Notes on the Design of Bioequivalence Study: Entecavir

Notes on the design of bioequivalence studies with products invited for submission 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 entecavir.

**Pharmacokinetics of entecavir**

Following oral administration, entecavir is rapidly absorbed and the peak plasma concentration is observed at about one hour post-dose. Relative to fasting conditions, the administration of a single dose of entecavir with a high-fat, high calorie meal slows the rate of absorption of entecavir. In addition, the maximum concentration ($C_{\text{max}}$) and extent of absorption (AUC) of entecavir decrease approximately 45% and 20%, respectively. For this reason administration without food is recommended in the dosing instructions of the labeling approved by the United States Food and Drug Administration, while the European Medicines Agency Summary of Product Characteristics recommends that for treatment of naive patients, entecavir can be taken with or without food, whereas in lamivudine refractory patients, entecavir is to be taken without food at least two hours before or after a meal. Single and multiple dose data suggest a greater than proportional increase in exposure after single dose administration, but roughly dose proportional exposure at steady-state. After reaching peak levels, entecavir plasma concentrations decrease in a bi-exponential manner with a terminal elimination half-life of approximately 128–149 hours.

**Guidance for the design of bioequivalence studies**

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

**Dose:** As entecavir is marketed as 0.5 and 1 mg tablets, and as a 0.05 mg/ml oral solution, and taking into account the suggested greater than dose proportional increase in exposure after single dose, a 1 mg dose using the highest tablet strength should be employed in the bioequivalence study. This should also be taken into account for the oral solution if a bioequivalence study is to be conducted for this dosage form i.e., a 1 mg dose should be employed in the bioequivalence study, if a biowaiver is not feasible due to differences in the amount of maltitol (380 mg/mL).

**Fasting/fed:** The bioequivalence study should be conducted in the fasting state.
**Subjects:** Healthy adult subjects should be used. It is not necessary to include patients in the bioequivalence study.

**Analytical considerations:** The measurement of entecavir in plasma is feasible (LLOQ = 50 pg/ml) and the use of the parent drug is considered to be more discriminative to differences in the biopharmaceutical performance of the drug products. Therefore, bioequivalence should be based on the determination of entecavir.

**Sample size:** Entecavir AUC and \( C_{\text{max}} \) in the fasting state have a low intra-subject variability (< 30%). Based on the information available in the literature (Jin J. et al. Bioequivalence evaluation of 2 tablet formulations of entecavir in healthy chinese volunteers: a single-dose, randomized-sequence, open-label crossover study. Arzneimittelforschung. 2012 62(3):113–116) the intra-subject coefficient of variation for \( C_{\text{max}} \) and AUC are approximately 10.6% and 5.6% for AUC, respectively. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study, but they should be employed cautiously since they are single observations and this study did not reflect the expected long half-life, only a half-life of 11.1–19.1 h.

**Washout:** In theory, given a terminal elimination half-life of 128-149 hours, a washout period of 7–8 weeks would be recommended. However, in practice, given the biphasic elimination behaviour of entecavir the investigator should determine what washout period is required to achieve plasma concentrations that will be less than 5% of the \( C_{\text{max}} \) that will be observed in a bioequivalence study. This period of time is expected to be considerably less than 7–8 weeks.

**Blood sampling:** The blood sampling should be more intensive between 0–2 hours after administration to properly characterize the \( C_{\text{max}} \) of entecavir. Considering the elimination half-life, it is sufficient to take blood samples up to 72 hours after administration for the characterization of entecavir pharmacokinetics.

**Parent or metabolite data for assessment of bioequivalence:** The parent drug is considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of entecavir.

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

- The 90% confidence interval of the relative mean \( \text{AUC}_{0-72h} \) 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%.

Information currently available to the PQTm suggests that the comparator product is not a highly variable drug product for AUC and \( C_{\text{max}} \) in the fasting state.

**Biowaiver:** With regard to the oral solution, a biowaiver for a bioequivalence study may be applicable if the amount of maltitol in the proposed product is similar to that of the comparator product (380 mg/mL), in line with the general guidelines of submission of documentation for WHO prequalification (see the "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability". In: Forty-ninth Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. Geneva, World Health Organization. WHO Technical Report Series, No. 992, 2015, Annex 7).