

Unit 4: Drug Product Performance, In Vivo: Bioavailability and Bioequivalence

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4.1 Drug product performance in vivo and the purpose of bioavailability studies

Unit 2 measured performance in the laboratory; this unit measures it in the body. The plasma concentration time curve after a dose of a product is its in vivo performance fingerprint: its height (C_{max}) reflects the rate of absorption, its area (AUC) the extent, and its timing (T_{max}) links the two. **Bioavailability** is the formal quantity: the rate and extent to which the active drug is absorbed from the product and becomes available in the systemic circulation. Bioavailability studies establish the absolute performance of a new drug (against an intravenous dose), compare formulations during development, prove that a changed or generic product performs like the established one (bioequivalence, the bulk of this unit), study food effects and interactions in vivo, and support modified release design.

4.2 Absolute and relative bioavailability

Absolute bioavailability (F) compares the product with an intravenous dose of the same drug, intravenous administration being by definition 100 percent available:

$$F = (\text{AUC oral} / \text{Dose oral}) / (\text{AUC IV} / \text{Dose IV})$$

If the doses are equal, F is simply AUC oral / AUC IV. F must lie between 0 and 1; a value above 1 signals an error in the study or the assay.

Relative bioavailability compares one product or formulation with another non intravenous reference (a standard formulation, a solution, or the innovator product):

$$\text{Relative BA} = (\text{AUC test} / \text{Dose test}) / (\text{AUC reference} / \text{Dose reference})$$

Worked example. A 100 mg tablet gives AUC = 480 mg·h/L; a 100 mg intravenous dose gives AUC = 600 mg·h/L. Absolute F = 480 / 600 = 0.80: eighty percent of the dose reached the systemic circulation, the missing fifth being incomplete absorption and first pass metabolism. A reformulated tablet giving AUC = 460 mg·h/L has relative bioavailability 460 / 480 = 0.958, or 95.8 percent of the reference. Whether 95.8 percent is close enough is decided by the bioequivalence statistics of section 4.4.

4.3 Methods for assessing bioavailability

- **Plasma level studies.** The standard method: serial blood samples after a single dose in healthy volunteers, assay of drug (and active metabolites where relevant), and calculation of AUC, C_{max} and T_{max}. AUC is measured to the last quantifiable concentration and extrapolated to infinity using the terminal rate constant, using sensitive, specific assays (usually LC-MS/MS).
- **Urinary excretion studies.** Used when plasma assay is difficult or the drug acts in the urinary tract: cumulative urinary excretion of unchanged drug is proportional to the amount absorbed, and the excretion rate profile reflects the absorption rate. It demands complete, timed collections and a drug excreted unchanged to a useful extent.

- **Pharmacodynamic (acute response) methods.** Where concentration cannot be measured, a quantifiable effect (blood pressure, blood glucose, pupil size) can stand in if precisely measured and related to concentration; inherently noisier, and a last resort.
- **In vitro methods as surrogates.** Dissolution (Unit 2) does not measure bioavailability, but with a validated IVIVC or under a BCS biowaiver (section 4.8) it can replace the in vivo study for defined cases.

Single dose studies in healthy volunteers are the norm, being the most sensitive design for detecting formulation differences; patient and multiple dose studies enter where safety or ethics require them.

4.4 Bioequivalence: the concept and the statistical rule

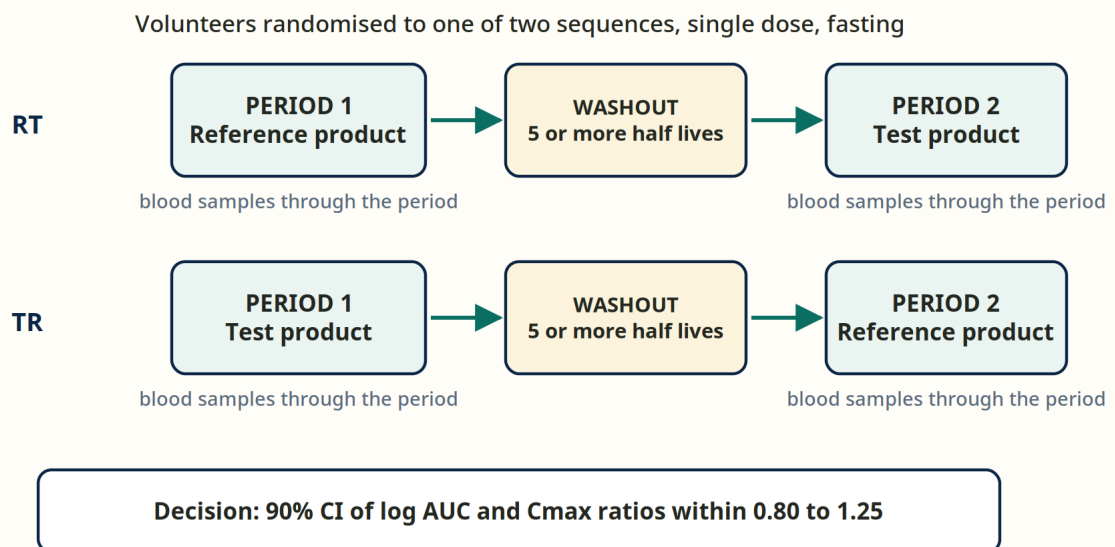
Two products are **bioequivalent** when the rate and extent of absorption of the active ingredient from them are not significantly different, so that they can be expected to be therapeutically equivalent and interchangeable. The comparison is made on log transformed AUC (extent) and C_{max} (rate), because pharmacokinetic data are log normally distributed and the analysis works on ratios. The decision rule used by regulators worldwide is:

