

Article

Impact of Postural Restrictions on Tetrabenazine Pharmacokinetics in Healthy Volunteers: A Randomized Crossover Study Emphasizing Variance Minimization Strategies in Good Clinical Practice-Guided Bioequivalence Research

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Abstract

Background: Tetrabenazine, a VMAT2 inhibitor used for hyperkinetic disorders, shows considerable pharmacokinetic variability due to extensive first-pass metabolism. Standardization of clinical trial conditions, including posture, may reduce variability and improve bioequivalence assessments. **Objective:** The aim of this study was to determine the impact of postural restriction on the pharmacokinetics of tetrabenazine and its active metabolite, dihydrotetrabenazine (HTBZ), under controlled conditions. **Methods:** A randomized, open-label, four-period replicate crossover study enrolled 72 healthy fasted adults who received a single 25 mg tetrabenazine dose under two conditions: 4 h semirecumbent posture versus unrestricted movement. Plasma drug concentrations were measured across 36 h using validated LC–MS/MS method. Pharmacokinetic parameters were estimated via non-compartmental analysis and compared with Wilcoxon signed-rank tests. **Results:** Postural restriction significantly increased tetrabenazine exposure (AUC_{0-t} : +16.4%, $p < 0.0001$) and half-life ($p = 0.002$), with a nonsignificant rise in C_{max} . For HTBZ, C_{max} decreased (−16.2%, $p = 0.018$), whereas AUC was unchanged. Parent-to-metabolite ratios increased by 24–29%. Replicate design analyses showed reduced intra-subject variability for tetrabenazine AUC with posture control (~24% vs. >28%). Simulation suggested that posture restriction could lower sample size requirements by 15–30% in two-period average bioequivalence trials. **Conclusions:** Maintaining a semirecumbent posture after dosing enhances tetrabenazine's bioavailability, attenuates early metabolite formation, and reduces pharmacokinetic variability. Incorporating posture control into bioequivalence trial protocols may optimize study design, reduce participant exposure, and align with ICH-GCP ethical principles.

Keywords: tetrabenazine; pharmacokinetics; posture; bioavailability; variability; GCP; BA/BE studies; dihydrotetrabenazine

1. Introduction

Bioavailability and bioequivalence (BA/BE) studies are cornerstone methodologies in clinical pharmacology, providing the evidence base for establishing therapeutic inter-



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changeability of drug products. The conduct of such trials under Good Clinical Practice (GCP) is not only a regulatory obligation but also an ethical imperative [1–3]. One of the critical tenets of ICH-GCP (E6) guidelines is the requirement to expose the smallest possible number of participants to investigational treatments while still generating scientifically valid outcomes (ICH-GCP E6[R2]) guidelines [2]. This principle demands rigorous control of pharmacokinetic (PK) variability, which, if uncontrolled, can artificially inflate sample sizes, prolong trial timelines, and increase participant risk without proportionate scientific benefit [4,5]. Regulatory agencies, including the US Food and Drug Administration (FDA, 2022) and the European Medicines Agency (EMA, 2018), consistently emphasize the identification, minimization, and management of variability sources as essential for reliable BE assessment [1,3,6].

A broad range of intrinsic and extrinsic factors contribute to PK variation, including genetics, metabolic enzyme activity, gastrointestinal physiology, and methodological or procedural inconsistencies across trials [4,5]. Among these, posture and physical activity following oral dosing represent potentially relevant but insufficiently characterized variables. While differences in gastrointestinal motility and regional blood flow associated with postural changes are well recognized in physiological studies [7–9], their direct influence on PK outcomes in BA/BE research has not been systematically standardized across clinical centers. Current practice often relies on broad instructions such as “rest” or “restricted activity,” which vary considerably in interpretation and implementation. Such lack of harmonization introduces additional, and avoidable, sources of variability that may compromise trial efficiency and precision.

The issue becomes particularly salient in the context of drugs with high intra-subject coefficient of variation (ISCV) and pronounced first-pass metabolism [10,11]. Tetrabenazine offers a compelling case study in this regard. As a vesicular monoamine transporter 2 (VMAT2) inhibitor, tetrabenazine is widely employed for the management of hyperkinetic movement disorders, including Huntington’s disease and tardive dyskinesia [12]. Its disposition is characterized by extensive hepatic first-pass metabolism, rapid conversion to its active metabolite dihydrotetrabenazine (HTBZ), and a relatively short elimination half-life [13]. These features confer considerable PK variability, often complicating dose optimization and consistency of therapeutic response. Given these properties, tetrabenazine represents an ideal probe compound for evaluating methodological refinements aimed at reducing ISCV in BA/BE research.

Emerging evidence supports the premise that posture can influence multiple determinants of oral drug absorption. For example, studies have demonstrated that upright or right-lateral decubitus positioning accelerates gastric emptying, leading to increased maximum plasma concentrations (C_{max}), while supine or left-lateral positioning can delay gastric emptying and reduce systemic exposure [9,14]. Moreover, physical activities such as repeated blood sampling, vital sign monitoring, or unrestricted water intake inherently involve changes in posture that may further accentuate inter- and intra-individual variability if not adequately standardized. Despite these insights, most BA/BE studies have not rigorously evaluated postural restrictions as part of trial design, leaving a gap in methodological optimization.

Reducing ISCV is not simply a statistical concern but has direct ethical and operational implications. Even modest reductions in ISCV can translate into substantial decreases in required sample size for crossover BE designs, thereby reducing unnecessary human exposure while maintaining statistical power [15]. This aligns directly with GCP principles that prioritize participant protection while ensuring scientific validity. Importantly, for highly variable drugs such as tetrabenazine, a reduction in ISCV by as little as 20% could

eliminate the need for multiple participants in a trial, thereby lowering overall costs, improving logistics, and accelerating timelines without compromising reliability.

