Notes on the Design of Bioequivalence Study: Methadone

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 methadone.

Pharmacokinetics of methadone

Methadone is well absorbed from the gastro-intestinal tract but undergoes fairly extensive first pass metabolism. Peak plasma concentrations are achieved between 1 and 7.5 hours. The half-life after a single oral dose is 12 – 18 (mean 15) hours, partly reflecting distribution into tissue stores, as well as metabolic and renal clearance. With regular doses, the tissue reservoir is already partly filled, and so the half-life is extended to 13 – 47 (mean 25) hours reflecting only clearance.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of methadone, 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 5 mg and 10 mg tablets, 5 mg/mL and 10 mg/mL oral liquid, as well as 5 mg/mL and 10 mg/mL concentrate for oral liquid. Each dosage form should be compared with its corresponding comparator product. In the case of the tablets, the highest strength to be developed should be tested, e.g., a single oral dose of one tablet of the test strength of 10 mg *versus* one tablet the comparator 10 mg tablet. In this case, the requirement for a BE study with the 5 mg strength can be waived if the conditions for the additional strength biowaiver are met. However, a BCS biowaiver could be possible if it is demonstrated that methadone is a highly soluble drug (see TRS1052, Annex 7 "WHO Guideline on Biopharmaceutics Classification System-based biowaivers" (2024)).

In the case of oral solutions or concentrates for oral solution, the in vivo demonstration of bioequivalence could be waived if the test product contains the API in the same molar concentration as the comparator product, and contains the same excipients that are known to affect bioavailability as the comparator product, if any, and in similar concentrations (see criteria in TRS1052, Annex 7 "WHO Guideline on Biopharmaceutics Classification System-based biowaivers" (2024)). Other excipients may differ qualitatively and quantitatively if methadone is demonstrated to be a BCS class I drug.

Fasted/fed: As the comparator product can be taken with or without food, a fasted state study is recommended.

Subjects: Healthy adult subjects should be used. It is not necessary to include patients in the bioequivalence study. A naltrexone blockade should be used to remove the risk of any opioid-related adverse events. Naltrexone should be administered well in advance of dosing to achieve adequate blockade of opioid receptors. The most common approach is to administer 50 mg of naltrexone at the following times: (1) 12 hours prior to dosing; (2) at the time of study drug dosing; and (3) 12 hours after the last dose of study drug.

Parent or metabolite data for assessment of bioequivalence: The parent drug is considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of methadone.

Sample size: Information on methadone currently available to the PQT/MED indicates that the intra-subject variability for methadone is around 10 – 12%. These data will facilitate the calculation of sufficient sample size for the crossover bioequivalence study.

Washout: Taking into account the elimination half-life of methadone in healthy volunteers (18 hours), a washout period of one or two weeks is considered sufficient to prevent carry over.

Blood sampling: As methadone t_{max} can range between 1 and 7.5 h, blood sampling should be intensive several hours after administration to cover the peak of methadone, e.g., pre-dose and at 0.33, 0.67, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.33, 3.67, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 7.50, 8.00, 8.50, 9.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00 and 72.00 hours post dose. It is not necessary to take blood samples over a longer time period, as this will only substantiate the elimination phase of methadone.

Analytical method: Information currently available to the PQT/MED indicates that it is possible to measure methadone 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) (LLOQ = 0.1 ng/ml). 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 methadone 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 80-125%.
- The 90% confidence interval of the relative mean C_{max} of the test to reference product should be within 80-125%.

Biowaiver: A BCS-based biowaiver for methadone is considered a possible alternative to a bioequivalence study, provided the requirements for granting a BCS-based biowaiver are met as outlined in the "WHO Guideline on Biopharmaceutics Classification System-based biowaivers" (2024) and the PQT/MED guidance "PQT/MED-specific Annotations for the WHO Guideline for Biopharmaceutics Classification System (BCS)-based Biowaiver Applications" (2025).