

Notes on the Design of Bioequivalence Study: Entecavir

Notes on the design of bioequivalence studies with products invited for submission 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 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_{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. On the other hand, the European Medicines Agency Summary of Product Characteristics recommends that for treatment of naive patients entecavir may be taken independent of food, whereas in lamivudine refractory patients entecavir is to be taken without food at least two hours before or two hours 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 considered:

Design: A single-dose cross-over design is recommended. It is noted that for the oral solution a biowaiver may be granted if requirements regarding the amount of maltitol are met.

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).

Fasted/fed: The bioequivalence study should be conducted in the fasted state.

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. Therefore, the data for the parent compound should be used to assess bioequivalence of entecavir.

Sample size: Entecavir AUC and C_{\max} in the fasted state display a low intra-subject variability (< 30%). Based on literature information (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_{\max} and AUC are approximately 10.6% and 5.6%, respectively. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study, but caution should be exercised since these values are derived from a single study with an unexpectedly short 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, due to the biphasic elimination behavior of entecavir a sufficiently long washout period should be employed to ensure pre-dose plasma concentrations of less than 5% of the C_{\max} observed in the 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_{\max} of entecavir. For example, samples should be taken at predose, 0.17, 0.33, 0.5, 0.67, 0.83, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 16.0, 24.0, 36.0, 48.0 and 72.0 h after drug administration. Considering the elimination half-life, it is sufficient to collect blood samples up to 72 hours after administration for the characterization of the absorption of entecavir.

Analytical considerations: Information currently available indicates that it is possible to measure entecavir in human plasma using LC-MS/MS analytical methodology (LLOQ = 50 pg/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 entecavir should meet the following bioequivalence standards in a single-dose crossover design study:

- The 90% confidence interval of the relative mean AUC_{0-72h} of the test to reference product should be within 80.00–125.00%
- The 90% confidence interval of the relative mean C_{\max} of the test to reference product should be within 80.00–125.00%.

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: *Fifty-seventh report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations*. Geneva, World Health Organization. WHO Technical Report Series, No. 1052, 2024, Annex 8).