The present investigation was therefore designed to systematically evaluate the influence of postural restriction after dosing on the pharmacokinetics of tetrabenazine and its active metabolite HTBZ in healthy volunteers. Specifically, we aimed to (i) quantify the effect of standardized posture on systemic exposure and intra-subject variability and (ii) assess the implications of such control strategies on sample size optimization in crossover BA/BE trials. By addressing this underexplored methodological factor, this study seeks to enhance the robustness, reproducibility, and ethical efficiency of GCP-compliant BA/BE evaluations.

2. Materials and Methods

2.1. Study Design

The investigation was conducted in healthy adult human volunteers as a randomized, open-label, four-period, two-sequence, two-treatment, fully replicated crossover clinical trial. The two treatment sequences were defined as RTRT and TRTR, where “R” represented the reference formulation and “T” the test formulation of tetrabenazine 25 mg (Piramal Enterprises Ltd., Mumbai, India). This replicate crossover model was specifically chosen as it allows for the precise estimation of intra-subject variability (ISCV), which is a critical parameter in bioequivalence assessments when evaluating highly variable drugs. In addition, the design enabled assessment of posture (restricted vs. unrestricted) as a procedural variable influencing pharmacokinetic outcomes.

All study procedures were conducted under controlled fasting conditions in healthy adult human volunteers. Washout intervals of at least 7 days, corresponding to more than five terminal elimination half-lives, were maintained between consecutive periods to avoid residual drug carryover. The clinical protocol adhered strictly to the ethical standards outlined in the Declaration of Helsinki (2024 revision) [16] and complied with regulatory requirements of the International Council for Harmonisation Good Clinical Practice (ICH-GCP E6[R2]) guidelines [2]. Ethical approval was obtained from the Shreenidhi Heart & Medical Hospital Ethics Committee, Gujarat, India (Protocol No. 0297-18) [17]. Written informed consent was obtained from all participants before enrolment.

2.2. Participant Eligibility

2.2.1. Screening Procedures

Potential participants underwent clinical screening within 28 days prior to the administration of the first dose. Screening evaluations included medical history, physical examination, vital signs, 12-lead electrocardiogram (ECG), routine clinical laboratory tests (haematology, clinical chemistry, and urinalysis), and chest radiography (valid within 6 months).

2.2.2. Inclusion Criteria

Participants were eligible if they met the following requirements:

- (a) Male or female healthy adults aged 18–45 years.
- (b) Residing primarily in Mehsana or other regions of Western India.
- (c) Body mass index (BMI) between 18.5 and 29.9 kg/m² with a body weight $\geq$ 50 kg.
- (d) Absence of clinically significant abnormalities in physical examination, ECG, chest X-ray, or laboratory evaluations.
- (e) No documented history of chronic diseases or psychiatric conditions.
- (f) Female participants were considered eligible if they were surgically sterilized for at least 6 months prior to screening, or if they agreed to use reliable contraceptive

methods throughout study participation. A negative serum pregnancy test was mandatory before enrollment.

- (g) All participants provided signed written informed consent, consistent with ICH-GCP recommendations.

2.2.3. Exclusion Criteria

Subjects were excluded if any of the following applied:

- (a) Known hypersensitivity to tetrabenazine or structurally related compounds.
- (b) Any significant systemic illness (cardiovascular, hepatic, renal, respiratory, neurological, or psychiatric).
- (c) History or evidence of conditions likely to affect drug absorption, distribution, metabolism, or excretion.
- (d) Use of monoamine oxidase inhibitors (MAOIs) or potent CYP2D6 inhibitors within 14 days prior to dosing.
- (e) History of alcohol abuse within the previous two years, or consumption of alcohol within 48 h of dosing.
- (f) Diagnosis of asthma, aspirin-induced asthma, nasal polyps, or a documented allergy to non-steroidal anti-inflammatory drugs (NSAIDs).
- (g) Abnormal ECG findings, including corrected QT interval (QTc) > 450 msec or irregular pulse rates.
- (h) Smoking ≥ 10 cigarettes/day, or any history of nicotine dependence.
- (i) Any abnormal laboratory values deemed clinically significant.
- (j) History of drug abuse or addiction.
- (k) Participation in another investigational study or blood donation (≥ 350 mL) within 90 days of dosing.
- (l) Positive test results for hepatitis B surface antigen, hepatitis C antibody, or HIV antibody.
- (m) Participants following medically prescribed restrictive diets or recent ingestion of grapefruit juice (within 72 h).
- (n) Lactating or breastfeeding women were excluded.

These eligibility criteria were consistent with regulatory guidance for bioequivalence studies involving healthy volunteers as recommended by agencies such as the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA) [3,6].

2.2.4. Randomization and Treatment Sequences

A total of 72 healthy participants meeting the eligibility requirements were randomized into one of the two treatment sequences using a computer-generated randomization schedule. The sequences were as follows: Sequence 1: RTRT; Sequence 2: TRTR.

Each subject received both the test (T) and reference (R) formulations of tetrabenazine 25 mg tablets under two postural conditions: Restricted posture (T1, R1): participants remained in a controlled semi-recumbent posture to assess positional influence on gastrointestinal transit and absorption; Unrestricted posture (T2, R2): participants were allowed free mobility under fasting conditions.

This replicate design with alternating posture conditions was expected to provide robust estimates of intra-subject pharmacokinetic variability and to allow direct evaluation of pharmacokinetic outcomes under different physiological circumstances.

