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

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. In particular, 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 and moxifloxacin.

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 Tmax 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

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 $AUC_{0-\infty}$ and C_{max} 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_{max} 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. The changes in rifapentine C_{max} and $AUC_{0-\infty}$ were dose linear, but disproportionate (more than proportional) from 150 to 600 mg. Two-fold increases in dose from 150 to 300 mg and from 300 to 600 mg resulted in 3.2- fold and 2.2-fold increases in $AUC_{0-\infty}$, respectively. Over the entire dose range studied, a 4-fold increase in dose resulted in a 7.2-fold increase in $AUC_{0-\infty}$. The dose-disproportionate increases in C_{max} with single, increasing doses of rifapentine were less pronounced. A 4-fold increase in dose from 150 to 600 mg resulted in a 5.2-fold increase in C_{max} (Keung et al. Single and multiple dose pharmacokinetics of rifapentine in man: part II. *Int J Tuberc Lung Dis*. 1999 May;3(5):437-44).

Pharmacokinetics of moxifloxacin

Following oral administration moxifloxacin is rapidly and almost completely absorbed. The absolute bioavailability amounts to approximately 91%. Following an oral dose, peak concentrations are reached within 0.5 - 4 h post administration. Moxifloxacin has no clinically relevant interaction with food including dairy products. The tablets may be taken independent of meals. Moxifloxacin is eliminated from plasma with a mean terminal half-life of approximately 12 hours.

Guidance for the design of bioequivalence studies:

Taking into account the pharmacokinetic properties of isoniazid, rifapentine and moxifloxacin, 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 the strength 75/300/100 mg only, this strength should be tested in vivo. As the comparator products for this fixed combination should be the monocomponent comparators, several units are necessary to reach the same dose, e.g. 4x75/300/100 mg tablets of the fixed combinations versus 3x100 mg of the isoniazid comparator, 8x150 mg of the rifapentine comparator and 1x400 mg of the moxifloxacin comparator.

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 and it does not affect significantly the bioavailability of moxifloxacin. 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. The data for the parent compound should be used to assess bioequivalence.

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, 0.17, 0.25, 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 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, rifapentine and moxifloxacin 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 two analytes (see ICH Harmonised Guideline M10 for more information).

Statistical considerations: The data for isoniazid, rifapentine and moxifloxacin 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 80.00 – 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%.