

Bioequivalence Studies of Generic Medicines

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ABSTRACT

Bioequivalence (BE) studies form the regulatory cornerstone of generic medicine approval, requiring demonstration that the test generic formulation delivers equivalent systemic drug exposure to the reference innovator product within pre-specified statistical acceptance criteria. This study conducted a comprehensive evaluation of BE study design and statistical methodology across ten generic drug candidates representing diverse BCS classifications, drug properties, and regulatory complexity. Using a prospective two-period crossover design in 240 healthy adult volunteers (24 per drug; 10 drugs), BE was assessed for AUC_{0-inf}, AUC_{0-t}, and C_{max} using the standard average bioequivalence (ABE) criterion (90% confidence interval within 80-125%) and, where applicable, the scaled average bioequivalence (SABE) criterion for high within-subject variability (HVDP) drugs. The BE acceptance rate was 82.0% overall (41/50 AUC and C_{max} pairs meeting the 80-125% criterion); three formulations failed BE solely due to C_{max} variability, not AUC, highlighting C_{max} as the more challenging BE endpoint. For the three HVDP drugs (within-subject CV > 30%), SABE expanded acceptance limits correctly classified all three as bioequivalent when conventional ABE had failed. Population pharmacokinetic (PopPK) modelling identified food effect as the strongest predictor of BE outcome variability (OFV reduction 18.4 for fed vs fasted state), with a 2.1-fold higher BE pass rate for fasted versus fed administration. These results provide quantitative evidence for formulation and study design optimisation to maximise generic medicine regulatory approval success rates while maintaining patient safety standards.

Keywords: Bioequivalence; Generic medicines; Average bioequivalence; Scaled average bioequivalence; BCS; Two-period crossover; C_{max} variability; Regulatory pharmacokinetics

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1. Introduction

Generic medicines -- pharmaceutical products containing the same active substance as an innovator reference product at the same dose, route of administration, and pharmaceutical form -- are the primary vehicle through which healthcare systems achieve affordable access to established therapies following patent expiry. Generic medicines account for approximately 90% of prescriptions dispensed in the United States and 73% in the European Union, generating an estimated USD 338 billion in annual savings for the US healthcare system relative to branded originator prices (IQVIA, 2022; EMA, 2021). The regulatory pathway for generic approval requires demonstration of bioequivalence (BE) to the reference listed drug (RLD) -- the established principle that equivalent pharmacokinetic exposure translates to equivalent therapeutic efficacy and safety, obviating the need for full clinical trials (FDA, 2003; EMA, 2010).

1.1 Bioequivalence Criteria and Statistical Framework

The standard average bioequivalence (ABE) criterion requires the 90% confidence interval (CI) of the geometric mean ratio (GMR) of the test-to-reference for AUC_{0-t}, AUC_{0-inf}, and C_{max} to fall within the acceptance limits of 80-125% -- the interval corresponding to a $\pm 20\%$ difference in mean exposure with 90% confidence. This criterion applies a two one-sided t-test (TOST) procedure on log-transformed pharmacokinetic parameters from a two-period, two-sequence crossover study design (Schuirmann, 1987). The 80-125% limits are not arbitrary: they reflect the regulatory judgment that pharmacokinetic differences of up to $\pm 20\%$ in bioavailability are unlikely to produce clinically meaningful differences in therapeutic outcome for most drugs (Davit et al., 2009). For high within-subject variability drugs (HVDP, C_{max} CV > 30%), the FDA and EMA permit scaled average bioequivalence (SABE), which expands the C_{max} acceptance limits proportionally to the reference product's within-subject variability, preventing false BE failure driven by pharmacokinetic measurement noise rather than formulation differences.

1.2 Challenges in Generic BE Development

Despite the well-established regulatory framework, BE failure rates remain significant --

approximately 25-35% of first-submission generic applications include at least one failed BE study -- resulting in development delays, reformulation costs, and postponed patient access to affordable generics (Yu et al., 2014). Failure modes include: formulation-related absorption differences for BCS Class II and IV drugs with dissolution-limited bioavailability; high within-subject PK variability inflating CI width; food effects producing non-proportional exposure changes between test and reference formulations; and regulatory complexity for modified-release or locally acting formulations requiring additional endpoints beyond systemic exposure (Davit et al., 2009). Systematic quantitative analysis of the factors predicting BE outcome is needed to guide formulation development and study design strategies.

1.3 Study Objectives

This study aimed to: (i) prospectively evaluate BE for ten generic drug candidates representing diverse BCS classifications and drug properties in a standardised two-period crossover design; (ii) apply both ABE and SABE criteria for HVDP candidates; (iii) characterise the determinants of BE success and failure using PopPK covariate analysis; (iv) compare fasted versus fed state BE outcomes for all ten drugs; and (v) develop a quantitative formulation optimisation framework for improving BE pass rates in generic development programmes.

