [1].

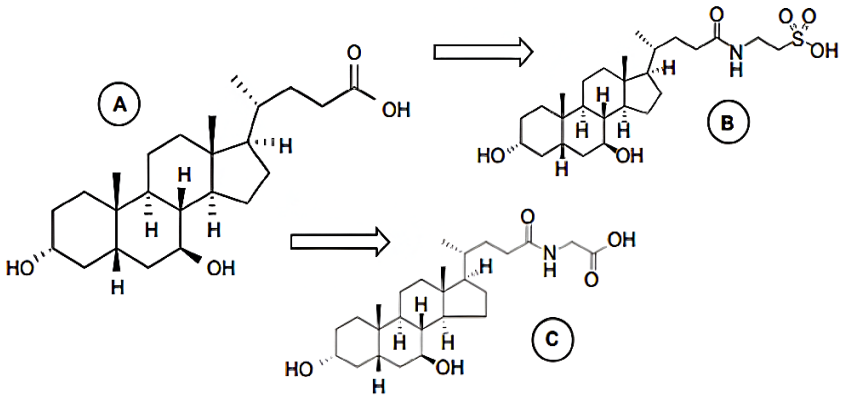

Figure 1. Chemical structure of UDCA [2].

Primary bile acids are synthesized hepatically and subsequently stored in the gallbladder. After secretion into the intestine, they undergo bacterial metabolism to form secondary bile acids such as UDCA. Upon hepatic uptake, UDCA is conjugated with glycine or taurine, forming the major metabolites glycoursodeoxycholic acid (GUDCA) and tauroursodeoxycholic acid (TUDCA). These conjugates are concentrated in the gallbladder and released into the duodenum, where they exert their physiological and pharmacological effects [3] [4].

UDCA regulates cholesterol homeostasis by reducing intestinal cholesterol absorption and promoting micellar disruption. In the liver, UDCA exhibits multiple complementary mechanisms, including cytoprotective, immunomodulatory, and choleretic actions. The hepatoprotective properties are attributed to antioxidant effects—mediated in part through increased concentrations of glutathione and thiol-containing proteins—and to inhibition of mitochondrial membrane depolarization and reactive oxygen species generation. UDCA activation of the glucocorticoid receptor suppresses NF-κB-dependent transcription, contributing to its anti-inflammatory action. Its ability to decrease biliary cholesterol saturation facilitates gradual dissolution of cholesterol gallstones [4].

Clinical and observational studies have demonstrated the benefits of UDCA as first-line therapy in conditions such as primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC), including cases of immune-mediated liver injury following immunotherapy [5] [6]. Gallstone disease remains prevalent and costly in South America, and UDCA is employed as a non-surgical alternative for

patients with radiolucent gallstones [2]. UDCA is approved for the treatment of PBC, for the dissolution of cholesterol gallstones in selected patients, and for the prevention of gallstone formation during rapid weight loss [3]-[7].

Following oral administration, UDCA is rapidly absorbed in the small intestine and undergoes extensive first-pass hepatic metabolism, primarily via conjugation with glycine or taurine. Only a small fraction appears in systemic circulation. The terminal half-life ranges from 3.5 to 5.8 days. Enterohepatic recirculation leads to the bacterial conversion of a fraction of UDCA to 7-keto-lithocholic acid, with approximately 80% of lithocholic acid excreted in feces and the remainder sulfated hepatically prior to biliary excretion [4]-[7].

Several clinical trials, including 16 randomized studies involving 1447 patients, have shown that UDCA improves biochemical markers such as bilirubin, ascites, and jaundice, with effects varying by disease severity and treatment duration [8]. Meta-analyses have also reported reductions in fasting plasma glucose, HbA1c, and insulin concentrations, suggesting beneficial effects on glucose homeostasis. Doses of 8 - 10 mg/kg/day appear most effective across studies involving 868 patients treated for 6 - 78 months [9]-[12]. While UDCA may provide some benefit in PSC, high-dose regimens have been associated with adverse events, leading to early termination of at least one clinical trial [9]-[12]. Common adverse effects include abdominal discomfort, diarrhea, nausea, pruritus, rash, and alopecia [3] [4] [13] [14].

The present bioequivalence study compares the rate and extent of absorption of a 600-mg oral dose (two 300-mg tablets) of a test UDCA formulation (Laboratorios LETI, S.A.V., Venezuela) with a reference formulation, Ursobilane® (Estedi S.L., Spain), in healthy adult male subjects under fed conditions. The study was conducted by ICBio Clinical Research Private Limited (India).

