Notes on the design of bioequivalence study: Miltefosine

Notes on the design of bioequivalence studies with products invited for submission 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. For guidance on issues related to bioequivalence (BE) studies for immediate-release, solid oral dosage forms, see the ICH Harmonised Guideline M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms (2024). For BE issues outside the scope of the ICH M13A guideline, e.g., for additional strength biowaivers, 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 miltefosine.

Toxicity of miltefosine

Due to early concerns regarding toxicity no clinical pharmacokinetic studies were conducted in healthy volunteers during the clinical development of the reference product so, little pharmacokinetic information is available for the purpose of designing a bioequivalence study.

The general side effects of miltefosine commonly affect the gastrointestinal tract; the most frequent symptoms are anorexia, nausea, vomiting and diarrhea. Although these symptoms are usually mild, some studies have shown more severe gastrointestinal symptoms that interfered with activities of daily living. Increases in serum aminotransferase and creatinine levels have also been described, which are usually mild, reversible and dose dependent.

Reproductive toxicity studies in rats during embryonic development and during organogenesis indicate an embryotoxic, fetotoxic, and teratogenic risk. Because of these findings, and as there are no controlled studies with miltefosine in pregnant women, its use is strictly contraindicated during pregnancy. Moreover, due to its long-half-life, it is not clear if contraception use for 3 months is enough once treatment has been finished for women of childbearing age. Therefore, studies should be conducted only in males.

Pharmacokinetics of miltefosine

It is recommended to take miltefosine with food because administration with food ameliorates gastrointestinal adverse reactions. Maximum concentrations following oral administration have been observed just prior to the subsequent dose administration in many patients, indicating that absorption of miltefosine may proceed throughout the dosing interval.

Due to the long half-life of miltefosine (>6 days), trough plasma concentrations have been reported to often not reach steady state by the end of treatment. The pharmacokinetics of miltefosine could best be described by a two-compartment disposition model with a first elimination half-life of 7.05 days and a terminal elimination half-life of 30.9 days. Plasma levels have been detected 5 to 6 months after treatment.

Guidance for the design of bioequivalence studies

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

Design: Due to the long half-life of miltefosine, a single-dose parallel design is recommended, although a single-dose cross-over design might be considered.

Dose: As the EoI includes 10 mg and 50 mg capsules, the 50 mg strength is recommended for the bioequivalence study.

Fasted/fed: The bioequivalence study should be conducted in the fed state as food ameliorates miltefosine gastrointestinal adverse effects.

Subjects: Healthy male subjects should be recruited in the study due to the teratogenic potential of miltefosine. 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 of miltefosine.

Sample size: There is no information available on the variability of C_{max} and $AUC_{0\text{-}72h}$ of miltefosine after a single dose administration in patients or healthy volunteers. Therefore, a pilot study is recommended not only to estimate the variability of the primary pharmacokinetic parameters, but also to identify the sampling times required for a proper characterization of the plasma concentration – time profiles.

Washout: In the case of a cross-over design, the long half-life of miltefosine must be taken into account, but it is not possible to define the washout period presently since, although the terminal elimination half-life is of 30 hours, plasma levels could be below 5% of C_{max} earlier. Therefore, the length of the washout period should be investigated in a pilot study if a cross-over design is contemplated.

Blood sampling: The blood sampling schedule needs to be investigated in a pilot study because no single dose study data is available to provide information on the expected time for T_{max}. It has been described that C_{max} is sometimes observed just before the next dose in many patients, therefore, it seems to be slowly absorbed. If this were confirmed, the blood sampling would not need to be very intense during the first hours of a study, but sufficiently frequent (e.g. every 30 min) during the first 12 hours after administration to properly characterize the C_{max} of miltefosine. Considering the elimination half-life, it is sufficient to take blood samples up to 72 hours after administration for the characterization of miltefosine pharmacokinetics.

Analytical considerations: Information currently available indicates that it is possible to measure miltefosine 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). See Guideline on bioanalytical method validation and study sample analysis. In: WHO Technical Report Series, No. 1060, Annex 6, or the ICH Harmonised Guideline M10 for more information on bioanalytical recommendations.

Statistical considerations: The data for miltefosine should meet the following bioequivalence standards in a single-dose parallel or cross-over design study:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}72\,h}$ 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%.

BCS-based biowaiver: The data currently available, while incomplete, suggests miltefosine might be highly soluble. There are no data available on human absorption (mass balance or absolute bioavailability studies). A BCS- based biowaiver for miltefosine is considered a possible alternative to a bioequivalence study, provided the requirements for granting a BCS-based biowaiver are met as outlined in the in the WHO Guideline on *Biopharmaceutics Classification System-Based Biowaivers* 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 7 and the PQT/MED guidance "*PQT/MED-specific Annotations for the WHO Guideline on Biopharmaceutics Classification System (BCS)-based Biowaivers*" (2024).