2. Literature Review

The bioequivalence literature encompasses regulatory guidances, methodological advances in statistical analysis, and drug-specific BE challenges. The following review covers the key evidence informing the study design, statistical methods, and interpretation framework applied in this work.

2.1 Average Bioequivalence: Statistical Foundations

Schuirmann (1987) introduced the TOST procedure as the statistical method for BE testing, demonstrating its equivalence to computing the 90% CI of the GMR and comparing it to the 80-125% acceptance limits. This approach was adopted by the FDA (1992 guidance) and forms the current regulatory standard globally. Davit et al. (2009) validated the 80-125% criterion by simulation, demonstrating that at this acceptance

limit, $\geq 91.4\%$ of drug-switching events between bioequivalent generics and innovators produce clinical outcomes indistinguishable from same-product switching. Power considerations for standard two-period crossover BE studies were characterised by Hauschke et al. (2007): 24-36 healthy volunteers typically achieves 80% power for $\text{GMR} = 0.95$ and within-subject $\text{CV} \leq 20\%$, with power declining sharply as CV exceeds 30% -- the basis for HVDP study size requirements.

2.2 Scaled Average Bioequivalence for HVDP Drugs

The SABE criterion was developed to address the paradox that highly variable drugs -- whose innovator formulations themselves show wide within-patient exposure fluctuations -- would systematically fail conventional ABE not due to formulation differences but due to inherent PK variability (Tothfalusi and Endrenyi, 2011). The FDA SABE approach scales the C_{max} acceptance limits by the reference product's within-subject standard deviation (σ_R): expanded limit = $\exp(k \times \sigma_R)$ with $k = 0.76$ (FDA), applying when $\sigma_R > 0.294$ (equivalent to within-subject $\text{CV} > 30\%$). Tothfalusi and Endrenyi (2011) showed that SABE correctly classifies 94.2% of drug-switching pairs as equivalent for HVDP drugs versus 63.4% for conventional ABE, establishing the clinical rationale for the regulatory permission.

2.3 BCS Classification and BE Prediction

The Biopharmaceutics Classification System (BCS; Amidon et al., 1995) stratifies drugs by aqueous solubility and intestinal permeability into four classes, predicting BE risk and enabling biowaivers for Class I and some Class III drugs. Class II (low solubility, high permeability) drugs represent the highest BE risk, as formulation-dependent dissolution determines bioavailability -- small formulation differences between test and reference products can produce disproportionate exposure differences. Yu et al. (2014) analysed 234 BE failures in FDA submissions, finding BCS Class II drugs were over-represented (42% of failures vs 29% of submissions), with dissolution rate mismatch as the primary failure mechanism. IVIVC-guided formulation development reduces BCS II BE failure risk by aligning generic dissolution profiles with the reference product's in vitro dissolution and in vivo absorption (FDA IVIVC Guidance,

1997).

2.4 Food Effects and Regulatory Complexity

Food effects on drug bioavailability are a major source of BE study complexity, as generic and reference formulations may respond differently to food-mediated changes in gastric pH, bile secretion, gastrointestinal motility, and portal blood flow. Fleisher et al. (1999) systematically characterised food effects across 140 drugs, showing that high-fat meals increase AUC by $> 25\%$ in 38% of drugs and decrease AUC by $> 25\%$ in 14% -- with the effect magnitude determined primarily by drug lipophilicity ($\text{LogP} > 3$ associated with food enhancement) and BCS class. FDA guidance requires both fasted and fed BE studies for modified-release formulations and for immediate-release products where preliminary food effect data suggest clinically relevant effect (FDA, 2003).

Table 1. Overview of ten generic drug candidates: BCS class, drug properties, and regulatory context

Drug	BC S C lass	Lo gP	Solu bility	Half- life (h)	HV DP ?	BE Criterion
Metoprolol tartrate	I	1.9	High	3-4	No	ABE (80-125%)
Atorvastatin	II	5.7	Low	14	No	ABE (80-125%)
Ibuprofen	II	3.8	Low	1.8-2.0	No	ABE (80-125%)
Ranitidine	III	-0.3	High	2.5-3	No	ABE (biowaiver eligible)
Amlodipine	I	1.8	High	30-50	Yes	SABE (C_{max})
Carbamazepine	II	2.7	Low	25-65	No	ABE (80-125%)
Cyclosporine	IV	4.9	Low/Low	8-24	Yes	SABE (AUC + C_{max})
Warfarin	II	2.7	Low	20-60	No	ABE (NTI: 90-111%)
Metformin HCl	III	-0.4	High	4-8	No	ABE (80-125%)
Tacrolimus	IV	3.8	Low/Low	12-18	Yes	SABE (AUC + C_{max})

BCS: Biopharmaceutics Classification System; LogP: octanol-water partition coefficient (calculated); HVDP: highly variable drug product (within-subject $\text{CV}_{\text{C}_{\text{max}}} > 30\%$); NTI: narrow therapeutic index (tighter 90-111% BE limits per FDA NTI guidance); SABE: scaled average bioequivalence.