2. Materials and Methods

2.1. Ethical Considerations

The study was conducted in accordance with the principles outlined in the ICMR Guidelines [15], the New Drugs and Clinical Trials Rules 2019 of India [16], the Declaration of Helsinki [17], the International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines [18], and the FDA Product-Specific Guidance for Bioequivalence Studies [19].

The protocol (ICBio/021/0823) was reviewed and approved by the ACE Independent Ethics Committee on 14 May 2024 (Version 00, dated 03 August 2023), and certified by the CDSCO/DGHS for ICBio Clinical Research Pvt. Ltd.

2.2. Study Design

This was an open-label, randomized, two-treatment, two-period, two-sequence, single-dose, crossover bioequivalence (BE) study under fed conditions comparing two 300 mg ursodeoxycholic acid (UDCA) formulations.

Test formulation (T): Ursodeoxycholic acid 300 mg tablets, Laboratorios Leti S.A.V., Venezuela; Batch EP-0416445-5; expiry 06/2026, and Reference formulation (R): Ursobilane® 300 mg tablets, Laboratorios ESTEDI S.L., Spain; Batch 0178T028; expiry 05/2025.

A total of 24 healthy male subjects were randomized to one of the two sequences (T-R or R-T). The randomization schedule was generated using SAS® 9.1.3 (SAS Institute Inc., Cary, NC, USA). Each subject received a single oral dose in each period. Subjects who received T in Period I received R in Period II, and vice versa.

The pre-screening period lasted 28 days. Participants were housed in the ICBio Clinical Research facility from 36 h pre-dose until 24 h post-dose in each period. The entire study was conducted from July 19 to August 18, 2024, including a 30-day washout, compliant with at least five elimination half-lives of UDCA [3] [4].

2.3. Subjects

Twenty-four healthy male volunteers who met the inclusion and exclusion criteria were enrolled. The mean age was 36.63 years, the mean weight 70.79 kg, the mean height 1.71 m, and the mean BMI 24.08 kg/m^2 (**Table 1**).

Table 1. Mean demographic characteristics of enrolled subjects (N = 24).

Variable	Mean	SD	Min	Max
Age (years)	36.63	6.01	22	45
Weight (kg)	70.79	9.16	60	99
Height (m)	1.71	0.063	1.57	1.84
BMI (kg/m^2)	24.08	2.71	19.37	29.73

Screening procedures included a complete medical history, physical examination, vital signs, liver transaminases, tests for hepatitis B and C, HIV, VDRL, 12-lead ECG, chest radiography, and urine toxicology including alcohol. Urine tests for drugs of abuse and alcohol were repeated on each check-in day. Eligible subjects were non-smokers or smokers who abstained from smoking for at least 10 hours before dosing. All subjects were capable of understanding and signing the informed consent form.

Exclusion criteria included any condition affecting the gastrointestinal tract or hematopoietic system, history of gastric or duodenal ulcer, gastrointestinal bleeding, or blood in stools, recent infection, major or minor surgery, hypersensitivity to UDCA or related drugs cardiovascular, renal, hepatic, metabolic, neurological, endocrine, psychiatric, or hematopoietic disorders, and use of medications interfering with UDCA metabolism or quantification.

2.4. Drug Administration

Screening was completed within 28 days before check-in. Subjects were housed from 36 h pre-dose to 24 h post-dose in each period. After a 10-hour overnight fast, a high-fat, high-calorie breakfast (1100 kcal) was served exactly 30 minutes prior to dosing, consistent with FDA fed-state requirements. Standardized meals totaling 2500 kcal/day were provided. Water was allowed ad libitum except 1 hour before and after dosing.

All subjects received a single oral dose of 600 mg UDCA (two 300 mg tablets) of either T or R according to the SAS-generated randomization schedule. Study personnel responsible for investigational product (IP) dispensing ensured compliance with randomization.

Blood Sampling

A total of 22 samples were obtained per period in baseline samples at −24.00, −18.00, −12.00, −06.00, and 0.00 h and post-dose samples at 0.334, 0.667, 1.00, 1.334, 1.667, 2.00, 2.334, 2.667, 3.00, 3.35, 4.00, 6.00, 8.00, 12.00, 24.00, 48.00, and 72.00 h.

The last three samples were collected by direct venipuncture. Pre-dose samples were 7 mL each; post-dose samples were 5 mL each.

2.5. Analytical Methodology

Venous blood samples were collected in pre-labeled K_2EDTA (ethylenediaminetetraacetic acid) vacutainers and were centrifuged at 4000 rpm for 10 min at 2˚C - 8˚C within 45 minutes of sample collection. Plasma was separated, labeled, and stored at −70˚C ± 15˚C in an appropriate container.

