Notes on the Design of Bioequivalence Study: Sofosbuvir

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 immediate release products containing sofosbuvir.

Pharmacokinetics of Sofosbuvir

Following oral administration, sofosbuvir is absorbed quickly and the peak plasma concentration was observed ~0.5–2 hour(s) post-dose, irrespective of the dose level. Relative to fasting conditions, the administration of a single dose of sofosbuvir with a standardized high fat meal slows the rate of absorption of sofosbuvir. Further, with food, the extent of absorption of sofosbuvir is increased approximately 1.8-fold, with little effect on peak concentration. For this reason, administration is recommended with food in the Summary of Product Characteristics approved by the European Medicines Agency (EMA), although it can be taken irrespective of meals according to the Product Labelling approved by the US Food and Drug Administration (US FDA). The median terminal half-life of sofosbuvir is 0.4 hours.

Guidance for the design of bioequivalence studies

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

Design: A single-dose cross-over design is recommended.

Dose: As the EoI includes Sofosbuvir 400 mg tablet and 100 mg tablet (preferably dispersible), the bioequivalence study should be conducted with the highest strength if the conditions for an additional strength biowaiver are fulfilled. Otherwise, bioequivalence should be shown for each strength.

When conducting bioequivalence studies, it is essential to administer the test and the comparator product according to their corresponding instructions for use. In those cases where the test and the comparator product are different dosage forms with different methods of administration (e.g., a tablet that should be taken with a glass of water vs. a dispersible tablet to be dispersed in 10 mL of water) the bioequivalence study should be conducted employing the intended method of administration of each product. It is considered incorrect to standardise the volume of liquid in all these cases (e.g., administering a glass of water after the intake of a dispersible tablet or rinsing the container where a dispersible tablet has been suspended with the remaining liquid of a glass of water) because this standardisation does not occur in real life conditions. The total volume of water employed during administration of a pediatric dispersible tablet should not exceed 50 mL.

Fasted/fed: It is preferred that the bioequivalence study be conducted in the fed state with a high-fat high-calorie meal as sofosbuvir exhibits a greater absorption in the presence of a high-fat meal. However, as the approved labelling for the posology for Sovaldi, the WHO prequalification comparator product, differs between the EMA and the US FDA, a bioequivalence study conducted under fasted conditions will also be acceptable if the comparator product is obtained from a market where the approved labeling indicates that it may be taken without regard to food.

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. The data for the parent compound should be used to assess bioequivalence of sofosbuvir.

Sample size: Sofosbuvir C_{max} in the fed state seems to be highly variable (54%), whereas AUC_{0-t} seems to have low variability (10%), based on the information available to PQT/MED. These data may facilitate the calculation of a sufficient sample size for the cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of sofosbuvir in the fed state of 1.5 h (range 0.3 – 9 h), a washout period of 7 days is considered sufficient to prevent carry-over.

Blood sampling: The blood sampling should be intensive the first four hours after administration to properly characterize the C_{max} of sofosbuvir. It is not necessary to take blood samples beyond 8−12 hours for the characterization of sofosbuvir pharmacokinetics.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure sofosbuvir in human plasma using LC-MS/MS analytical methodology. 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). See Guideline on bioanalytical method validation and study sample analysis. In: WHO Technical Report Series, No. 1060, Annex 6, or the ICH Harmonised Guideline M10 for more information on bioanalytical recommendations.

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

- The 90% confidence interval of the relative mean AUC_{0-t} 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%.

Information currently available to PQT/MED indicates that the comparator product is a highly variable drug product. Therefore, if the Applicant suspects that the variability of C_{max} or AUC_{0-t} is high (CV>30%), the applicant may prefer to employ a full replicate design study in order to widen the acceptance range of C_{max}. and/or AUC_{0-t}. For more information on replicate study designs and widening the acceptance limits of average bioequivalence based on the intra-subject variability of the comparator product, refer to Section 7.9.3 of Annex 8, TRS 1052 and PQT/MED guidance document “Application of reference-scaled criteria for AUC in bioequivalence studies conducted for submission to PQT/MED”.