

## Notes on the design of bioequivalence study: Isoniazid/Rifapentine

Notes on the design of bioequivalence studies with products invited to be submitted 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. 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 isoniazid, rifapentine, moxifloxacin and pyrazinamide.

### **Pharmacokinetics of isoniazid**

In the fasted state, isoniazid is rapidly and almost completely absorbed. Peak plasma concentrations are reached in approximately 1 to 2 hours. The bioavailability of isoniazid is reduced significantly and T<sub>max</sub> delayed (3 hours) when administered with food. The administration with food decreases the bioavailability of isoniazid 46% in C<sub>max</sub> and 23% in AUC. Isoniazid is usually administered under fasted conditions.

The half-life of isoniazid in fast acetylators is 1 to 2 hours, while in slow acetylators it is 2 to 5 hours.

### **Pharmacokinetics of rifapentine**

Maximum concentrations were observed from 4 to 6 hours after administration of a 600 mg rifapentine dose.

The administration of rifapentine with a high fat meal (850 total calories: 33 g protein, 55 g fat and 58 g carbohydrate) increased AUC<sub>0-∞</sub> and C<sub>max</sub> by 43% and 44%, respectively, over that observed when administered under fasting conditions. The administration of rifapentine (900 mg single dose), concomitant with a low fat, high carbohydrate breakfast, led to an increase of rifapentine bioavailability by 47% in C<sub>max</sub> and 51% in AUC.

Rifapentine half-life is approximately 15 hours (13.2 – 14.1 hours) and it was similar across the 150-600 mg dose range.

### **Guidance for the design of bioequivalence studies:**

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

**Design:** A single dose crossover design is recommended.

**Dose:** The EoI includes a 300 mg/300 mg tablet so this strength should be studied in a BE study in comparison to a combination of mono-component comparator products.

**Fasting/fed:** The bioequivalence study should be conducted in the fed state as food increases the bioavailability of rifapentine, although it decreases the bioavailability of isoniazid. As a low-fat high-carbohydrate breakfast increases  $C_{\max}$  and AUC as much as or more than a high-fat high-calorie breakfast, a standard breakfast (non-high-fat breakfast, 550 Kcal) is recommended since it is more similar to the meal composition of patients. However, a high-fat high-calorie breakfast is also acceptable in those cases where the fed study is to be submitted also to other regulatory agencies where demonstration of bioequivalence is required both in the fasting and the fed state. In such cases, both studies (in fasting and fed state) should be submitted.

**Subjects:** Healthy adults 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, bioequivalence should be based on the determination of isoniazid and rifapentine.

**Sample size:** Isoniazid pharmacokinetic parameters,  $C_{\max}$  and  $AUC_{0-t}$ , in the fed state seem to possess higher variability than those of rifapentine. Therefore, the sample size calculation should be based on the intra-subject variability of isoniazid  $C_{\max}$ , which could be highly variable (30-40%) based on information available to the PQT/MED. These data will facilitate the calculation of a sufficient sample size for the bioequivalence study.

**Washout:** Taking into account the half-lives of these drugs, a wash-out period of at least 7 days is considered enough to prevent carry-over.

**Blood sampling:** The blood sampling should be intensive up to 6 hours after administration. For example, samples should be taken at pre-dose, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, 16.0, 24.0, 36.0, 48.0, and 72.0 h after drug administration. Isoniazid does not need to be measured in samples after 24 h.

**Analytical considerations:** Information currently available indicates that it is possible to measure isoniazid and rifapentine 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 each analyte should be validated in the presence of the other three analytes ((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 isoniazid and rifapentine should meet the following bioequivalence standards in a single-dose crossover design study:

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