Of the 72 randomized participants, 54 completed all four treatment periods without major protocol deviations and were included in the per-protocol (PP) dataset used for pharmacokinetic and posture-effect analyses. Participants who discontinued prematurely or deviated significantly from the protocol (e.g., non-compliance with fasting, posture

restriction violations, or prohibited medication use) were excluded from the PP analysis but contributed to the safety dataset.

2.3. Dosing Procedure and Posture Conditions

During each study period, participants were administered a single oral dose of tetrabenazine 25 mg with 240 mL of water following an overnight fast of at least 10 h, in compliance with bioequivalence study requirements outlined by the US FDA (2003), EMA (2010), and ICH M10 (2022) guidance documents [3,6,18].

Post-dose posture was carefully controlled to evaluate the impact of positional restriction on the pharmacokinetic profile of tetrabenazine. Two conditions were implemented:

- Restricted Posture (T1, R1): Participants were required to remain in a semi-recumbent position at a 30–45° incline for the first 4 h post-dosing. Ambulation and other physical movements were prohibited unless medically indicated. Compliance with this restriction was ensured by real-time observation by trained study personnel as well as by posture logs, in accordance with standardized clinical trial monitoring procedures.
- Unrestricted Posture (T2, R2): Under this condition, participants were permitted free physical activity within the clinical research facility during the first 4 h post-dosing. No postural restrictions were imposed.

To minimize variability in fluid intake, water consumption was prohibited from 1 h before dosing until 1 h after administration. Standardized meals, consistent with regulatory recommendations for fed/fasting bioequivalence trials, were provided beginning at 4 h post-dose.

Each subject participated in both posture conditions across the four study periods. The first treatment period was conducted under postural restriction (semi-recumbent 30–45° angle), while the second was conducted without restriction (upright seated position). The same sequence was mirrored in the replicate periods to maintain design symmetry. Thus, each subject received all treatments (restricted and unrestricted) twice, ensuring intra-subject comparisons were based on identical participants across posture conditions.

2.4. Blood Sampling and Handling

For pharmacokinetic profiling, 25 venous blood samples were collected from each subject per study period. Each sample comprised 3 mL of blood, drawn at the following pre-specified intervals: pre-dose (0 h), 0.083, 0.167, 0.333, 0.500, 0.750, 1.000, 1.250, 1.500, 1.750, 2.000, 2.333, 2.667, 3.000, 3.500, 4.000, 5.000, 6.000, 8.000, 10.000, 12.000, 16.000, 20.000, 24.000, and 36.000 h post-dose.

Blood was collected in K₂EDTA vacutainers to prevent coagulation. Immediately after collection, tubes were placed upright in racks immersed in an ice-cold water bath until centrifugation. Plasma separation was achieved by centrifugation at 3000 rpm for 5 min at a controlled temperature below 10 °C. The resulting plasma aliquots were promptly transferred to pre-labeled polypropylene tubes and stored at −70 °C ± 10 °C until bioanalysis. These procedures were performed in strict accordance with Good Clinical Practice (ICH E6[R2]) [2] and Good Laboratory Practice (OECD, 1998) standards [19].

2.5. Bioanalytical Methodology

Quantification of tetrabenazine and its active metabolite dihydrotetrabenazine (HTBZ) was performed at Lambda Therapeutic Research Ltd., Ahmedabad, India, utilizing a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method in line with US FDA (2018) and EMA (2011) bioanalytical validation guidance.

Sample Preparation: Plasma samples underwent Solid-Phase Extraction (SPE) using C18 cartridges, a well-established protocol for analyte purification from complex biological

matrices. A deuterated internal standard (tetrabenazine-d7) was employed to ensure precision in quantification and correct for potential matrix effects.

Analytical Conditions: Chromatographic separation was achieved on a C18 reversed-phase column, employing a binary mobile phase comprising acetonitrile and an aqueous buffer system optimized for retention and peak resolution. Detection was performed via API 6500 triple quadrupole MS/MS system with positive ionization mode.

Calibration and Linearity: The validated linearity range for tetrabenazine was 5.124 pg/mL to 2520.488 pg/mL, while HTBZ levels were quantifiable within 0.212 ng/mL to 253.169 ng/mL. All validation parameters, including accuracy, precision, recovery, matrix effect, and stability, were within acceptance thresholds defined by ICH M10 and FDA 2018 guidance [20].

2.6. Pharmacokinetic Assessment

Pharmacokinetic (PK) parameters were calculated using non-compartmental analysis (NCA) implemented in Phoenix WinNonlin software (v6.4, Certara, Princeton, NJ, USA). Primary PK endpoints: Maximum observed plasma concentration ($C_{\max}$) and area under the plasma concentration–time curve from dosing to the last measurable concentration (AUC_{0-t}). Secondary PK endpoints: Time to reach maximum concentration ($T_{\max}$) and terminal elimination half-life ($t_{1/2}$). Apparent oral clearance (CL/F) and apparent volume of distribution (Vd/F) were calculated according to standard equations:

$$CL/F = \frac{Dose}{AUC_{0-t}}, Vd/F = \frac{CL/F}{\lambda_z}.$$

It was normalized by the fraction of the dose that reaches systemic circulation (bioavailability, F). Dose was 25 mg tetrabenazine (parent drug). Since the systemic dose of HTBZ is unknown, CL/F and Vd/F were not derived for the metabolite. All PK parameters were computed for each subject under both postural conditions (restricted vs. unrestricted). Both arithmetic means with standard deviations and geometric means with coefficients of variation (%CV) were computed to account for potential non-normal distribution of exposure data. Variability estimates included the intra-subject coefficient of variation (ISCV), derived from the replicate TRTR/RTTR design, under both posture-restricted and unrestricted conditions.

