Notes on the Design of Bioequivalence Study: Azithromycin

Notes on the design of bioequivalence studies with products invited to be submitted 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.

The current notes should be read and followed in line with the general guidelines of submission of documentation for WHO prequalification. In particular, please consult the "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability" in: *Fifty-seventh report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations,* Geneva, World Health Organization, 2024. WHO Technical Report Series, No. 1052, Annex 8.

Below, additional specific guidance is provided on the invited immediate release products containing azithromycin.

Pharmacokinetics of azithromycin

Peak plasma concentrations are attained 2 to 3 hours after taking the medicinal product. The terminal plasma elimination half-life closely reflects the elimination half-life from tissues of 2 – 4 days. Azithromycin tablet and suspension can be taken irrespective of meals.

Guidance for the design of bioequivalence studies

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

Design: A crossover design is recommended.

Dose: The EoI for treatment of neglected tropical diseases includes azithromycin 500 mg tablet. The bioequivalence study for this product should be conducted with this strength and dose.

The EoI for treatment of bacterial infections in children includes azithromycin orodispersible multiparticulates (minitablets or sprinkles) 50 mg per unit dose or scored dispersible tablet 100 mg, as preferred; and dispersible tablets 50 mg as alternative, the corresponding dose of the comparator suspension should be administered to match the strength of the test product (50 mg for minitablets or sprinkles / 50 mg for dispersible tablets and 100 mg for the scored dispersible tablet). The requirement for a bioequivalence study with the 50 mg dispersible tablet could be waived if the conditions for an additional strength biowaiver with respect to the 100 mg scored dispersible tablet are fulfilled.

The comparator product should be administered in a fashion consistent with its labelling. It is acceptable to rinse the container with an additional volume of water (e.g., 10 mL) but additional water beyond that should not be employed. The test product should be administered according to their intended method of administration / labelling. Orodispersible multiparticulates may be administered without water, or a small amount of water (e.g., 20 – 40 mL) may be administered to facilitate swallowing, as indicated in the proposed labeling. Dispersible tablets 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 total volume of water employed should not exceed 50 mL.

Fasting/fed: The bioequivalence study should be conducted in the fasted state as azithromycin can be taken irrespective of meals.

Subjects: Healthy adult subjects should be recruited. It is not necessary to include patients in the bioequivalence study.

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

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for azithromycin is around 33%. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study.

Washout: Taking into account the elimination half-life of azithromycin of 2 – 4 days, a washout period of 21 days is considered sufficient to prevent carry over.

Blood sampling: The blood sampling should be intensive for the first four hours after administration to properly characterize the C_{max} of azithromycin. Blood samples for the characterization of azithromycin pharmacokinetics should be taken for 72 h post-dose in order to determine AUC_{0-t}. For example, blood samples might be taken at pre-dose, 0.5, 1.0, 1.5, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 24.0, 48.0, 72.0 after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure azithromycin in human plasma using LC-MS/MS analytical methodology. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the Cmax in most profiles of each formulation (test or comparator).

Statistical considerations: The data for azithromycin should meet the following bioequivalence standards in a single-dose, crossover design study:

- The 90% confidence interval of the relative mean AUC_{0-t} of the test to comparator product should be within 80 – 125%
- The 90% confidence interval of the relative mean C_{max} of the test to comparator product should be within 80 – 125%.