The 90 percent confidence interval for the ratio of the geometric means (test / reference) of AUC and of C_{max} must lie entirely within **0.80 to 1.25** (that is, 80.00 to 125.00 percent).

The interval is asymmetric on the ordinary scale because it is symmetric on the log scale ($\log 0.80 = \text{minus } \log 1.25$), which is why the famous limits are 80 and 125 rather than 80 and 120. Two features deserve emphasis. First, it is the whole confidence interval that must fit inside the limits, not the point estimate: a study with a perfect mean ratio of 1.00 but wide variability can fail, so the study must be powered adequately, in practice enough subjects for the drug's within subject variability (commonly 12 to 36, more for highly variable drugs). Second, T_{max} is usually compared descriptively, because it is a discrete sampled quantity. Narrow therapeutic index drugs are held to tighter limits by several regulators, and highly variable drugs may use widened C_{max} limits under defined conditions; the 80 to 125 percent rule on log transformed data remains the reference standard used in section 4.6.

4.5 Design and evaluation of bioequivalence studies

Bioequivalence: Two Period Crossover Design



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Figure: The standard 2 x 2 crossover. Each volunteer receives both products in randomised order, so each subject is their own control and the comparison is made within subjects, which is what gives the design its power.

The standard design is the **single dose, two period, two sequence crossover** in healthy volunteers under fasting conditions. Volunteers are randomised to sequence RT (reference, then test) or sequence TR (the reverse). The periods are separated by a **washout** of at least five half lives, so no drug carries over. Blood samples are taken on a schedule dense around Tmax and long enough to characterise AUC (sampled AUC should cover at least 80 percent of the extrapolated total). The crossover is chosen because within subject variability is much smaller than between subject variability, so far fewer subjects are needed than in a parallel design; its analysis (ANOVA on log transformed data) separates formulation, period, sequence and subject effects. Other designs have defined places: **parallel designs** for drugs with very long half lives; **replicate designs** (each product given twice) for highly variable drugs, which measure the reference's own within subject variability and support scaled acceptance limits; **fed studies** alongside fasting ones where food affects the product or labelling recommends dosing with food; and **multiple dose studies** where single dose data cannot represent use, now uncommon. Standardisation is part of the design: fasting state, water volume, posture, activity, diet and sampling times are all fixed by protocol, so the study exposes formulation differences, not lifestyle differences.

4.6 A bioequivalence example, evaluated

Worked example. A 24 volunteer fasting crossover study compares a generic tablet (test) with the innovator tablet (reference), 100 mg. Log transformed analysis gives: AUC geometric mean ratio = 1.02, 90 percent confidence interval 0.95 to 1.10; Cmax ratio = 0.98, interval 0.92 to 1.08; median Tmax 2.0 hours for both.

Judgement: the AUC interval (0.95 to 1.10) lies entirely within 0.80 to 1.25, and so does the Cmax interval (0.92 to 1.08); Tmax is the same. The products are bioequivalent and the generic may be approved as substitutable. Contrast the near miss: had the Cmax interval been 0.78 to 1.05, the study would fail on Cmax even though the point estimate (about 0.90) looks acceptable, because the lower bound falls outside 0.80. The interval, not the average, carries the decision.

4.7 Study submission and the drug review process

A bioequivalence study is a regulated document, not just an experiment. The sponsor submits the full study report (protocol and ethics approval, clinical and analytical reports, individual subject data, the statistical analysis, and certificates for the test and reference products including batch numbers, potency and dissolution profiles) within the marketing application, in India to the Central Drugs Standard Control Organisation (CDSCO) under the Drugs and Cosmetics Act and the New Drugs and Clinical Trials Rules, and elsewhere within an abbreviated application relying on the innovator's data. Reviewers check the design against the guideline (subjects, washout, sampling, assay validation, handling of dropouts and outliers), may audit the clinical and bioanalytical sites, and can require the raw data. Approval of the application is approval of the study: the generic is licensed as bioequivalent; section 4.8 defines the cases where the study itself may be omitted.