A sensitive and selective LCMS/MS method to quantify ursodeoxycholic acid was developed and validated using ursodeoxycholic acid D4 as internal standards (Vivian Life Sciences). The calibration curve (CC) of the internal standard (ISTD) showed a linearity range for UDCA of 0.010 to 10.034 µg/ml, as per method SOP N˚MVR-087-02-23 at the bioanalytical laboratory of ICBio Research Pvt. Ltd., Bangalore. The method with a linearity range of 0.010 - 20.000 µg/ml was validated to determine the UDCA endogenous levels.

Liquid-liquid extraction was carried out by adding 50 µL of ISTD dilution to all the samples. Ursodeoxycholic acid was selectively isolated from plasma by the solid phase extraction method. Estimation was done by the mass spectrometric method and high performance liquid chromatography (HPLC) using a BDS Hypersil C18, 4.6 × 100 mm, 5 µm column (Shimadzu). The mass spectrometer was operated in positive electrospray mode. Identifications were based on multiple reaction monitoring transitions; m/z 391.30 - 391.30 for UDCA drug and 395.30 - 395.30 for UDCA D4 IS, m/z 448.40 - 448.40. The inter-batch calibration standard for UDCA showed a precision range of 5.26% to 7.37%, with accuracy from 102.57% to 106.68%, and UDCA D4 IS 5.77% to 10.94% precision range with accuracy 96.67% to 116.67%. Inter-batch precision and accuracy for UDCA met

acceptance criteria [20] [21].

2.6. Statistical Analysis

Sample size was based on intra-subject CV% from published data [22] [23]. The highest reported intra-subject CV% was ~24.08% for C_{max}. Assuming CV% ≤26% for C_{max} and AUC, and a true ratio between 98% and 102%, 21 evaluable subjects provided 80% power at $\alpha = 0.05$. To allow for dropouts, 24 subjects were enrolled.

Pharmacokinetic analysis was performed on subjects completing both periods using SAS® 9.1.3. Primary PK Parameters (per FDA guidance [19]): C_{max} and $AUC_{0\text{-}72}$.

Secondary PK Parameters T_{max}, $t_{½}$ and K_{el} and N points of terminal phase C_{max} and $AUC_{0\text{-}72}$ were ln-transformed and analyzed using a generalized linear model (GLM) ANOVA with fixed effects (treatment, period) and random effect (subject nested in sequence).

Bioequivalence was concluded if the 90% confidence intervals for T/R geometric mean ratios of C_{max} and $AUC_{0\text{-}72}$ fell within 80% - 125% [24].

2.7. Descriptive Statistics

For UDCA (unconjugated) and total UDCA (conjugated + unconjugated), descriptive statistics included the mean, SD, CV%, geometric mean, median, and range.

2.8. Ratio Analysis and Confidence Intervals

Least-square means (LSMs) of ln-transformed parameters were compared (T-R). Anti-logged LSM differences produced geometric mean ratios (T/R). Schuirmann's two one-sided t-tests generated 90% CIs for BE determination.

2.9. Safety Assessments

Safety was evaluated through adverse event monitoring, vital signs, screening/end-of-study laboratory tests, ECG at screening, and urine drug testing before check-in for each period.

3. Results

3.1. Pharmacokinetics Results

Pharmacokinetic (PK) analyses were completed for 22 of the 24 randomized subjects. Subjects 17 and 23 were classified as dropouts because they did not attend Period II. PK parameters are presented in **Table 2**. A non-compartmental analysis was performed to estimate the peak plasma concentration (C_{max}), the area under the plasma concentration-time curve from zero to 72 hours ($AUC_{0\text{-}72}$), time to reach peak concentration (T_{max}), elimination rate constant (λ_z/K_{el}), and half-life ($T_{1/2}$). These parameters were calculated for baseline-corrected and uncorrected unconjugated UDCA (**Table 2**) and for total UDCA (conjugated + unconjugated)

(Table 3) for both test and reference formulations.

Table 2. UDCA unconjugated, uncorrected, and corrected (N = 22).