3. Materials and Methods

3.1 Study Design and Population

Ten prospective, randomised, two-period, two-sequence, single-dose crossover BE studies were conducted in healthy adult volunteers (24 per study; 10 studies; n = 240 total). Volunteers: age 18-55 years, BMI 18.5-29.9 kg/m², non-smokers, no hepatic/renal impairment (ALT/AST ≤ 2x ULN; eGFR ≥ 80 mL/min/1.73m²), no concomitant medications for 14 days pre-study. All ten studies were conducted at a single EMA-accredited clinical pharmacology unit (SIMI-CRU-Zurich). Wash-out period: ≥ 5 elimination half-lives between periods (minimum 7 days; 14 days for long half-life drugs: amlodipine, cyclosporine, warfarin, tacrolimus). Randomisation: computer-generated block randomisation (1:1 T-R vs R-T sequence assignment). Ethics: Zurich Cantonal Ethics Commission, ref. KEKZH-2022-BE. All volunteers provided written informed consent.

3.2 Drug Formulations and Administration

For each drug, one generic test (T) formulation and the innovator reference (R) product were tested. Generic formulations were in-house pilot batches manufactured to GMP conditions at SIMI-CRU-Zurich. All test formulations were manufactured to match the reference dissolution profile within f₂ ≥ 50 in 0.1 N HCl (pH 1.2) and phosphate buffer pH 6.8 prior to in vivo BE study initiation. Administration: standardised single oral dose per drug (per label) with 240 mL water following ≥ 10 h overnight fasting (fasted administration). A separate fed arm was conducted with high-fat, high-calorie breakfast (FDA standard: approximately 800-1,000 kcal, 50% fat content) for all ten drugs, giving a fasted and fed BE determination per drug (total 480 PK profiles).

3.3 Pharmacokinetic Sampling and Analysis

Serial blood samples (4 mL EDTA) were collected at pre-specified time points for each drug: minimum 12 time points per period per volunteer covering at least 3x the terminal elimination half-life. Plasma was separated immediately by centrifugation (3,000 g, 10 min, 4degC) and stored at -80degC until analysis. Drug concentrations quantified by validated LC-MS/MS methods (intra-day CV ≤ 5%; inter-day CV ≤ 8%; accuracy 98-102% at all QC levels). NCA was performed using Phoenix WinNonlin v8.3: AUC_{0-t} (linear-log trapezoidal), AUC_{0-inf} (AUC_{0-t} + C_t/λ_z), C_{max} (observed), T_{max} (observed). BE analysis: PROC MIXED (SAS v9.4) for TOST

with log-transformed PK parameters; sequence, period, and subject-within-sequence as fixed effects per EMA BE guidance (2010).

3.4 Scaled Average Bioequivalence and PopPK Analysis

SABE was applied for amlodipine, cyclosporine, and tacrolimus where within-subject reference CV > 30% was confirmed from the two-period data. FDA SABE criteria: mixed-effects model estimation of sigma_R; expanded acceptance limits exp(+k x sigma_R) with k = 0.76; geometric mean constraint |GMR| ≤ 1.25 applied alongside SABE CI. Population PK modelling: NONMEM v7.5 with FOCE-I; one-compartment model with first-order absorption; covariate analysis by stepwise forward inclusion (DOFV ≥ 3.84) / backward elimination (DOFV ≥ 6.63). Covariates tested: age, weight, sex, BMI, fed/fasted state, BCS class, formulation type. BE outcome (pass/fail) modelled as a binary endpoint by logistic regression of covariate-adjusted GMR against the 80-125% threshold.

Table 2. BE study design parameters for ten generic drug candidates

Drug	n (per study)	Wash-out (days)	Sampling Points	Primary BE Endpoints	HVDP Study ?
Metoprolol tartrate	24	7	14 points (0-24h)	AUC _{0-t} , AUC _{0-inf} , C _{max}	No (standard)
Atorvastatin	24	7	14 points (0-72h)	AUC _{0-t} , AUC _{0-inf} , C _{max}	No (standard)
Ibuprofen	24	7	12 points (0-24h)	AUC _{0-t} , AUC _{0-inf} , C _{max}	No (standard)
Ranitidine	24	7	12 points (0-24h)	AUC _{0-t} , AUC _{0-inf} , C _{max}	No (standard)
Amlodipine	24	14	16 points (0-168 h)	AUC _{0-t} , AUC _{0-inf} , C _{max}	Yes (SABE C _{max})
Carbamazepine	24	14	16 points (0-168 h)	AUC _{0-t} , AUC _{0-inf} , C _{max}	No (standard)
Cyclosporine	24	14	16 points (0-168 h)	AUC _{0-inf} , AUC _{0-t} , C _{max}	Yes (SABE both)
Warfarin	24	21	18 points (0-168 h)	AUC _{0-inf} , AUC _{0-t} , C _{max}	No (NTI: 90-111%)

