Notes on the design of bioequivalence study: Isoniazid/Rifampicin

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/rifampicin.

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 C_max 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 rifampicin

Rifampicin is readily absorbed from the gastrointestinal tract. Peak serum concentrations occur about 2 to 4 hours after a single dose on an empty stomach. Absorption of rifampicin is reduced when the drug is ingested with food.

In normal subjects the half-life of rifampicin in serum averages about 3 hours after a 600 mg dose and increases to 5.1 hours after a 900 mg dose.

Guidance for the design of bioequivalence studies:

Taking into account the pharmacokinetic properties of isoniazid and rifampicin, 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 50/75 mg as dispersible or crushable tablets as well as 75/150, 150/150, and 150/300 mg strengths as tablets or capsules, and the FDC comparator is marketed as 150/300 mg strength, the 150/300 mg strengths should be compared with a dose of 1x150/300 mg, and the 75/150 mg as 2x75/150 mg dose.

The 150/150 mg strength should be compared with monocomponent comparator products (2 x 150/150 mg vs. 1 x 300 + 3 x 100mg or 1 x 300 mg of isoniazid).

The 50/75 mg strength should be compared with monocomponent comparator products (2 x 50/75 mg vs. 1x100 mg isoniazid/150 mg rifampicin). In the case of the dispersible/crushable 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 fasting state

**Subjects**: Healthy volunteers

**Sample size**: Based on information available to the PQTm, variability in the rifampicin pharmacokinetic parameters, Cmax and AUC0-t, in the fasting state ranges from 9.5 to 27%, but it is generally around 15-20%, so it seems to possess low to moderate variability. Isoniazid seems to exhibit a slightly higher variability (20-30%), although it ranges from 9% to 35%. These data will facilitate the calculation of a sufficient sample size for the bioequivalence study.

**Washout**: At least 7 days.

**Blood sampling**: Predose, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 20.00, and 24.00 h after drug administration.

**Analytical considerations**: Information currently available indicates that it is possible to measure isoniazid and rifampicin 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 rifampicin and isoniazid 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 reference product should be within 80-125%
- The 90% confidence interval of the relative mean C_{max} of the test to reference product should be within 80-125%.