2.7. Statistical Analysis

Statistical assessments were performed using SAS (9.4, SAS Institute Inc., Cary, NC, USA) and Phoenix WinNonlin (v6.4, Certara, Princeton, NJ, USA). $C_{\max}$ and AUC_{0-t} values were log-transformed and analyzed using a mixed-effects ANOVA model, with sequence, period, treatment, and posture as fixed effects and subject nested within sequence as a random effect. Bioequivalence was assessed by calculating test/reference ratios and corresponding 90% confidence intervals (CIs) for log-transformed PK parameters, following FDA guidelines [6]. $T_{\max}$ and $t_{1/2}$ were compared using the non-parametric Wilcoxon signed-rank test. Statistical significance was defined as a two-sided p -value < 0.05 . ISCV estimates were further applied in sample size re-estimation and simulation studies to explore the influence of posture restriction on study efficiency and overall subject exposure.

3. Results and Discussion

3.1. Subject Disposition and Baseline Characteristics

A total of 72 healthy adult participants were enrolled and randomized into two treatment sequences (RTTR and TRTR). All subjects received tetrabenazine (25 mg) under both postural conditions across four periods. Of these, 54 subjects completed all study peri-

ods and were included in the per-protocol (PK-evaluable) population, while all 72 were included in the safety set. The demographic characteristics of enrolled population was homogeneous, with mean age 32.7 ± 6.4 years, height 166.9 ± 4.0 cm, weight 70.5 ± 9.0 kg, and BMI 25.2 ± 2.6 kg/m² among completers. No clinically meaningful differences were observed between sequence or posture groups, supporting within-subject comparability in this replicate crossover design. Participant disposition, including enrollment, randomization, and completion, is shown in Figure 1 (Flow Chart).

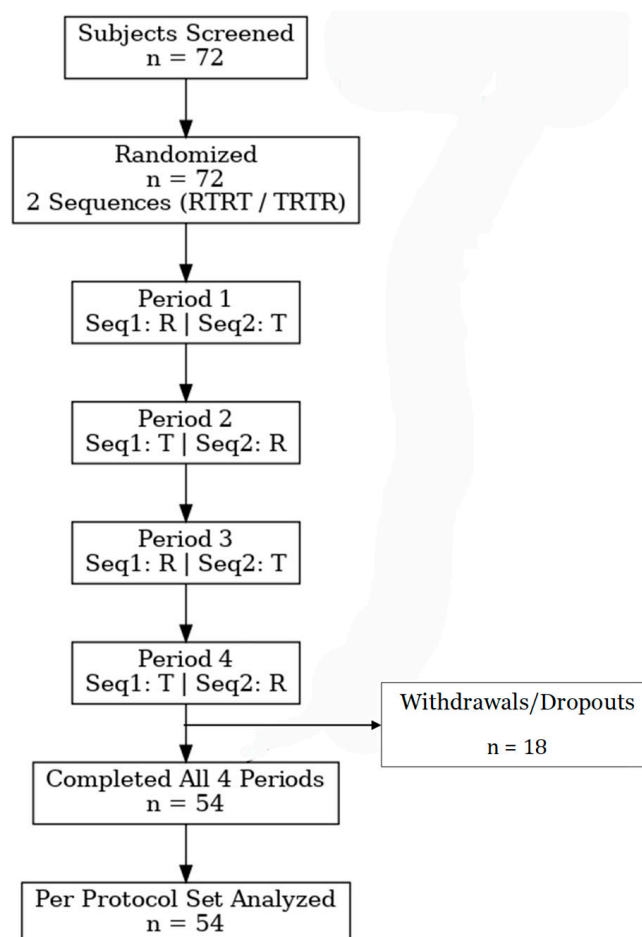


Figure 1. Participants Disposition.

The pharmacokinetic parameters of tetrabenazine and its primary metabolites are summarized in Table 1, with arithmetic means, geometric means, and coefficients of variation (%CV) provided to describe central tendency and interindividual variability (see also Figures 1 and 2). This approach is consistent with the FDA Bioequivalence Guidance for Industry (2014), which recommends reporting both measures of central tendency and variability to facilitate reproducibility and regulatory interpretation.

Table 1. The effects of posture on the pharmacokinetics of tetrabenazine and HTBZ metabolite.

Parameter (n = 54)	With Postural Restrictions	Without Postural Restrictions	p-Value	% Change
Tetrabenazine				
C _{max} (pg/mL)	404.78 ± 83.53	378.69 ± 73.73	0.121	6.90%
C _{max} , Geometric Mean (CV%)	251.02 (151.65%)	252.42 (143.07%)		
T _{max} (h)	0.573 ± 0.035	0.582 ± 0.045	0.484	−1.70%
AUC _{0–t} (pg.h/mL)	817.64 ± 91.22 *	702.50 ± 74.43	<0.0001	16.40%
AUC _{0–t} , Geometric Mean (CV%)	581.09 (87.77%) *	535.88 (80.52%)		
t½ (h)	8.31 ± 0.82	7.06 ± 0.51	0.002	17.70%
λ _z (1/h)	0.105 ± 0.040	0.117 ± 0.051	0.271	+19.7
CL/F (L/h)	49.4 ± 24.0	53.4 ± 25.8	0.133	+15.3%
Vd/F (L)	527.0 ± 285.4	509.0 ± 241.2	0.840	+12.7%
Dihydro-tetrabenazine (HTBZ) Metabolite				
C _{max} (ng/mL)	88.28 ± 4.35 *	105.36 ± 5.60	0.018	−16.20%
C _{max} , Geometric Mean (CV%)	82.87(32.20%) *	97.27(39.10%)		
T _{max} (h)	1.13 ± 0.08	1.02 ± 0.07	0.347	10.80%
AUC _{0–t} (ng.h/mL)	534.12 ± 66.90	566.34 ± 69.36	0.739	−5.70%
AUC _{0–t} , Geometric Mean (CV%)	415.15(92.0%)	443.44 (90.0%)		
t½ (h)	8.19 ± 0.55	8.39 ± 0.63	0.082	−2.40%
% Ratio Tetrabenazine/Metabolite				
C _{max} Ratio	0.52 ± 0.13 *	0.42 ± 0.11		
AUC _{0–t} Ratio	0.22 ± 0.06 *	0.17 ± 0.04		