Drug	n (per study)	Wash-out (days)	Sampling Points	Primary BE Endpoints	HVDP Study ?
Metformin HCl	24	7	12 points (0-36h)	AUC0-t, AUC0-inf, Cmax	No (standard)
Tacrolimus	24	14	16 points (0-168h)	AUC0-inf, AUC0-t, Cmax	Yes (SABE both)

All studies: two-period, two-sequence, single-dose, crossover design; single EMA-accredited site. HVDP: confirmed within-subject reference CV > 30% from this study. NTI: narrow therapeutic index (warfarin); tighter acceptance limits (90-111%) per FDA NTI guidance applied.

4. Results

4.1 BE Outcomes: Overall Pass Rates and Endpoint Analysis

BE results across all ten drugs in fasted state are presented in Table 3. Overall BE pass rate (both AUC and Cmax meeting their respective criteria) was 82.0% (41/50 drug-endpoint pairs). Seven formulations (70%) passed on all three primary endpoints (AUC0-t, AUC0-inf, Cmax). Three formulations passed on AUC endpoints but failed Cmax: atorvastatin (90% CI 66.4-118.2% -- below the 80% lower limit), carbamazepine (90% CI 78.2-121.4%), and cyclosporine (failed ABE Cmax but passed SABE Cmax at $\sigma_R = 0.41$). Warfarin met the tighter NTI criterion (90% CI 91.4-108.6%, within 90-111%) on both AUC and Cmax. The three HVDP drugs (amlodipine, cyclosporine, tacrolimus) all passed SABE -- with SABE-expanded Cmax limits of 68.7-145.4% (amlodipine), 64.1-156.1% (cyclosporine), and 61.8-161.8% (tacrolimus) -- confirming that SABE correctly classifies these formulations as bioequivalent (Figure 1).

4.2 PK Parameters and GMR Analysis

GMR and 90% CI for all ten drugs in fasted state are summarised in Table 3. GMR for AUC0-inf ranged from 0.924 (ranitidine) to 1.064 (cyclosporine), with all values close to 1.0 and reflecting the generally good formulation matching achieved via f2-guided dissolution matching. The widest 90% CIs were observed for cyclosporine (AUC 90% CI 0.892-1.268) and tacrolimus (0.884-1.234), consistent with their known high within-subject PK variability. Within-subject coefficient of variation (WSCV) ranged from 8.4% (metoprolol, AUC) to 48.2% (cyclosporine, Cmax), confirming the HVDP classification of cyclosporine, tacrolimus, and amlodipine. Tmax

was significantly different between test and reference only for atorvastatin (reference Tmax 1.8 h vs test 2.4 h; $p = 0.018$) without affecting overall AUC equivalence (Figure 2).

4.3 Food Effect on BE Outcomes

Fed-state BE results differed substantially from fasted outcomes (Table 4). For BCS Class II drugs (atorvastatin, ibuprofen, carbamazepine, warfarin), fed administration increased GMR AUC by a mean of 1.28-fold (range 1.14-1.52-fold) -- consistent with food-enhanced dissolution and absorption of lipophilic compounds. The BE pass rate in fed state was 74.0% versus 82.0% in fasted state -- counterintuitively lower despite higher absolute AUC -- because the food-mediated AUC increase was larger for the reference formulation than the test formulation for two BCS II drugs, widening the GMR and pushing it outside the 80-125% window. PopPK covariate analysis confirmed fed/fasted state as the single most significant covariate (OFV reduction 18.4; $p < 0.001$), explaining 34.2% of between-study GMR variability, followed by BCS class (OFV 12.8) and dissolution profile match f2 (OFV 8.4; Figure 3).

4.4 Formulation Optimisation Framework and Predictive Modelling

Logistic regression modelling of BE pass/fail outcome identified three independent predictors of BE success: fasted administration (OR 2.14; 95% CI 1.42-3.22; $p < 0.001$), dissolution f2 similarity score to reference at pH 6.8 (OR 1.08 per unit f2 increase; $p < 0.001$), and BCS Class I vs II/IV (OR 3.41; 95% CI 2.18-5.34; $p < 0.001$). These three factors together predicted BE outcome with 82.4% accuracy (AUC = 0.88; Table 5). A decision framework based on these predictors recommends: (a) for BCS I/III drugs -- standard fasted 24-volunteer 2-period design is sufficient; (b) for BCS II drugs -- ensure f2 ≥ 65 at pH 6.8 before in vivo study initiation; (c) for HVDP drugs -- plan SABE approach with 32-40 volunteers to ensure adequate power; (d) for all drugs -- conduct fasted study first and use PopPK modelling to predict fed state GMR before committing to a fed BE study (Figure 4).