600 mg (300 mg × 2 tablets—Dose)				
	Test (T) UDCA Mean ± SD		Reference (R) UDCA Ursobilane® Mean ± SD	
PK Parameters	Unconjugated UDCA (Baseline Uncorrected)	Unconjugated UDCA (Baseline Corrected)	Unconjugated UDCA (Baseline Uncorrected)	Unconjugated UDCA (Baseline Corrected)
C_{max} (μg/mL)	4.86 ± 1.91	4.75 ± 1.92	4.55 ± 1.57	4.46 ± 1.57
$AUC_{0\text{-}72}$ (μg*hr/mL)	53.59 ± 13.67	43.70 ± 11.85	49.73 ± 9.24	40.47 ± 8.16
T_{max} (hrs)	5.00 (2.66 - 6.00)	5.00 (2.66 - 6.00)	4.00 (2.66 - 8.00)	4.00 (2.66 - 8.00)
K_{el} (hrs^{-1})	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01
$T_{1/2}$ (hrs)	29.07 ± 15.84	21.80 ± 11.18	35.56 ± 36.53	25.03 ± 23.30

Note: For mean along with minimum and maximum values represented. Data are presented as mean ± standard deviation. C_{max} = Maximum concentration, $AUC_{0\text{-}t}$ = Area under the plasma concentration-time curve from time 0 to the last measurable concentration, K_{el} = Elimination rate constant, T_{max} = Time to reach C_{max}, $T_{1/2}$ = time required for plasma concentration to decrease by 50%, PK: Pharmacokinetic.

Table 3. Total UDCA (conjugated and unconjugated) (N = 22).

600 mg (300 mg × 2 tablets—Dose)				
	Test (T) Mean ± SD		Reference (R) UDCA Ursobilane® Mean ± SD	
PK Parameters	Total UDCA (Baseline Uncorrected)	Total UDCA (Baseline Corrected)	Total UDCA Ursobilane® (Baseline Uncorrected)	Total UDCA Ursobilane® (Baseline Corrected)
C_{max} (μg/mL)	9.07 ± 2.41	7.49 ± 2.43	8.95 ± 2.87	7.48 ± 2.73
$AUC_{0\text{-}72}$ (μg*hr/mL)	246.32 ± 67.62	108.50 ± 54.04	250.82 ± 82.57	115.70 ± 75.67
T_{max} (hrs)	6.00 (2.66 - 8.00)	6.00 (2.66 - 8.00)	6.00 (3.00 - 8.00)	6.00 (3.00 - 8.00)
K_{el} (hrs^{-1})	0.01 ± 0.00	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.03
$T_{1/2}$ (hrs)	43.3 ± 14.45	38.00 ± 52.98	48.66 ± 23.88	31.38 ± 23.68

Note: For the mean along with the minimum and maximum values represented. Data are presented as mean ± standard deviation. C_{max} = Maximum concentration, $AUC_{0\text{-}t}$ = Area under the plasma concentration-time curve from time 0 to the last measurable concentration, K_{el} = Elimination rate constant, T_{max} = Time to reach C_{max}, $T_{1/2}$ = time required for plasma concentration to decrease by 50%. PK: Pharmacokinetic.

3.2. Statistical ANOVA Results

The ANOVA results for log-transformed C_{max} and $AUC_{0\text{-}72}$ confirmed that, for both unconjugated and total UDCA, there were no statistically significant effects of treatment, sequence, or period between the test and reference formulations (Table 4 and Table 5).

The geometric mean ratios (Test/Reference) and their corresponding 90% confidence intervals for log-transformed PK parameters demonstrated that bioequivalence was achieved. For unconjugated UDCA, the 90% CI for C_{max}

(103.42%; 90.34% - 118.40%) and $AUC_{0\text{-}72}$ (105.09%; 94.95% - 116.30%) were within the acceptance limits of 80% - 125%. For total UDCA (conjugated + unconjugated), the 90% CI for C_{max} (100.27%; 89.50 - 112.33%) and $AUC_{0\text{-}72}$ (96.96%; 80.49% - 116.80%) also fell within the predefined bioequivalence interval (Table 6).

Table 4. ANOVA unconjugated UDCA (Baseline corrected, P value).

	Sequence	Period	Treatment
C_{max} (μg/mL)	0.914	0.116	0.672
$AUC_{0\text{-}72}$ (μg*hr/mL)	0.582	0.162	0.408

Table 5. ANOVA total UDCA, conjugated and non-conjugated (Baseline corrected, P value).

	Sequence	Period	Treatment
C_{max} (μg/mL)	0.628	0.364	0.967
$AUC_{0\text{-}72}$ (μg*hr/mL)	0.474	0.468	0.777

Table 6. UDCA unconjugated and total (conjugated and unconjugated) in Plasma.