All values are expressed as mean ± SEM. * *p* < 0.05 as compared to without postural restrictions using Wilcoxon signed test. λ_z = terminal elimination rate constant; CL/F = apparent oral clearance; Vd/F = apparent volume of distribution. Values scaled for AUC_{0–t} in ng.h/mL (CL/F and Vd/F reduced by × 10³ for interpretability). CL/F and Vd/F were not derived for HTBZ as no systemic dose or metabolic fraction (*f_m*) is known; metabolite results describe exposure and elimination parameters only.

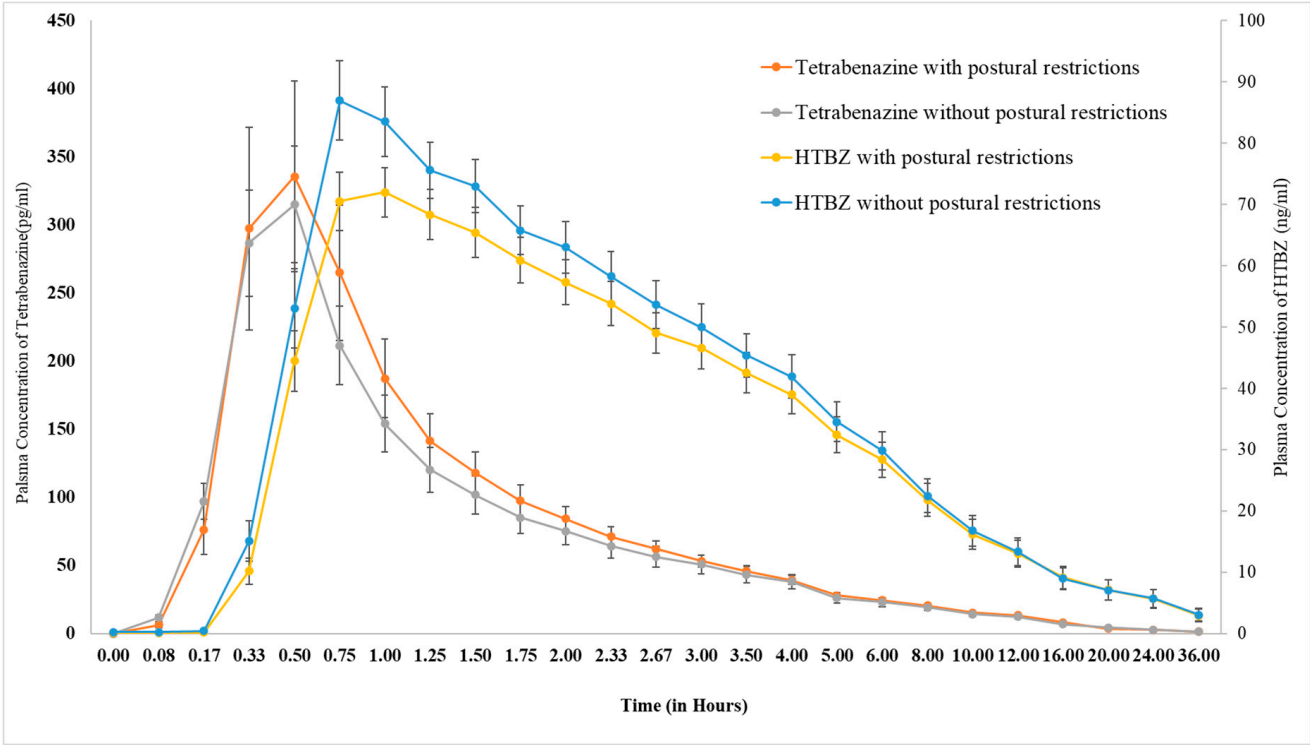


Figure 2. Mean plasma concentrations of tetrabenazine, and HBTZ metabolite with and without postural restrictions.

3.2. Pharmacokinetics of Tetrabenazine

In the present study, postural positioning significantly modulated the pharmacokinetic (PK) profile of tetrabenazine (TBZ). When subjects were maintained in a semirecumbent posture under conditions of physical restriction, the peak plasma concentration ($C_{\max}$) of tetrabenazine was elevated (404.78 ± 83.53 pg/mL) relative to the unrestricted condition (378.69 ± 73.73 pg/mL), resulting in a 6.9% increase ($p = 0.121$) (Table 1, Figure 2). Although this difference did not achieve statistical significance, it suggests a trend toward enhanced early absorption under restricted positioning. Time to maximum concentration ($T_{\max}$) remained nearly identical between groups (0.573 ± 0.035 h vs. 0.582 ± 0.045 h), indicating minimal disruption in the rate of absorption onset.