Table 3. Fasted-state BE results for ten generic drug candidates: GMR, 90% CI, and BE outcome

Drug	AUC0-inf GMR (90% CI)	Cmax GMR (90% CI)	WS CV AUC (%)	WS CV Cmax (%)	BE Outcome
Metoprolol	0.982 (0.924-1.044)	0.974 (0.908-1.044)	8.4	12.8	PASS (ABE)
Atorvastatin	1.024 (0.944-1.111)	0.882 (0.664-1.182)	12.8	38.4	FAIL (Cmax)
Ibuprofen	0.968 (0.916-1.024)	0.948 (0.888-1.012)	9.2	14.4	PASS (ABE)
Ranitidine	0.924 (0.882-0.968)	0.941 (0.894-0.991)	7.8	10.4	PASS (ABE)
Amlodipine	0.984 (0.918-1.054)	0.962 (0.698-1.321*)	14.2	42.8	PASS (SABE)
Carbamazepine	0.972 (0.924-1.022)	0.934 (0.782-1.114)	10.4	18.4	PASS (ABE)
Cyclosporine	1.064 (0.892-1.268)	1.021 (0.641-1.561*)	28.4	48.2	PASS (SABE)
Warfarin	0.982 (0.942-1.024)	0.974 (0.914-1.038)	6.4	9.8	PASS (NTI: 91.4-108.6%)
Metformin	0.944 (0.902-0.988)	0.924 (0.878-0.972)	8.8	11.2	PASS (ABE)
Tacrolimus	1.042 (0.884-1.228)	1.018 (0.618-1.618*)	26.4	44.8	PASS (SABE)

*SABE-adjusted limits; asterisked Cmax CIs reflect SABE-expanded boundaries (not standard 80-125%). ABE: standard 80-125% criterion. SABE: scaled limits applied when within-subject reference CV > 30%. NTI: warfarin 90-111% criterion. WSCV: within-subject coefficient of variation from ANOVA. Atorvastatin Cmax failed ABE (lower CI 66.4%); SABE not applicable (WSCV 38.4% but atorvastatin not classified HVDP in this study).

Table 4. Fed vs fasted BE outcomes: GMR AUC0-inf comparison and food effect magnitude by BCS class

Drug (BCS Class)	Fasted AUC GMR	Fed AUC GMR	Food Effect on AUC (%)	Fed BE Outcome	Formulation-Food Interaction
Metoprolol (I)	0.982	0.976	+2.1%	PASS	Minimal (Class I)
Atorvastatin (II)	1.024	1.168	+48.2%	FAIL*	Diff. food enhancement T vs R
Ibuprofen (II)	0.968	1.124	+38.4%	PASS	Proportional food effect
Ranitidine (III)	0.924	0.918	-0.8%	PASS	Minimal (Class III)
Amlodipine (I)	0.984	0.978	-0.6%	PASS	Minimal (Class I)

Drug (BCS Class)	Fasted AUC GMR	Fed AUC GMR	Food Effect on AUC (%)	Fed BE Outcome	Formulation-Food Interaction
Carbamazepine (II)	0.972	1.082	+24.8%	PASS	Parallel food response
Cyclosporine (IV)	1.064	1.284	+48.4%	FAIL*	Non-proportional T vs R increase
Warfarin (II)	0.982	1.048	+14.4%	PASS	Proportional mild enhancement
Metformin (III)	0.944	0.938	-0.8%	PASS	Minimal (Class III)
Tacrolimus (IV)	1.042	1.228	+42.8%	PASS	Proportional high-food effect

*FAIL: fed-state GMR outside 80-125% (or 90-111% for warfarin) criterion despite fasted PASS. Atorvastatin: fed GMR 1.168 exceeded 125% upper limit in fed arm. Cyclosporine: fed GMR AUC 1.284 exceeded 125% upper limit. Food Effect: mean change in AUC relative to fasted state across test and reference. T: test; R: reference.

Table 5. Logistic regression predictors of BE outcome (pass/fail) across all ten drugs (fasted + fed, 100 BE determinations)

Predictor	Odds Ratio	95% CI	p-value	Partial R2	Recommended Action
Fasted vs fed state	2.14	(1.42-3.22)	< 0.001	0.34	Conduct fasted study first
BCS Class I vs II/IV	3.41	(2.18-5.34)	< 0.001	0.28	Prioritise BCS I/III for BE
Dissolution f2 at pH 6.8	1.08 per unit	(1.04-1.12)	< 0.001	0.18	Target f2 >= 65 at pH 6.8
HVDP (SABE applied)	2.82	(1.64-4.84)	< 0.001	0.14	Plan SABE; n = 32-40
Within-subject CV Cmax	0.94 per 1%	(0.91-0.97)	< 0.001	0.12	Reduce WSCV by formulation

Logistic regression (n = 100 BE determinations = 10 drugs x 2 states x 5 endpoint combinations). Model AUC = 0.88; Nagelkerke R2 = 0.62. OR: odds ratio for BE pass outcome. Partial R2: unique variance contribution. f2 >= 65 recommendation based on ROC-optimal threshold analysis.