4.8 The biopharmaceutics classification system (BCS)

The BCS classifies drugs by aqueous **solubility** and intestinal **permeability**, each high or low against defined criteria (highly soluble: highest dose dissolves in 250 mL or less across the physiological pH range; highly permeable: absorption of 85 percent or more). The four classes:

Class	Solubility	Permeability	Behaviour and examples
I	High	High	Rapid, complete absorption; gastric emptying often rate limiting. Examples: propranolol, metoprolol, paracetamol

Class	Solubility	Permeability	Behaviour and examples
II	Low	High	Dissolution rate limiting; bioavailability improved by particle size reduction, solubilisation. Examples: carbamazepine, glibenclamide, ketoconazole
III	High	Low	Permeation rate limiting; absorption variable, transporter sensitive. Examples: atenolol, cimetidine, metformin
IV	Low	Low	Both steps limiting; poor and variable bioavailability. Examples: furosemide, hydrochlorothiazide

The regulatory application is the **biowaiver**: for immediate release products of class I drugs (and, under current harmonised guidance, defined class III cases) dissolving very rapidly, an in vivo bioequivalence study may be waived on dissolution data, because the dosage form cannot plausibly change absorption controlled by gastric emptying. **Methods** behind the classification are the solubility determination across pH and the permeability determination of section 4.9.

4.9 Permeability: in vitro, in situ and in vivo methods

- **In vitro methods.** Cell monolayers grown on filter inserts: **Caco-2** cells (human colon carcinoma cells differentiating into enterocyte like cells with transporters and tight junctions) are the standard, giving apparent permeability in both directions, whose ratio exposes efflux. **PAMPA** (parallel artificial membrane permeability assay) measures passive permeability only, through a lipid impregnated filter, at high throughput. Excised intestinal tissue in diffusion (Ussing) chambers keeps the tissue architecture intact.
- **In situ methods.** The **single pass intestinal perfusion** in anaesthetised animals perfuses a defined intestinal segment at a known rate and calculates effective permeability from the disappearance rate, with blood supply and nerves intact. The closed loop variant injects drug into a tied off segment and measures what remains.
- **In vivo methods.** Human absorption is estimated from absolute bioavailability corrected for first pass, from urinary recovery, from radiolabel mass balance studies, or from marker compounds: metoprolol is the classic high permeability reference, atenolol or mannitol the low permeability references.

4.10 Generic biologics: biosimilar drug products

A biological drug (a protein made by living cells, Unit 5) cannot be copied exactly: the manufacturing process defines the product, and a second manufacturer's version will differ in detail however carefully made. Such follow on products are called **biosimilars**, not generics, and are not shown bioequivalent by the plasma curve method of this unit, because blood levels of a protein do not capture its structure or immunogenicity. Approval instead rests on a stepwise comparability exercise: analytical and structural comparison with the reference biologic, functional assays, comparative pharmacokinetic and pharmacodynamic studies where a marker exists, a comparative clinical study in a sensitive indication, and immunogenicity assessment, the totality of evidence substituting for identity.

4.11 Clinical significance, special concerns and generic substitution

Bioequivalence is a statistical statement about average behaviour in a study population, and its clinical use needs qualifications. **Clinical significance**: an approved bioequivalent generic can be substituted for the innovator with confidence for the great majority of drugs, because the 80 to 125 percent interval, on top of manufacturing controls, keeps exposure differences within the variation patients already experience between doses, days and meals. **Special concerns** arise for narrow therapeutic index drugs (warfarin,

phenytoin, ciclosporin, levothyroxine), where small exposure changes matter and regulators apply tighter limits and clinicians monitor levels after a switch; for modified release products, whose profiles must match at several points, not only in AUC and Cmax; for drugs with nonlinear kinetics, where equivalence at one dose does not automatically prove it at another; and for patient factors (age, disease, interacting medicines) no volunteer study reproduces. **Generic substitution**, dispensing an approved generic in place of the prescribed brand, is the everyday consequence of the system: it lowers cost and widens access, and its safety rests on the study, the statistics, the review and manufacturing quality. Substitution between two different generics, each proven against the innovator but not against each other, is acceptable in the same framework because both are anchored to the same reference; repeated switching for narrow index drugs is nevertheless discouraged.

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