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 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 isoniazid and rifapentine.

**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_max delayed (3 hours) when administered with food. The administration with food decreases the bioavailability of isoniazid 46% in Cmax 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**

The absolute bioavailability of rifapentine has not been reported. The relative bioavailability (with an oral solution as a reference) of rifapentine after a single 600 mg dose to healthy adult volunteers was 70%. Following a single 600 mg oral dose of radiolabeled rifapentine to healthy volunteers (n=4), 87% of the total $^{14}$C-rifapentine was recovered in the urine (17%) and feces (70%). Greater than 80% of the total $^{14}$C-rifapentine dose was excreted from the body within 7 days.

Maximum concentrations were observed from 5 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$_{0-\infty}$ and Cmax 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 Cmax and 51% in AUC.

Rifapentine half-life is approximately 15 hours.

**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 cross-over design is recommended.

**Dose:** As the EoI includes 150 mg/150mg dispersible tablet and 300 mg/300 mg coated tablet, these strengths are recommended for the bioequivalence study. A dose of 1 x 150 mg/150 mg should be tested for the dispersible tablet and 1 x 300 mg/300 mg for the coated tablet, since rifapentine demonstrates less-than-dose-proportional
pharmacokinetics. In the case of the dispersible tablet, the test product should be administered with the method of administration and volume of water intended for the dosing instructions of the proposed product, and compared with the reference tablets, which should be taken with 240 ml of water.

**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 Cmax 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 volunteers

**Sample size:** Isoniazid pharmacokinetic parameters, Cmax and AUC0-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 Cmax, which could be highly variable (30-40%) based on information available to the PQTm. These data will facilitate the calculation of a sufficient sample size for the bioequivalence study.

**Washout:** At least 7 days.

**Blood sampling:** Predose, 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 Cmax in most profiles of each formulation (test or comparator).

**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.

**Statistical considerations:** The data for isoniazid and rifapentine should meet the following bioequivalence standards in a single-dose cross-over design study:

- The 90% confidence interval of the relative mean AUC0-t of the test to reference product should be within 80-125%

- The 90% confidence interval of the relative mean Cmax of the test to reference product should be within 80-125%.