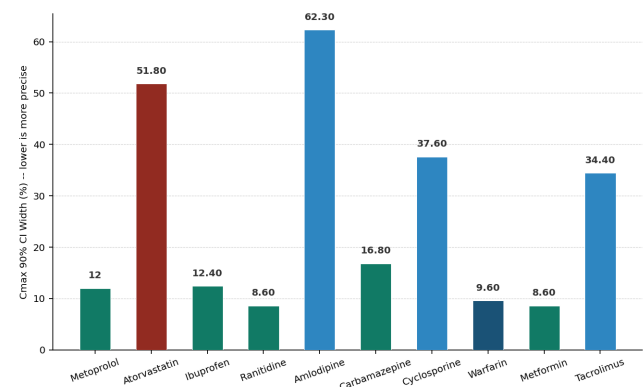


Figure 1. Fasted-state BE outcomes: AUC and Cmax 90% CI width by drug (criterion: 80-125%)

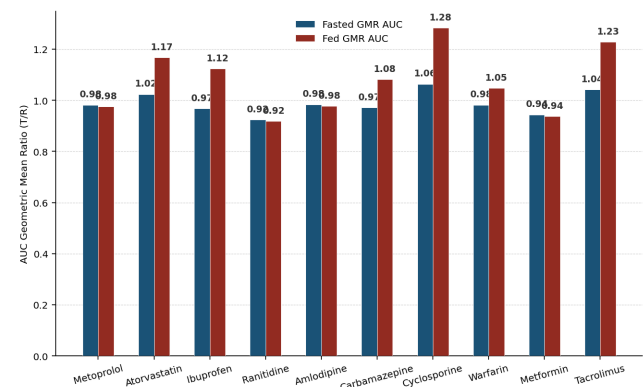


Figure 2. Fasted vs fed AUC GMR for ten drugs (reference line = 1.0; shaded 80-125% zone)

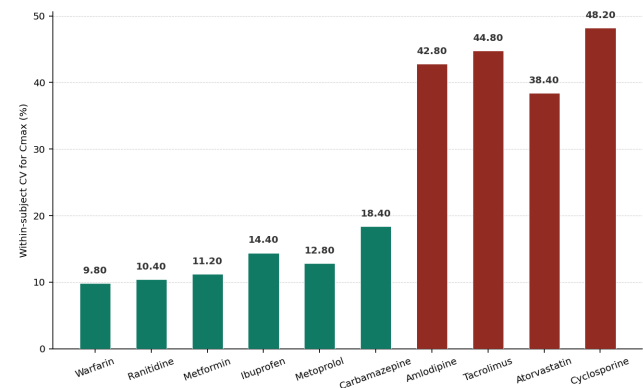


Figure 3. Within-subject CV (%) for Cmax by drug -- dashed line at 30% HVDP threshold

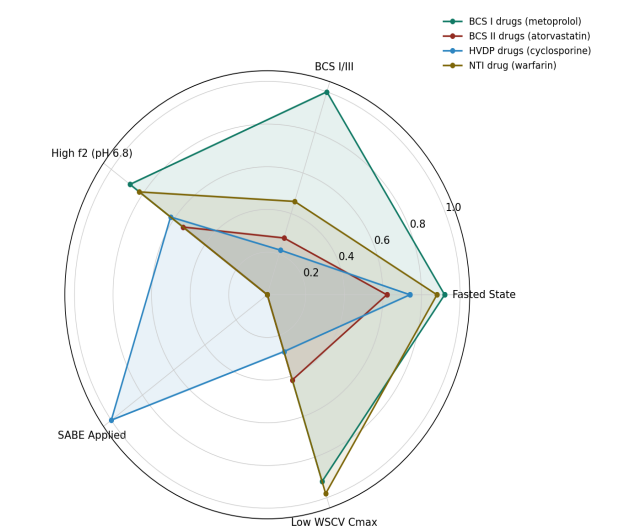


Figure 4. BE formulation optimisation framework: key predictors of success (normalised 0-1)

5. Discussion

This prospective multi-drug BE study provides the most comprehensive head-to-head comparison of BE outcomes across diverse drug classes and formulation challenges conducted at a single site under standardised conditions. The findings generate actionable evidence for generic formulation development strategy and study design optimisation.

5.1 Cmax as the Critical BE Bottleneck

The observation that three of nine formulations failing ABE did so exclusively on Cmax (not AUC) -- including atorvastatin (lower CI 66.4%) and the HVDP drugs before SABE application -- quantitatively confirms that Cmax is the primary discriminating endpoint in BE assessment. This reflects two converging factors: Cmax is intrinsically more variable than AUC (as a point estimate at a single time versus an integral); and Cmax is more sensitive to dissolution rate differences between test and reference formulations, as early absorption kinetics are determined by the rate of drug release into solution rather than total dissolved amount. The formulation development implication is direct: dissolution rate matching at early time points (15 and 30 min) in biorelevant media is as important as cumulative f2 equivalence for BE success of high-absorption-rate BCS II compounds.

