Notes on the Design of Bioequivalence Study: Mebendazole

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-first Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations.* Geneva, World Health Organization, 2017. WHO Technical Report Series, No. 1003, Annex 6.

Below, additional specific guidance is provided on the invited immediate release products containing mebendazole.

Pharmacokinetics of mebendazole

Following oral administration, <10% of the dose reaches the systemic circulation due to incomplete absorption and extensive pre-systemic metabolism (first-pass effect). However, as absorption does occur, systemic levels obtained following administration of a product need to be assessed for reasons of safety. To this end, pharmacokinetic bioequivalence trials are considered the best way to assess the biopharmaceutical quality of a multisource mebendazole product, thus avoiding the need to conduct full clinical trials to establish the safety and efficacy of the proposed mebendazole product.

Maximum plasma concentrations are generally seen 2 – 4 hours after administration. Administration with a high fat meal increases the bioavailability of mebendazole, but the overall effect of food on the amount of drug remaining in the gastrointestinal tract is not expected to be substantial.

Orally administered mebendazole is extensively metabolised primarily by the liver. Plasma concentrations of its major metabolites (amino and hydroxylated amino forms of mebendazole) are substantially higher than those of mebendazole. Mebendazole, the conjugated forms of mebendazole, and its metabolites likely undergo some degree of enterohepatic recirculation and are excreted in the urine and bile. The apparent elimination half-life after an oral dose ranges from 3 – 6 hours in most patients.

Guidance for the design of bioequivalence studies

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

Study design: A single-dose replicate 4x2 cross-over design is recommended due to the high residual variability observed in 2x2 cross-over studies, to estimate intra-subject variability more accurately and to widen the acceptance range for C_{max} and AUC_{0-t} if the high variability of the comparator product is confirmed in a replicate design.

Dose: As the EoI includes only mebendazole 500 mg chewable tablets, this strength should be employed in the bioequivalence study. During treatment mebendazole tablets can be administered whole or chewed but, for the bioequivalence study, both products should be administered whole (unchewed).

Fasted/fed: The instructions for administration of mebendazole in its labeling indicates that mebendazole can be taken without regard to food intake. In the clinical studies conducted in pediatric patients with soil transmitted helminth infections, the majority of these patients were administered mebendazole tablets with food. In addition, as a high fat meal increases systemic exposure 2.6 fold for AUC and 4-fold for C_{max}, a study in the fed state is recommended. However, a fasting state study may also be acceptable.

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 drug is considered to best reflect the rate of release of the drug from the dosage form to the site of action in the lumen of the gastrointestinal tract. The data for the parent compound should be used to assess bioequivalence.

Sample size: The intra-subject variability (residual error) observed in a fasting state 2x2 cross-over study was 54.6% for AUC_{0-t} and 31.6% for C_{max}. Similarly, high intra-subject CV values have been observed in a 2x2 cross-over study in the fed state: 40.1% for C_{max} and 37.6% for AUC_{0-t}. However, in a 4x2 replicate cross-over design the intra-subject variability of the reference was reduced to 23.7% for C_{max} and 21.3% for AUC_{0-t}. Therefore, a replicate design is recommended. These data may facilitate the calculation of the sample size for a single-dose cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of approximately 6 h, a washout period of seven days is sufficient to prevent carry-over.

Blood sampling: The blood sampling for mebendazole should be intensive for the first six hours after administration to properly characterize the C_{max} of mebendazole, which is observed 4 hours after administration (range: 1 – 6 h). It is not necessary to take blood samples beyond 32 hours. For example, samples can be taken pre-dose and at 0.50, 1.00, 1.50, 2.000, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.33, 4.67, 5.00, 5.33, 5.67, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 18.00, 24.00 and 32.00 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure mebendazole 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), e.g. LOQ of at least 0.25 ng/ml for a fasting study and 1 ng/ml for a fed study.

Statistical considerations: The data for mebendazole should meet the following bioequivalence standards in a single-dose crossover design study:

- The 90% confidence interval of the relative mean AUC_T of the test to comparator product should be within 80.00 – 125.00%
- The 90% confidence interval of the relative mean C_{max} of the test to comparator product should be within 80.00 – 125.00%.

Information currently available to PQT/MED suggests that the comparator product is a highly variable drug product for both C_{max} and AUC_{0-t}. Therefore, the applicant may prefer to employ a full replicate design study to estimate variability more accurately and to widen the acceptance range for C_{max} and/or AUC_{0-t}.. For more information on replicate study designs and widening the acceptance range of average bioequivalence based on the intra-subject variability of the comparator product, refer to Section 7.9.3 of Annex 6, TRS 1003, and PQT/MED guidance document "*Application of reference-scaled criteria for AUC in bioequivalence studies conducted for submission to PQT/MED*".