Notably, the area under the plasma concentration–time curve from time zero to the last measurable concentration (AUC_{0-t}) was significantly greater in the restricted posture (817.64 ± 91.22 pg·h/mL) compared to the unrestricted state (702.50 ± 74.43 pg·h/mL), with a statistically significant difference ($p < 0.05$) (Table 1, Figure 2). This represents a 16.4% increase in systemic exposure, underscoring a meaningful alteration in overall drug availability. Furthermore, the elimination half-life ($t_{1/2}$) of tetrabenazine was prolonged under restriction, increasing from 7.06 ± 0.51 h to 8.31 ± 0.82 h—an extension of 17.7% ($p = 0.002$). Such a change implies either reduced clearance or altered distribution kinetics, potentially mediated by posturally induced physiological shifts in splanchnic blood flow or hepatic enzyme activity.

In addition to changes in exposure and half-life, the apparent oral clearance (CL/F) and volume of distribution (V_d/F) of tetrabenazine were determined to further assess the impact of posture on drug disposition. Under restricted posture, the mean CL/F was 49.4 ± 24.0 L/h, which was modestly lower than in the unrestricted state (53.4 ± 25.8 L/h), representing an approximate 15% reduction that did not reach statistical significance ($p = 0.133$). The apparent V_d/F demonstrated a small, nonsignificant increase (527 ± 285 L vs. 509 ± 241 L, $p = 0.840$), consistent with the observed prolongation in elimination half-life. These findings support the notion that restricted posture leads to a mild reduction in systemic clearance, likely secondary to decreased hepatic perfusion. Given that tetrabenazine is a high-extraction, flow-limited compound metabolized predominantly by carbonyl reductase, its clearance is expected to vary in proportion to hepatic blood flow. As the absolute bioavailability (F) following oral dosing is unknown, the parameters are expressed as apparent values (CL/F and V_d/F).

These pharmacokinetic differences suggest that postural restriction influenced systemic exposure primarily through changes in hepatic or intestinal blood flow rather than through gastric emptying. The unchanged $T_{\max}$ between postures supports the view that the rate of absorption was unaffected. Tetrabenazine undergoes rapid first-pass metabolism by carbonyl reductase and exhibits a high hepatic extraction ratio; hence, its clearance is largely blood flow-limited. It has been reported that portal vein blood flow decreases in semi-recumbent or supine positions relative to upright posture [21–23]. A reduction in splanchnic blood flow under restricted posture could therefore diminish hepatic extraction, leading to higher systemic exposure and prolonged half-life. Since tetrabenazine displays linear pharmacokinetics and no evidence of metabolic saturation, the altered exposure pattern is plausibly attributed to blood flow redistribution rather than enzyme saturation or gastric motility effects [14,23,24].

3.3. Pharmacokinetics of Dihydratetrabenazine (HTBZ)

The active metabolite, α -dihydratetrabenazine (HTBZ), exhibited a divergent pharmacokinetic response to postural restriction. $C_{\max}$ for HTBZ was significantly lower under restriction (88.28 ± 4.35 ng/mL) compared to the unrestricted condition (105.36 ± 5.60 ng/mL);

$p < 0.05$) (Table 1, Figure 3). This reduction in peak metabolite concentration aligns with the notion of attenuated presystemic conversion of the parent compound. $T_{\max}$ was slightly delayed in the restricted group (1.13 ± 0.08 h vs. 1.02 ± 0.07 h), possibly reflecting altered intestinal transit dynamics or modified metabolic onset. AUC_{0-t} also showed a downward trend (534.12 ± 66.90 vs. 566.34 ± 69.36 ng·h/mL), though it did not reach statistical significance. Elimination half-life remained largely unchanged between conditions (8.19 ± 0.55 h vs. 8.39 ± 0.63 h), suggesting that systemic elimination kinetics of HTBZ are less sensitive to postural influence once formed.

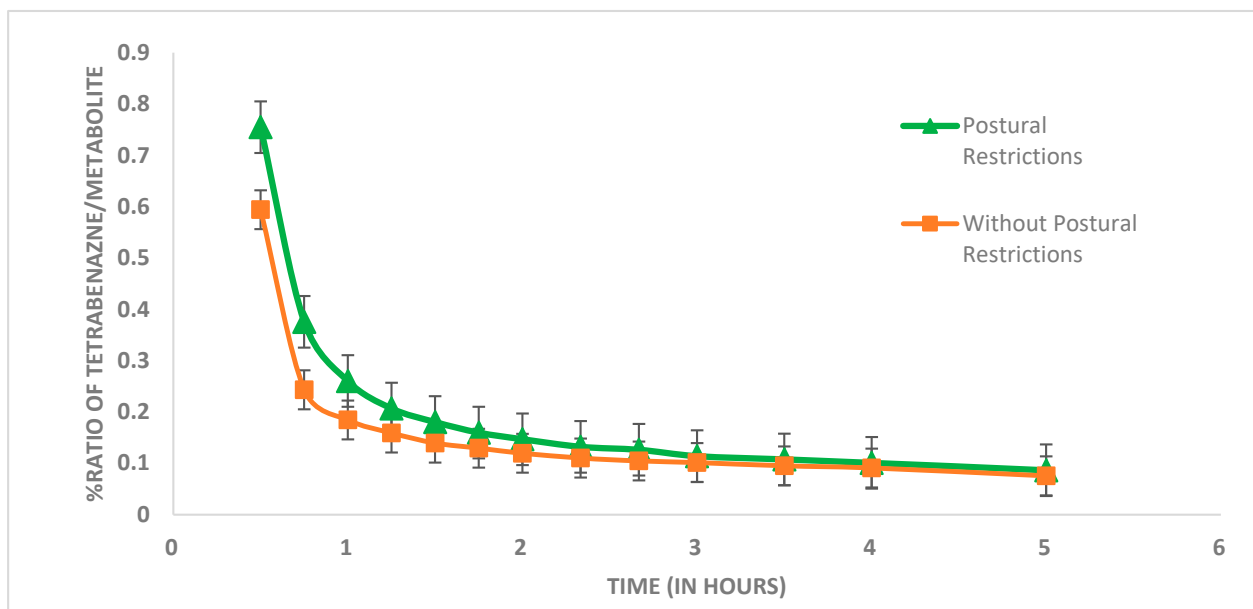


Figure 3. The ratio of plasma concentration of Tetrabenazine to that of its HTBZ metabolite following the administration of Tetrabenazine (25 mg).