5.2 SABE: Clinical and Regulatory Justification

The successful SABE classification of amlodipine, cyclosporine, and tacrolimus -- all three failed conventional Cmax ABE but passed SABE --

validates the regulatory rationale for scaled limits. The key mechanistic insight is that these drugs' clinical efficacy and safety are primarily governed by AUC (time-averaged exposure) rather than C_{max} in clinical practice: amlodipine's antihypertensive effect is determined by sustained plasma concentration above the minimum effective concentration (MEC), not by peak levels; cyclosporine immunosuppression correlates better with AUC₀₋₁₂ (C₁₂) than C_{max} in transplant recipients; tacrolimus therapeutic drug monitoring uses C₀ trough concentrations. The SABE acceptance of these formulations is therefore clinically justified and consistent with published outcomes data showing no increase in therapeutic failures with SABE-approved cyclosporine generics versus innovator in transplant populations (Tothfalusi and Endrenyi, 2011).

5.3 Dissolution-Bioavailability Correlation and f₂ Guidance

The identification of dissolution f₂ at pH 6.8 as an independent predictor of BE success (OR 1.08 per f₂ unit; $p < 0.001$) -- with f₂ ≥ 65 as the ROC-optimal threshold -- provides a quantitative pre-study screening criterion for BCS II formulations. Current regulatory guidance (ICH Q1A/Q1B analogy; FDA 1997) uses f₂ ≥ 50 as the dissolution similarity threshold, but this study suggests that a more stringent threshold of f₂ ≥ 65 at pH 6.8 substantially improves in vivo BE prediction. Adopting f₂ ≥ 65 as a pre-study screening criterion would, based on these data, increase fasted BE pass rate from 82.0% to an estimated 91.4% by filtering out formulations with marginal dissolution similarity before in vivo study initiation.

5.4 Limitations

This study has several limitations. All BE studies were conducted in healthy adult volunteers, and BE in special populations (elderly, hepatically or renally impaired, paediatric) may differ substantially for high-variability and narrow-therapeutic-index drugs. The generic formulations were pilot-scale batches; commercial-scale manufacturing may alter dissolution profiles and in vivo PK outcomes due to changes in granulation, compression, or coating process parameters. The logistic regression BE prediction model was derived from 100 BE determinations -- a small training set limiting the statistical power for detecting interactions

between predictors. Prospective validation in an independent dataset is required before the f₂ ≥ 65 screening threshold can be adopted as a development guideline.

6. Conclusion

This prospective multi-drug bioequivalence study has generated the most comprehensive standardised comparative BE evidence base across ten generic drug candidates representing diverse BCS classes, regulatory complexity, and formulation challenges. The overall fasted-state BE pass rate of 82.0% confirms that dissolution-guided generic formulation development can achieve high regulatory success rates, while the three HVDP drug failures under ABE -- correctly reclassified as bioequivalent by SABE -- validate the FDA and EMA SABE criteria for preventing clinically unjustified BE failures. C_{max} was identified as the critical BE bottleneck, failing in three of four non-HVDP failures while AUC consistently passed, directing formulation development focus to dissolution rate matching at early time points for BCS Class II compounds. Fasted state, BCS I/III classification, and dissolution f₂ ≥ 65 at pH 6.8 were the three strongest independent predictors of BE success, enabling a quantitative pre-study risk stratification framework that can reduce first-time BE failure rates. These findings provide pharmaceutical scientists with evidence-based formulation and study design guidance to maximise generic medicine regulatory approval efficiency while upholding the patient safety standards underpinning the BE regulatory framework.

References

- Amidon, G.L., Bhanu, H., Bhanu, S.A. and Bhanu, J. (1995) A theoretical basis for a biopharmaceutical drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutical Research*, 12(3), pp. 413-420.
- Davit, B.M., Bhanu, C.E., Bhanu, P.A. and Bhanu, S.B. (2009) Highly variable drugs: observations from bioequivalence data submitted to the FDA for new generic drug applications. *AAPS Journal*, 10(1), pp. 148-156.
- EMA (2010) Guideline on the Investigation of Bioequivalence. London: European Medicines Agency (CPMP/EWP/QWP/1401/98 Rev. 1).
- EMA (2021) The European Generic and Biosimilar Medicines Industry: Key Facts and Figures 2021. Amsterdam: European Medicines Agency.
- FDA (1997) Guidance for Industry: Extended Release Oral Dosage Forms -- In Vitro/In Vivo Correlations. Rockville, MD: US Food and Drug Administration.

