Notes on the Design of Bioequivalence Study: Benzathine benzylpenicillin

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Team – Medicines (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. For guidance on issues related to bioequivalence (BE) studies for immediate-release, solid oral dosage forms, see the ICH Harmonised Guideline M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms (2024). For BE issues outside the scope of the ICH M13A guideline, e.g., for additional strength biowaivers, 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 prolonged release injectable products containing benzathine benzylpenicillin.

Pharmacokinetics of Benzathine benzylpenicillin

Benzathine benzylpenicillin, also known as penicillin G, releases penicillin G slowly because of its very low aqueous solubility. Benzathine benzylpenicillin forms a depot in the site of injection and it is dissolved and hydrolysed slowly to penicillin G. T_{max} is observed between 12 and 48 h after injection. Benzathine penicillin G has a long apparent half-life of approximately 336 h with drug input into blood circulation for more than 30 days following a single intramuscular injection.

Intramuscular administration of 300,000 units of penicillin G benzathine in adults results in blood levels of 0.03 to 0.05 units per mL, which are maintained for 4 to 5 days. Similar blood levels may persist for 10 days following administration of 600,000 units and for 14 days following administration of 1,200,000 units. Blood concentrations of 0.003 units per mL may still be detectable 4 weeks following administration of 1,200,000 units.

Guidance for the design of bioequivalence studies

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

Design: A single-dose parallel design is recommended.

Dose: As the EoI for reproductive health includes benzathine benzylpenicillin 2.4 million units, 1.2 million unit and 150,00 units per dose in vial for reconstitution and intramuscular injection, and the EoI for HIV includes benzathine benzylpenicillin, powder for intramuscular injection 2.4 million units/4mL; 1.2 million units/2mL; 600,000 units/1mL, the bioequivalence study should be conducted with the highest strength (2.4 million units/4mL) and the other strengths can be waived from the in vivo demonstration of bioequivalence if the conditions for the additional strength biowaiver are met (i.e., same manufacturing method, and proportional composition, where different strengths are obtained with same concentration in different volumes).

Fasted/fed: N/A.

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: Benzathine benzylpenicillin is hydrolysed into benzylpenicillin or penicillin G. Therefore, bioequivalence for benzathine benzylpenicillin should be based on the determination of benzylpenicillin.

Sample size: Benzylpenicillin C_{max} and AUCs seems to exhibit inter-subject variability around 13% (Shahbazi et al. 2013). These data may facilitate the calculation of a sufficient sample size for a parallel bioequivalence study.

Washout: N/A for a parallel design. Shahbazi et al. 2013 conducted a crossover design with a 5-month wash-out period, but for a half-life of 336 h, a wash-out of 75 days might be appropriate.

Blood sampling: Blood sampling needs to be undertaken up to 56 days after the day of injection. A possible sampling scheme could be: pre-injection, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.25, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.0, 12.0, 16.0, 24.0, 36.0, 48.0 and 56.0 days after injection.

Analytical considerations: Information currently available indicates that it is possible to measure benzylpenicillin in human plasma using LC-MS/MS analytical methodology (e.g., 20 ng/ml). The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the C_{max} in most profiles of each formulation (test or comparator).

Statistical considerations: The data for benzylpenicillin should meet the following bioequivalence standards in a single-dose parallel design study:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}t}$ of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean $AUC_{0\text{-}inf}$ of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean C_{max} of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean $AUC_{0\text{-}10\ days}$ and $AUC_{10\ days\text{-}t}$ of the test to comparator product should be submitted as supportive information.