Notes on the Design of Bioequivalence Study:
Amoxicillin

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Unit – Medicines Assessment Team (PQT/MED) are issued to aid manufacturers with the development of their product dossier. Deviations from the approach suggested below can be considered acceptable if justified by sound scientific evidence.


Below, additional specific guidance is provided on the invited products, containing amoxicillin 125 mg and 250 mg scored tablets.

**Pharmacokinetics of amoxicillin**

Amoxicillin is stable in the gastric acid secretion and is rapidly absorbed from the gastrointestinal tract after oral administration. The time to peak plasma concentration ($T_{\text{max}}$) is approximately one hour.

In the range 250 to 3000 mg the bioavailability is linear in proportion to dose (measured as $C_{\text{max}}$ and AUC). The absorption is not influenced by simultaneous food intake.

Amoxicillin has a mean elimination half-life of approximately one hour.

**Guidance for the design of bioequivalence studies:**

Taking into account the pharmacokinetic properties of amoxicillin, the following guidance with regard to the study design should be taken into account:

**Study design:** A cross-over design is recommended.

**Dose:** As the EoI includes two strengths: 125 and 250 mg (scored) tablets, the highest strength should be used in the bioequivalence study if the conditions for a biowaiver for the low strength are fulfilled. Otherwise both strengths should be tested.

**Fasting/fed:** The bioequivalence study should be conducted in the fasting state.

For administration, the test product should be dispersed in a small amount of liquid suitable for the intended paediatric population (e.g. 20 – 40 ml) and a similar small amount of water should be used to rinse the container. Additional water should not be administered in order to mimic the real conditions of use. The method of administration should be consistent with the proposed labelling for the product.

The reference product should be administered in a fashion consistent with its labelling, using a volume of water appropriate for a pediatric population. For example, for Amoxil/Clamoxyl powder for oral suspension in sachet it is indicated that the content of the sachet be suspended in 10 – 20 ml of water and administered. It is acceptable to rinse the container with an additional similar volume of water but, additional water beyond that should not be employed.
**Subjects:** Healthy adult subjects should be recruited. It is not necessary to include patients in the bioequivalence study.

**Sample size:** Based on the information available to PQT, amoxicillin $C_{\text{max}}$ exhibits low to moderate intra-subject variability (10-20%), whereas $\text{AUC}_{0-t}$ exhibits low variability (10-13%) in the fasting state. These data will facilitate the calculation of a sufficient sample size for the bioequivalence study.

**Washout:** Seven (7) days.

**Blood sampling:** Pre-dose, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.0, 7.00, and 8.00 h after drug administration.

**Analytical method:** Information currently available indicates that it is possible to measure amoxicillin in human plasma using LC-MS/MS analytical methodology. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the $C_{\text{max}}$ in most profiles of each formulation (test or comparator).

**Parent or metabolite data for assessment of bioequivalence:** The parent drug is considered to best reflect the biopharmaceutical quality of the proposed product. The data for the parent compound should be used to assess bioequivalence.

**Statistical considerations:** The data for amoxicillin should meet the following bioequivalence standards in a single-dose cross-over design study:

- The 90% confidence interval of the relative mean $\text{AUC}_{0-t}$ of the test to reference product should be within 80–125%.

- The 90% confidence interval of the relative mean $C_{\text{max}}$ of the test to reference product should be within 80–125%.