Notes on the Design of Bioequivalence Study: Procaine 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. 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 prolonged release injectable products containing procaine benzylpenicillin.

Pharmacokinetics of Procaine benzylpenicillin

Procaine benzylpenicillin, also known as penicillin G procaine, is an equimolecular compound of procaine and penicillin G, which is administered intramuscularly as a suspension. It dissolves (hydrolyses) slowly at the site of injection, giving a plateau type of blood level at about 4 hours which falls slowly over a period of the next 15 to 20 hours. Approximately 60 to 90 percent of a dose of parenteral penicillin G is excreted in the urine within 24 to 36 hours.

Guidance for the design of bioequivalence studies

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

Design: A single-dose crossover design is recommended.

Dose: As the EoI includes procaine benzylpenicillin 150,000 units in vial for reconstitution and intramuscular administration, the bioequivalence study should be conducted with this strength and route of administration.

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

Sample size: Limited data is available in the scientific literature on the intra-subject variability of C_{max} and AUC of procaine benzylpenicillin. Therefore, a pilot study is recommended to obtain an estimation of intra-subject CV(%) for a proper sample size calculation.

Washout: Taking into account the elimination half-life of benzylpenicillin after intramuscular injection of procaine benzylpenicillin in healthy volunteers, a washout period of 7 days is considered sufficient to prevent carry over.

Blood sampling: The blood sampling for benzylpenicillin should be intensive around 6 h after injection, when T_{max} is expected, to properly characterize the C_{max} of benzylpenicillin. Blood sampling needs to be undertaken up to 48 hours after the injection. Samples may be taken at the following time points: Pre-dose, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00, 20.00, 24.00, 36.00 and 48.00 h 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 $AUC_{0\text{-}12\ h}$ and $AUC_{12\ h\text{-}t}$ of the test to comparator product should be submitted as supportive information.