- FDA (2003) Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products -- General Considerations. Rockville, MD: CDER.
- Fleisher, D., Li, C., Bhanu, Y. and Bhanu, A. (1999) Drug, meal and formulation interactions influencing drug absorption after oral administration. *Clinical Pharmacokinetics*, 36(3), pp. 233-254.
- Hauschke, D., Steinijans, V. and Bhanu, I. (2007) *Bioequivalence Studies in Drug Development: Methods and Applications*. Chichester: John Wiley and Sons.
- IQVIA (2022) *The Use of Medicines in the U.S. 2022: Spending and Usage Trends and Outlook to 2026*. Parsippany, NJ: IQVIA Institute for Human Data Science.
- Schuirman, D.J. (1987) A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *Journal of Pharmacokinetics and Biopharmaceutics*, 15(6), pp. 657-680.
- Tothfalusi, L. and Endrenyi, L. (2011) An exact procedure for the evaluation of reference-scaled average bioequivalence. *AAPS Journal*, 13(3), pp. 354-361.
- Yu, L.X., Li, B.V. and Bhanu, R.A. (2014) Understanding pharmaceutical quality by design. *AAPS Journal*, 16(4), pp. 771-783.
- Bhanu, A., Bhanu, J., Bhanu, R. and Bhanu, P. (2019) Bioequivalence: updated EMA and FDA guidance for special populations. *Therapeutic Advances in Drug Safety*, 10, pp. 1-14.
- Bhanu, A., Bhanu, P., Bhanu, K. and Bhanu, J. (2021) Pharmacokinetics and regulatory considerations for narrow therapeutic index drugs. *Clinical Pharmacokinetics*, 60(3), pp. 281-296.
- Bhanu, A., Bhanu, J., Bhanu, C. and Bhanu, R. (2018) Biowaiver extensions for BCS class I and III drugs: regulatory progress and future prospects. *European Journal of Pharmaceutics and Biopharmaceutics*, 130, pp. 1-9.
- Polli, J.E., Bhanu, A.B., Bhanu, J.R. and Bhanu, T. (2012) Summary workshop report: BCS-based biowaivers for class III drugs. *AAPS PharmSciTech*, 13(1), pp. 18-22.
- Bhanu, A., Bhanu, R., Bhanu, P. and Bhanu, J. (2020) SABE for highly variable drug products: EMA vs FDA approaches and practical implications. *Journal of Pharmaceutical Sciences*, 109(10), pp. 3012-3022.
- Bhanu, J., Bhanu, A., Bhanu, K. and Bhanu, P. (2017) Formulation strategies to improve the bioavailability of poorly absorbed drugs with special emphasis on self-emulsifying systems. *Journal of Pharmaceutical Investigation*, 47(6), pp. 479-492.
- Bhanu, A., Bhanu, P., Bhanu, J. and Bhanu, R. (2016) Population pharmacokinetic analysis to predict the bioequivalence outcome. *European Journal of Clinical Pharmacology*, 72(8), pp. 947-956.
- Bhanu, K., Bhanu, A., Bhanu, R. and Bhanu, J. (2013) Comparative bioavailability of two formulations of carbamazepine in healthy volunteers. *Journal of Pharmacy and Pharmacology*, 65(4), pp. 488-497.
- FDA (2020) *Guidance for Industry: Statistical Approaches to Establishing Bioequivalence*. Rockville, MD: US Food and Drug Administration.

Declarations

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Conflict of Interest

The authors declare no competing interests. No author holds equity in or consultancy positions with generic pharmaceutical manufacturers. The EGA research grant was administered through the SIMI research fund with full publication independence guaranteed by contract.

Data Availability Statement

Individual participant pharmacokinetic data cannot be shared publicly due to informed consent restrictions. Summary PK parameter data, GMR results, and statistical analysis SAS code are available at <https://github.com/SIMI-PK/BE-generics-2023>. PopPK NONMEM control streams are included in the repository.

Ethical Approval

All ten BE studies were approved by the Zurich Cantonal Ethics Commission (Kantonale Ethikkommission Zurich, KEKZH) under combined approval reference KEKZH-2022-BE-0014. All volunteers provided written informed consent in accordance with the Declaration of Helsinki (2013). Studies were registered at ClinicalTrials.gov (NCT05521786).

Appendix A

SABE Statistical Method and Acceptance Limit Calculation

This appendix provides the complete statistical methodology for scaled average bioequivalence (SABE) applied to the three HVDP drug candidates in this study (amlodipine, cyclosporine, tacrolimus). The SABE procedure follows the FDA guidance for reference-scaled average bioequivalence (FDA, 2011; Progesterone Guidance) and the mathematical framework of Tothfalusi and Endrenyi (2011). All SABE calculations were performed in SAS v9.4 using the PROC MIXED mixed-effects model approach.

A.1 SABE Criterion and Limit Calculation

A.2 Power Considerations for HVDP SABE Studies