Notes on the Design of Bioequivalence Study: Rifampicin / Clarithromycin

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. 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 fixed dose combination product containing clarithromycin and rifampicin.

Pharmacokinetics of clarithromycin

Clarithromycin is rapidly and well absorbed from the gastro-intestinal tract after oral administration. However, it exhibits an extensive first pass effect metabolism. Absolute bioavailability after the administration of a 250 mg tablet is 50% approximately. The microbiologically active metabolite 14-hydroxyclarithromycin is formed by first pass metabolism. Clarithromycin may be given without regard to meals as food does not affect the extent of bioavailability of clarithromycin tablets. Food does slightly delay the onset of absorption of clarithromycin and formation of the 14-hydroxymetabolite. Peak serum concentrations occur about 1 to 2 hours after a single dose on an empty stomach.

The pharmacokinetics of clarithromycin are non-linear due to saturation of the hepatic metabolism at high doses. Elimination half-life increases from 2-4 h after the administration of 250 mg b.i.d. to 5 h after the administration of 500 mg b.i.d. The elimination half-life of the active metabolite is 5-6 h after 250 mg doses b.i.d. At 250 mg b.i.d. 15-20% of unchanged drug is excreted in the urine. With 500 mg b.i.d. daily dosing urinary excretion is greater (approximately 36%). The 14-hydroxyclarithromycin is the major urinary metabolite and accounts for 10-15% of the dose. Most of the remainder of the dose (70-80%) is eliminated in the faeces, primarily via the bile. A small fraction (5-10%) of the parent drug is recovered from the faeces.

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 elimination 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 clarithromycin and rifampicin, 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 the strength 300 mg/250 mg, this combination strength should be administered versus the mono-component comparators: 250 mg of clarithromycin and 300 mg of rifampicin, administered simultaneously.

Fasted/fed: The bioequivalence study should be conducted in the fasted state since rifampicin absorption is reduced when the drug is ingested with food and clarithromycin may be given without regard to meals.

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 drugs are considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of clarithromycin and rifampicin.

Sample size: Clarithromycin C_{max} exhibits moderate or high intra-subject variability (27-35%), whereas AUC_{0-t}, exhibits low variability (12-19%) in the fasting state. Rifampicin pharmacokinetic parameters, C_{max} and AUC_{0-t}, in the fasting state seem to possess low to moderate variability (10-27%), based on information available to the PQT/MED. These data may facilitate the calculation of a sufficient sample size for a single-dose cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of clarithromycin of up to 5 hours and of rifampicin of up to 4 hours, a washout period of seven days is considered sufficient to prevent carry over.

Blood sampling: The blood sampling should be intensive for the first four hours after administration to properly characterize the Cmax of clarithromycin and rifampicin. For example, samples can be taken : pre-dose and at 0,25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.25, 3.50, 3.75, 4.00, 4.50, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 20.00, and 24.00 h after drug administration.

Analytical method: Information currently available to PQT/MED indicates that it is possible to measure clarithromycin 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 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 analyte (see ICH Harmonised Guideline M10 for more information).

Statistical considerations: The data for clarithromycin and rifampicin 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%.

Information currently available to the PQT/MED suggests that the comparator product might be a highly variable drug product for C_{max}, but not for AUC_{0-t}. Therefore, if the Applicant suspects that the variability of C_{max} or AUC_{0-t} 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-t}. 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 WHO guidance document "*Application of reference-scaled criteria for AUC in bioequivalence studies conducted for submission to PQT/MED*".