Notes on the Design of Bioequivalence Study: Nitrofurantoin

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 nitrofurantoin.

Pharmacokinetics of nitrofurantoin

Nitrofurantoin should always be taken with food or milk because a meal improves absorption, which is important for optimal efficacy, and it also minimizes its side effects. Orally administered nitrofurantoin exhibits t_{max} around 2 – 4.5 h when administered with food and an elimination half-life of about 1.5 – 1.7 h.

Guidance for the design of bioequivalence studies

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

Design: A crossover design is recommended.

Dose: The EoI for treatment of bacterial infections in children includes nitrofurantoin orodispersible multiparticulates (minitablets or sprinkles) 5 mg per unit dose, preferred; dispersible tablets 5 mg,10 mg (scored) as alternative. In principle, the corresponding dose of the comparator suspension should be administered to match the strength of the test product (5 mg for minitablets or sprinkles / 5 mg for dispersible tablets and 10 mg for the scored dispersible tablet). The requirement for a bioequivalence study with the 5 mg dispersible tablet could be waived if the conditions for an additional strength biowaiver with respect to the 10 mg scored dispersible tablet are fulfilled. However, in the case that the bioanalytical method is not able to detect 5% of the mean C_{max}, multiple units of the test product and the corresponding dose of the comparator suspension can be used (e.g., 25 mg).

The comparator product should be administered in a fashion consistent with its labelling. It is acceptable to rinse the container with an additional volume of water (e.g., 10 mL) but additional water beyond that should not be employed. The test product should be administered according to its intended method of administration / labelling. Orodispersible multiparticulates may be administered without water, or a small amount of water (e.g., 20 – 40 mL) may be administered to facilitate swallowing, as indicated in the proposed labeling. Dispersible tablets should be dispersed in a small amount of liquid suitable for the intended paediatric population (e.g., 20 – 40 ml) and a similar small amount of water should be used to rinse the container. Additional water should not be administered in order to mimic the real conditions of use. The total volume of water employed should not exceed 50 mL.

Fasting/fed: The bioequivalence study should be conducted in the fed state as nitrofurantoin should be taken with food. Either a high-fat, high-calorie meal or a low-fat, low-calorie meal, e.g., a meal of approximately 500 kcal with approximately 25% of calories from fat, may be administered. Subjects should start the meal 30 minutes before administration of the drug product and completely consume the meal within 30 minutes.

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 biopharmaceutical quality of the product. The data for the parent compound should be used to assess bioequivalence of nitrofurantoin

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for nitrofurantoin is around 13% when administered with food. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study.

Washout: Taking into account the elimination half-life of nitrofurantoin of 1.5 – 1.7 hours, a washout period of 7 days is considered sufficient to prevent carry over.

Blood sampling: The blood sampling should be intensive for the first seven hours after administration to properly characterize the C_{max} of nitrofurantoin. Blood samples for the characterization of nitrofurantoin pharmacokinetics do not need to be taken after 16 h post-dose in order to determine AUC_{0-t}. For example, blood samples might be taken at pre-dose, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 10.0, 12.0 and 16.0 hours after drug administration.

Analytical considerations: A lower limit of quantitation of 5 ng/mL has been described in the literature (Patel et al. Acta Pharm. 2013 Jun;63(2):141-58. doi: 10.2478/acph-2013-0012), but this LLOQ may be insufficient to detect 5% of the C_{max} when administering 5 mg of nitrofurantoin (i.e., around 1.25 ng/mL). Therefore, if the bioanalytical method is not able to reach a LLOQ of 1.0 ng/mL, the administered dose should be increased.

Statistical considerations: The data for nitrofurantoin 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 comparator product should be within 80 – 125%
- The 90% confidence interval of the relative mean C_{max} of the test to comparator product should be within 80 – 125%.