Crucially, the parent-to-metabolite exposure ratios provide strong evidence of altered metabolic fate. The $C_{\max}$ ratio of TBZ:HTBZ was significantly higher under restriction (0.52 ± 0.13 vs. 0.42 ± 0.11 ; $p < 0.05$), as was the AUC_{0-t} ratio (0.22 ± 0.06 vs. 0.17 ± 0.04 ; $p < 0.05$) (Table 1, Figure 2). These increased ratios indicate a shift in metabolic efficiency—specifically, reduced first-pass conversion of tetrabenazine to HTBZ. These results further support a blood flow limited clearance mechanism, wherein reduced hepatic perfusion under restricted posture transiently decreases the metabolic conversion of tetrabenazine to HTBZ [23,25,26]. The data imply that postural restriction may transiently reduce hepatic extraction ratio, potentially through diminished portal blood flow redistribution or altered substrate availability during absorption.

Because HTBZ is a secondary metabolite formed in-vivo, and its absolute formation dose and bioavailability cannot be reliably determined, apparent clearance (CL/F) and apparent volume of distribution (V_d/F) were not calculated. Instead, exposure ratios (AUC and $C_{\max}$) were used as surrogate indicators of metabolic formation efficiency. This approach provides a more physiologically appropriate assessment of how postural changes influence hepatic conversion without assuming an arbitrary metabolite dose or systemic availability.

3.4. Geometric Means and Interindividual Variability

Analysis of geometric means and associated coefficients of variation (%CV) further highlighted the high intrinsic variability in tetrabenazine pharmacokinetics. Despite mean $C_{\max}$ values being similar between conditions (251.02 pg/mL restricted vs. 252.42 pg/mL

unrestricted), both exhibited extremely high variability (CV: 152% and 143%, respectively) (Table 1). This level of variability exceeds typical thresholds used in bioequivalence assessments and underscores the challenges in reliably predicting systemic exposure for this compound. AUC_{0-t} geometric means were higher under restriction (581.09 pg·h/mL) compared to unrestricted (535.88 pg·h/mL), with slightly reduced variability (88% vs. 81%). For HTBZ, geometric mean C_{max} was significantly lower in the restricted posture (82.87 ng/mL vs. 97.27 ng/mL), with moderate inter-subject variability (CV: 32% vs. 39%).

The persistent high variability in tetrabenazine PK parameters—even under standardized postural conditions—has critical implications for clinical trial design. Thus, future trials should consider both physiological controls (e.g., posture) and pharmacogenetic stratification to mitigate noise in exposure metrics.

3.5. Intra-Subject Variability and Implications for Bioequivalence Study Design

Employing a replicate crossover design (TRTR/RTRT), we estimated intra-subject coefficient of variation (ISCV) for key PK parameters of both tetrabenazine and HTBZ under restricted (T1, R1) and unrestricted (T2, R2) conditions (Table 2). For tetrabenazine, ISCV for AUC_{0-t} was moderate (24.4% for test, 23.2% for reference), whereas C_{max} demonstrated high intra-individual fluctuation (50–55%), which is likely attributable to rapid absorption and elimination kinetics rather than enterohepatic recycling. In contrast, HTBZ exhibited substantially lower variability—ISCV for C_{max} ranged between 26% and 31%, and for AUC_{0-t} , between 12% and 13%—indicating more consistent metabolite kinetics across dosing periods.

Table 2. Intra-subject coefficient of variation (ISCV%) of tetrabenazine and dihydrotetrabenazine (HTBZ) pharmacokinetic parameters under restricted (T1/R1) and unrestricted (T2/R2) postural conditions. (*n* = 54).

Analyte	Endpoint	Comparison	N	ISCV (%)	95% CI (Lower–Upper)
Tetrabenazine	C_{max} (pg/mL)	Test (T1 vs. T2)	54	49.9	41.2–63.5
		Reference (R1 vs. R2)	54	55.1	45.4–70.6
	AUC_{0-t} (pg·h/mL)	Test (T1 vs. T2)	54	24.4	20.4–30.3
		Reference (R1 vs. R2)	54	23.2	19.4–28.9
	T_{max} (h)	Test (T1 vs. T2)	54	35.5	29.6–44.6
		Reference (R1 vs. R2)	54	56.2	46.3–72.0
HTBZ	$t_{1/2}$ (h)	Test (T1 vs. T2)	53	30.5	25.5–38.2
		Reference (R1 vs. R2)	49	51.5	42.2–66.6
	T_{max} (h)	Test (T1 vs. T2)	54	37.1	30.9–46.6
		Reference (R1 vs. R2)	54	41.2	34.2–51.9
	C_{max} (ng/mL)	Test (T1 vs. T2)	54	26.6	22.2–33.1
		Reference (R1 vs. R2)	54	30.6	25.6–38.3
	AUC_{0-t} (ng·h/mL)	Test (T1 vs. T2)	54	12.5	10.5–15.4
		Reference (R1 vs. R2)	54	12.9	10.8–15.9
	$t_{1/2}$ (h)	Test (T1 vs. T2)	54	14.7	12.3–18.2
		Reference (R1 vs. R2)	53	8.0	6.7–9.9