Statistical Evaluation in 22 healthy adult human subjects				
PK Parameters	Unconjugated UDCA Baseline corrected		Total UDCA (conjugate and unconjugated Baseline corrected	
	C_{max} (μg/mL)	$AUC_{0\text{-}72}$ (μg*hr/mL)	C_{max} (μg/mL)	$AUC_{0\text{-}72}$ (μg*hr/mL)
Test (T)				
Geometric Mean	4.4289	42.0586	7.0919	96.8423
CV%	39.62	30.05	36.39	52.15
N	22	22	22	22
Reference (R)				
Geometric Mean	4.2325	39.7135	7.0335	99.1574
CV%	33.38	19.99	37.27	57.59
N	22	22	22	22
Test LSM	4.4002	41.7999	7.0944	95.8722
Reference LSM	4.2546	39.7764	7.0753	98.8781
T/R ratio (%)	103.42	105.09	100.27	96.96
90% Lower CI	90.34	94.95	89.50	80.49
90% Upper CI	118.40	116.30	112.33	116.80
p-values				
Period	0.3641	0.4688	0.3641	0.4688
Treatment	0.9676	0.7778	0.9676	0.7778
Sequence	0.6280	0.4741	0.6280	0.4741
Intra CV (%)	22.01	36.81	22.01	36.81
Power	89.62	49.27	89.62	49.27

There were no significant differences between the PK parameters of both UDCA formulations ($p > 0.05$). These findings confirm that the test product meets the regulatory bioequivalence criteria [13].

3.3. Plasma Concentration Profiles

Mean UDCA plasma concentrations following oral administration of 600 mg (2 × 300 mg tablets) for up to 72 hours post-dose are shown in Table 7 and Table 8 and are graphically represented in Figures 2-5, using both arithmetic and logarithmic scales.

Table 7. Plasma concentration UDCA unconjugated corrected.

22 healthy subjects								
TIME (h)	Plasma Concentration Test (T)	Plasma Concentration Reference (R)	Plasma SD (T)	Plasma SD (R)	Ln plasma (T)	Ln SE (T)	Ln plasma (R)	Ln SE (R)
0	0.0208	0.0131	0.027	0.016	0	0	0	0
0.3	0.0509	0.0406	0.040	0.036	0	0	0	0
0.6	0.1590	0.2024	0.249	0.261	0	0	0	0
1	0.2730	0.3426	0.418	0.451	0	0	0	0
1.3	0.5015	0.5674	0.623	0.609	0	0	0	0
1.6	0.7383	0.8046	0.775	0.729	0	0	0	0
2	1.0342	0.9645	0.964	0.737	0.033	0	0	0
2.3	1.3651	1.2569	1.064	0.788	0.311	0.062	0.228	0
2.6	1.6037	1.7469	1.067	0.919	0.472	0.065	0.557	0
3	2.1355	2.1116	1.279	0.874	0.758	0.246	0.747	0
3.5	2.6258	2.8158	1.586	1.191	0.965	0.461	1.035	0.175
4	3.4869	3.5631	2.044	1.645	1.249	0.715	1.270	0.498
6	3.7194	3.3221	1.463	1.625	1.313	0.380	1.200	0.175
8	1.4825	1.3055	0.660	0.788	0.393	0	0.266	0
12	1.2733	0.9600	0.798	0.453	0.241	0	0	0
24	0.3952	0.3196	0.163	0.141	0	0	0	0
48	0.2369	0.2649	0.178	0.211	0	0	0	0
72	0.3305	0.1786	0.350	0.143	0	0	0	0

Ln = neperian logarithm; SE = standard error.

Table 8. Plasma concentration of UDCA total (conjugated and unconjugated) corrected.

22 healthy subjects								
TIME (h)	Plasma [Test] (T)	Plasma [Reference] (R)	Plasma SD (T)	Plasma SD (R)	Ln plasma (T)	Ln SE (T)	Ln plasma (R)	Ln SE (R)
0	0.131	0.274	0.308	0.430	0	0	0	0
0.3	0.422	0.302	0.599	0.526	0.862	0	0	0
0.6	0.490	0.447	0.659	0.565	0.711	0	0	0
1	0.698	0.645	0.612	0.613	0.359	0	0	0
1.3	1.094	1.033	1.059	0.913	0.090	0.058	0.032	0
1.6	1.187	1.240	1.151	1.092	0.171	0.141	0.215	0.088
2	1.480	1.417	1.379	1.126	0.0392	0.321	0.348	0.119

Continued

2.3	1.891	1.843	1.310	1.176	0.637	0.270	0.611	0.162
2.6	2.375	2.731	1.649	1.630	0.865	0.500	1.004	0.489
3	3.102	3.227	1.905	1.791	1.132	0.645	1.171	0.583
3.5	3.806	4.244	2.030	1.639	1.336	0.708	1.445	0.494
4	4.664	5.279	2.448	2.714	1.639	0.895	1.663	0.998
6	6.535	6.643	2.517	2.788	1.877	0.923	1.893	1.025
8	4.508	3.692	1.883	1.996	1.505	0.633	0.306	0.691
12	3.405	2.913	1.962	1.592	1.225	0.674	1.069	0.465
24	0.384	0.844	0.639	1.405	0	0	0	0.340
48	1.157	1.393	1.366	1.389	0	0.312	0.331	0.328
72	1.407	1.047	1.685	1.452	0	0.522	0.046	0.373

Ln = neperian logarithm; SE = standard error.

