Notes on the Design of Bioequivalence Study: Ledipasvir

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 ledipasvir.

Pharmacokinetics of ledipasvir

Following oral administration of ledipasvir/sofosbuvir to hepatitis C-infected patients, ledipasvir median peak plasma concentration is observed at 4.0 hours post-dose. Ledipasvir AUC is dose-proportional over the dose range of 3 to 100 mg. Relative to fasting conditions, the administration of a single dose of ledipasvir/sofosbuvir with a moderate-fat or high-fat meal does not significantly affect the exposure to ledipasvir. The combination of sofosbuvir and ledipasvir can be administered without regard to food.

The median terminal half-life of ledipasvir in healthy volunteers following administration of ledipasvir/sofosbuvir in the fasted state is 47 hours.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of ledipasvir, 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 ledipasvir 90 mg tablet, the bioequivalence study should be conducted with this product vs. the fixed combination of sofosbuvir and ledipasvir as comparator product.

Fasted/fed: Sofosbuvir/ledipasvir is recommended to be taken with or without food. Due to the low solubility of ledipasvir, the comparator product employs a complex manufacturing process in order to enhance the dissolution and absorption of ledipasvir. Due to the low solubility of this API and the complexity of the manufacturing process employed, there is an increased risk that the in vivo performance of such a product will be impacted differently by varying gastrointestinal (GI) conditions between the fasted and fed conditions. Therefore, for such drug products, bioavailability differences (i.e., test/comparator ratios) related to differences in formulation and/or manufacturing process may not be detected with a single BE study, i.e., results from a fasting BE study may not be extrapolated to predict fed BE study outcome or vice versa, thus both fasting and fed BE studies should be conducted, unless

otherwise justified (e.g., by demonstrating the use of a formulation with the same or similar excipient composition and the same manufacturing process as in the comparator product)..

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 ledipasvir.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability in the fasted state for ledipasvir is approximately 45%. These data may facilitate the calculation of the sample size for the crossover bioequivalence studies.

Washout: Taking into account the elimination half-life of ledipasvir in healthy volunteers (approximately 47 hours), a washout period of 14 days is considered sufficient to prevent carry-over

Blood sampling: The blood sampling should be intense between 3 and 5 hours after drug administration to properly characterize the Cmax of ledipasvir. It is not necessary to take blood samples beyond 72 hours.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure ledipasvir 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). The bioanalytical method for the analyte should be validated in the presence of sofosbuvir (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).

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

- The 90% confidence interval of the relative mean $AUC_{0\text{-}72\ h}$ 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 $AUC_{0\text{-}72h}$ 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 $AUC_{0\text{-}72\ h}$. For more information on replicate study designs and widening the limits of average bioequivalence, 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”.