The reduction in ISCV under posture restriction had direct consequences for sample size requirements in bioequivalence trials. Simulations based on observed variability (Table 3) revealed that restriction reduced ISCV by approximately 20% for the test formulation, translating into fewer participants needed to achieve adequate power. For example, for tetrabenazine AUC_{0-t} , the required sample size decreased from 38 to 27 subjects when accounting for a 10% attrition rate (Table 4). This represents a 29% reduction in enrollment burden—a substantial efficiency gain in clinical trial operations.

Table 3. Effect of posture restrictions on intra-subject variability (endpoint-wise) and projected sample size requirements.

Endpoint	Observed ISCV (%CV)	Required <i>n</i> (total) (N1)	ISCV if Posture Restricted (−20%)	New <i>n</i> (Total)(N2)	Subjects Saved (N1−N2)
Tetrabenazine C _{max}	28%	46	22%	38	8
Tetrabenazine AUC _{0–t}	25%	37	20%	31	6
HTBZ C _{max}	24%	35	19%	29	6
HTBZ AUC _{0–t}	22%	32	17%	27	5

Assumptions: two-period crossover; α = 0.05; power = 90%; BE 80–125%; T/R ratio 0.95. Calculations illustrative, dependent on final ISCV estimates.

Table 4. Impact of posture restrictions on trial size (formulation-wise planning; tetrabenazine AUC_{0–t}).

Formulation	ISCV Used for Planning	Planned N (No Dropout)	Planned N (+10% Attrition)	ISCV if Posture Re- strictions (20%)	Planned N (No Dropout)	Planned N (+10% Attrition)	Subjects Saved
Test (T1 vs. T2)	24.40%	34	38	20%	24	27	11
Reference (R1 vs. R2)	23.20%	32	36	20%	24	27	9

Notably, the reduction in variability was asymmetric: the test product benefited more from posture standardization than the reference, whose ISCV remained relatively stable. This discrepancy may reflect formulation-specific interactions with gastrointestinal motility, where the restricted posture preferentially enhances dissolution or transit of the test formulation. Such differential sensitivity highlights the importance of physiological standardization not only for accuracy but also for fairness in bioequivalence evaluation—a principle that is emphasized in EMA’s guideline on the investigation of bioequivalence [3].

3.6. Clinical and Ethical Implications

The enhancement in systemic tetrabenazine exposure under semirecumbent restriction has dual clinical ramifications. On one hand, increased parent drug bioavailability may improve therapeutic efficacy in indications such as chorea associated with Huntington’s disease, where CNS vesicular monoamine transporter 2 (VMAT2) inhibition is the primary mechanism of action [13,27]. On the other hand, excessive exposure could elevate the risk of adverse events, including sedation, depression, and QT prolongation—particularly in metabolically sensitive individuals. Therefore, uncontrolled postural variation in clinical practice may contribute to unpredictable dose–response relationships, reinforcing the need for standardized administration guidelines.

The hemodynamic mechanism proposed here underscores the physiological sensitivity of flow limited drugs to posture, reinforcing the importance of standardized positioning during clinical dosing and sampling. From an ethical and regulatory standpoint, posture standardization represents a low cost, non-invasive intervention that enhances trial precision while reducing participant burden. By decreasing required sample sizes without compromising statistical rigor, such controls align with the principles of the 3Rs in clinical research specifically reduction in human subject exposure [28]. Implementing posture controls in bioequivalence studies may thus serve both scientific integrity and ethical responsibility. Future studies incorporating IV microdosing, hepatic blood flow assessments, or dynamic imaging techniques could better delineate the contribution of splanchnic circulation and tissue partitioning to the observed postural effects. Despite these limitations,

the current findings provide mechanistic evidence that posture-induced hemodynamic changes can modulate the disposition of blood flow-limited drugs.

4. Conclusions

This study demonstrates that posture is a practical and controllable factor that substantially influences the pharmacokinetics of tetrabenazine. Standardizing a four-hour semi-recumbent posture after dosing increased systemic exposure to the parent compound, reduced early metabolite formation, and lowered intra-subject coefficient of variation; these changes translated into smaller sample-size requirements and improved trial efficiency in simulation. Given tetrabenazine's rapid carbonyl reductase-mediated first-pass metabolism and its high extraction ratio, the observed pattern of effects, together with an unchanged T_{max} , is most consistent with posture-related reductions in splanchnic or portal blood flow rather than with altered gastric emptying or metabolic saturation.

From a regulatory and ethical standpoint, posture standardization offers a low-cost, non-invasive means to improve assay sensitivity and data reproducibility in GCP-compliant bioavailability and bioequivalence studies, particularly for drugs with high first-pass metabolism or BCS Class IV properties. To confirm mechanism and generalizability, future studies should couple intensive pharmacokinetic sampling with direct physiological measurements, such as scintigraphic or ^{13}C breath tests for gastric emptying, Doppler or MRI assessment of splanchnic and portal flow, and evaluation in additional highly extracted compounds; such work will determine whether posture protocols should be recommended in formal regulatory guidance.

Overall, incorporating standardized postural control into BA/BE study protocols represents a tangible improvement in study quality, with potential benefits for scientific rigor, participant welfare, and resource efficiency.

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