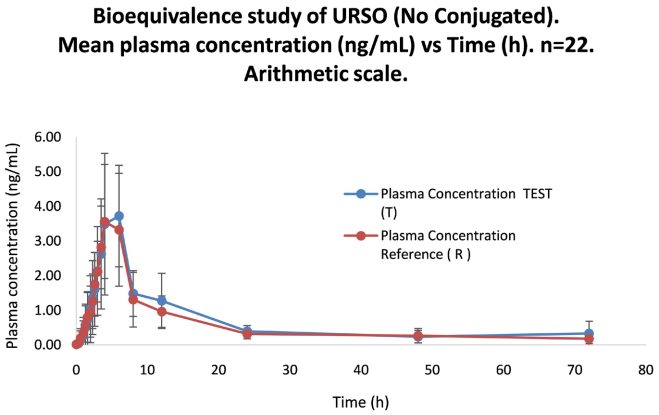

Figure 2. Ursodeoxycholic acid 600 mg dose (2 tablets of 300 mg), mean plasma concentration vs time (h) profile for each formulation is presented on a linear scale, following a single oral dose in the fed condition. The blue line indicates UDCA 600 mg, test product of Laboratorios Leti S.A.V, and the red line indicates Ursobilane® 600 mg, reference product of Laboratorio Estedi S.L., Spain.

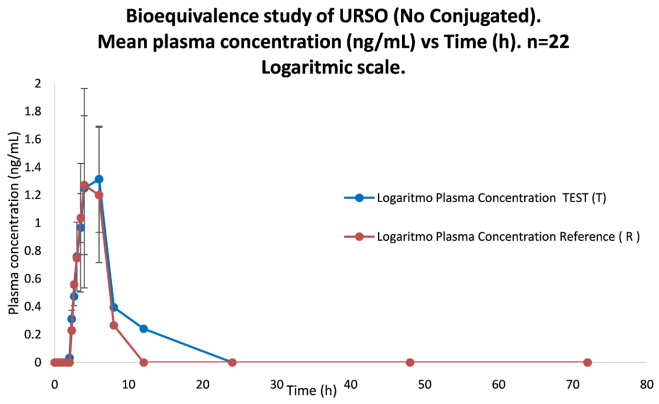

Figure 3. The ursodeoxycholic acid 600 mg dose (2 tablets of 300 mg), mean plasma concentration vs time (h) profile for each formulation is presented on a logarithmic scale, following a single oral dose in the fed condition. The blue line indicates UDCA 600 mg, Test product of Laboratorios Leti S.A.V., and the red line indicates Ursobilane® 600 mg, Reference product of Laboratorio Estedi S.L., Spain.

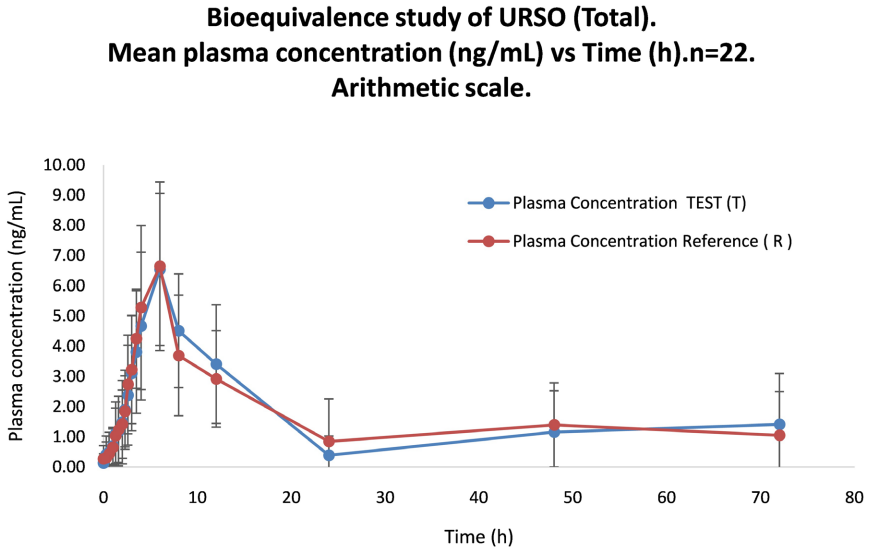

Figure 4. Ursodeoxycholic acid 600 mg dose (2 tablets of 300 mg) total (conjugated and unconjugated) mean plasma concentration vs time (h) profile for each formulation is presented on a linear scale, following a single oral dose in fed condition. The blue line indicates UDCA 600 mg, Test product of Laboratorios Leti S.A.V., and the red line indicates Ursobilane® 600 mg, Reference product of Laboratorio Estedi S.L., Spain.

