Notes on the design of bioequivalence study: Benzathine benzylpenicillin

Notes on the design of bioequivalence studies with products invited to be submitted to the WHO Prequalification Team – Medicines (PQTm) 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 immediate release products, containing benzathine benzylpenicillin (for reconstitution for i.m. injection).

Pharmacokinetics of benzathine benzylpenicillin

Benzathine benzylpenicillin or Penicillin G benzathine has an extremely low aqueous solubility (0.02%) and, thus, the drug is slowly released from intramuscular injection sites. The drug is hydrolyzed to penicillin G or benzylpenicillin. This combination of hydrolysis and slow absorption results in blood serum levels much lower but much more prolonged than other parenteral penicillins. 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. With normal kidney function, the drug is excreted rapidly by tubular excretion.

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 cross-over design is recommended, although a parallel design is also possible.

**Dose:** As the EoI includes Benzathine benzylpenicillin (for reconstitution for i.m. injection) at three strengths (i.e. 2,400,000 IU/dose, 1,200,000 IU/dose, 150,000 IU/dose) and the reference product strengths are of the same concentration and only differ in the administered volume (1 mL size, containing 600,000 units per syringe, 2 mL size, containing 1,200,000 units per syringe, and 4 mL size, containing 2,400,000 units per syringe, any dose can be administered as long as it is the same for test and reference if the applied product also follows the same strength formulation strategy.

**Fasting/fed:** The bioequivalence study may be conducted in the fasting (or fed) state.

**Subjects:** Healthy volunteers
**Sample size:** The intra-subject variability of benzylpenicillin Cmax and AUC\textsubscript{0-t} seems to be approximately 5% and 12%, respectively. The inter-subject variability was reported to be 13.2% for AUC\textsubscript{0-t} and 9.0% for Cmax. These data will facilitate the calculation of a sufficient sample size for the bioequivalence study.

**Washout:** 70-90 days in case of a cross-over design.

**Blood sampling:** Predose, 1.00, 2.00, 4.00, 8.00, 12.00, 24.00 (1 day), 48.00 (2 days), 120.00 (5 days), 240.00 (10 days), 360.00 (15 days), 480.00 (20 days), 600.00 (25 days), 720.00 (30 days), 840.00 (35 days), 960.00 (40 days) and 1080 hours (45 days) after drug administration.

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

**Parent or metabolite data for assessment of bioequivalence:** The parent prodrug is not measurable, therefore, the active metabolite benzylpenicillin should be used to assess bioequivalence.

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

- The 90% confidence interval of the relative mean AUC\textsubscript{0-\infty} of the test to reference product should be within 80-125%

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

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