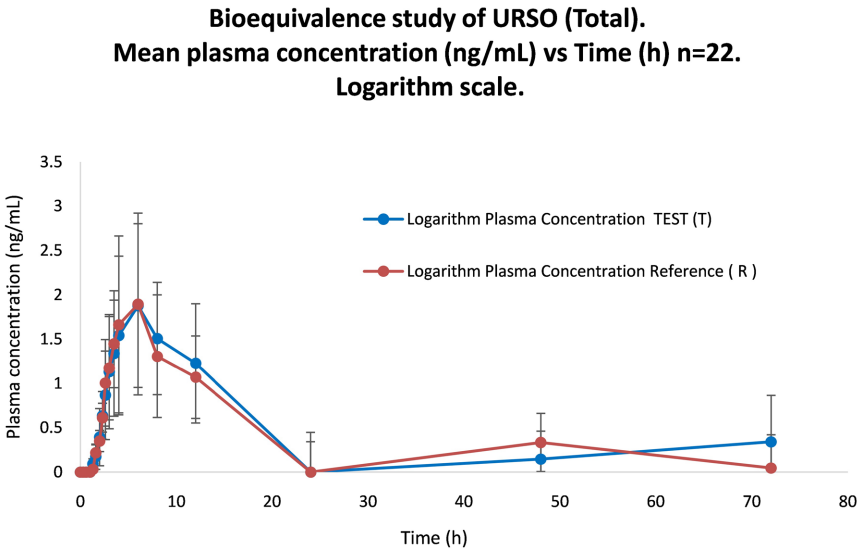

Figure 5. Ursodeoxycholic acid 600 mg dose (2 tablets of 300 mg) total, (conjugated and unconjugated) mean plasma concentration vs time (h) profile for each formulation is presented on a logarithmic scale, following a single oral dose in the fed condition. The blue line indicates UDCA 600 mg, Test product of Laboratorios Leti S.A.V, and the red line indicates Ursobilane® 600 mg, Reference product of Laboratorio Estedi S.L., Spain.

3.4. Tolerability and Safety

All subjects were monitored for clinical well-being and vital signs during the study. Measurements were recorded at check-in (−36 h), at pre-dose (−24 h, −18 h, −12 h, and within 2 h prior to dosing), at 3, 7, and 11 hours post-dose, at check-out for each period, and during all scheduled ambulatory visits.

No adverse events (AEs) or serious adverse events (SAEs) were reported during the study. Post-study safety evaluations were completed for all subjects who attended Period II, except for subjects 17 and 23, who were withdrawn as dropouts. All clinical laboratory parameters were within normal limits.

4. Discussion

This study compared the bioequivalence of two formulations of ursodeoxycholic acid (UDCA) 600 mg, one dose (2 tablets of 300 mg) from Laboratorios Leti S.A.V. and the reference product Ursobilane® 600 mg (2 tablets of 300 mg) from Laboratorio Estedi S.L., Spain, in healthy subjects under fed conditions. UDCA is indicated for adults for the dissolution of cholesterol gallstones, provided the gallbladder is functioning, and for primary biliary cholangitis (PBC). The recommended dosage for gallstone dissolution is 10 mg/kg/day, divided into 2 - 3 doses, and for PBC, the daily recommended dose is 13 - 15 mg/kg/day taken with meals [3] [4].

In this BE study, no significant differences were found in the main pharmacokinetic (PK) parameters, C_{max} and $AUC_{0\text{-}72}$, between Ursobilane® and the UDCA formulation from Laboratorios Leti S.A.V. after a single oral dose under fed conditions. The geometric mean ratio (GMR) and 90% confidence interval (CI) for unconjugated UDCA were C_{max}: 103.42% (90.34% - 118.40%) and $AUC_{0\text{-}t}$: 105.09% (94.95% - 116.30%); and for total UDCA (conjugated and unconjugated) C_{max}: 100.27% (89.50% - 112.33%) and $AUC_{0\text{-}72}$: 96.96% (80.49% - 116.80%). All values were within the accepted bioequivalence limits of 80% - 125%. The T/R ratios for both unconjugated and total UDCA fell consistently within the regulatory BE acceptance interval.

Other PK parameters were also comparable. For unconjugated UDCA, T_{max} was 5.00 h for the test product and 4.00 h for the reference, while $T_{1/2}$ values were 21 ± 11 h and 25.03 ± 23 h, respectively. For total UDCA, T_{max} was 6.00 h for both formulations, with $T_{1/2}$ of 38 ± 52 h for the test product and 31.38 ± 23 h for the reference.

The findings of this study are consistent with the pharmacokinetic characteristics of UDCA reported in earlier literature. A key comparative reference corresponds to a clinical evaluation in healthy subjects that assessed the bioavailability of four different UDCA formulations under standardized conditions, reporting marked variability between products. In that analysis, substantial differences were observed in the main pharmacokinetic parameters: the mean AUC ranged from 46.66 to 68.99 μmol/1·6 h^{-1} across formulations, while C_{max} values varied between 16.63 and 24.29 nmol/mL. Additionally, T_{max} showed notable dispersion, ranging from 1.82 to 3.39 h. These findings highlight that UDCA products may exhibit heterogeneous absorption profiles depending on their pharmaceutical characteristics, underscoring the relevance of establishing bioequivalence to ensure consistent therapeutic performance [22].

These results demonstrated that UDCA formulations are not uniformly

bioavailable, highlighting the relevance of formulation characteristics in determining systemic exposure. In contrast to the variability described in the previously referenced work, where marked differences in AUC, C_{max}, and T_{max} were observed among the evaluated ursodeoxycholic acid formulations, the present study demonstrated consistent pharmacokinetic behavior between the test and reference products under fed conditions.

The tight 90% CI ranges and near-superimposable GMRs in our study reflect high formulation consistency and absorption similarity. This supports the notion that tablet formulations, particularly those manufactured under standardized processes, tend to exhibit superior intra-subject reproducibility compared with capsule formulations. This is consistent with previously reported findings showing markedly higher AUC reproducibility for tablets than for capsules (0.97 and 0.88 vs. 0.32 and 0.15, respectively) [22].

Furthermore, the T_{max} values observed in this study were slightly longer than those reported in previous evaluations of UDCA formulations [22]. This difference is expected because the referenced trial was conducted under fasting conditions, whereas the present study was conducted under fed conditions, which typically delay gastric emptying and prolong T_{max} for bile acids. Despite the fed-state delay, the extent of absorption (AUC) remained robust and comparable between formulations in the current investigation, reinforcing the bioequivalence conclusion.

Overall, the consistency of PK parameters between test and reference UDCA formulations in this study aligns with the literature and confirms that both products demonstrate adequate and comparable systemic exposure following oral administration.

The therapeutic use of UDCA is well established for primary biliary cholangitis and cholesterol gallstone dissolution. Gallstones and cholelithiasis are common causes of hospital admission in several Latin American countries [25], with approximately 50% requiring surgical management. In Chile, laparoscopic cholecystectomy represents 40% of all cholecystectomies performed within the National Health Service [26]. There is growing interest in UDCA for symptomatic gallstones, especially for patients who are poor surgical candidates [27]. A randomized clinical trial reported that the use of 300 mg - 600 mg of UDCA compared with placebo resulted in a significantly decreased proportion of patients developing gallstones within 12 months after gastrectomy for gastric cancer—4.3% in the 600 mg group vs 16.7% in the placebo group [28]. Another study evaluated the effect of UDCA as a single or multiple dose regimen and reported similar effects on liver biochemistry and biliary enrichment in cholestatic liver disease [29].

In many Latin American regulatory agencies, drugs that successfully pass bioequivalence assessments receive favorable consideration during approval processes. Thus, BE studies play an essential role in ensuring therapeutic equivalence across generic formulations, expanding access to effective treatment options for patients with gallstone disease and PBC [30] [31].

5. Limitations

This study included only male subjects, which may limit the generalizability of the PK findings to female populations, particularly given known sex-related physiological differences that may influence bile acid kinetics.

6. Conclusion

The 90% confidence intervals for C_{max} and AUC_{0-72} for baseline-corrected unconjugated UDCA and total (conjugated and unconjugated) UDCA fell within the accepted bioequivalence limits of 80% - 125%. Therefore, the test product is bioequivalent to the reference product.

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Author's Contribution

EP, AI, JCh, AT, and XS performed the statistical analysis, interpretation, writing, and review of the manuscript.

Declaration of Patient Consent

All volunteers provided written informed consent after being fully informed about the study before screening.

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

Conflicts of Interest

All authors are Laboratorios Leti S.A.V employees. The authors have no other potential conflicts of interest relevant to this study.

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