Notes on the Design of Bioequivalence Study: Para-aminosalicylic acid

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 para-aminosalicylic acid.

Pharmacokinetics of para-aminosalicylic acid

The absorption of PAS salts including NaPAS and KPAS is rapid and complete following oral administration, which usually produces higher PAS concentrations than a PAS acid formulation. PAS acid is poorly soluble in acidic environments, tends to be slowly released while still in the stomach, and is therefore readily acetylated during first-pass metabolism. Compared to the PAS acid, NaPAS, KPAS, and CaPAS formulations are more water soluble, and more easily absorbed and more easily saturate the N-acetyltransferase-1 (NAT1) acetylation capacity of the gut and liver.

Following an oral dose administration of p-Aminosalicylate sodium, equivalent to 4 g of aminosalicylic acid, peak plasma concentration is reached within 0.5 – 1 hour.

P-aminosalicylic acid (as sodium salt) administration with food results in 1.5 and 1.7-fold higher PAS C_{max} and $AUC_{0\text{-}inf}$, respectively, compared to its administration when fasting. In addition to the better absorption when given with food, intolerance to PAS might be less.

The elimination half-life of PAS varies from about 0.5 to 2.5 hours depending on the PAS formulation and administration with or without food or antacid.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of para-aminosalicylic acid the following guidance with regard to the study design should be taken into account:

Design: A single-dose, crossover design is recommended. However, a waiver of the bioequivalence study might be possible if it is shown that the product is an oral solution at the time of administration and the excipients are similar to those of the comparator product. See for example the PQT/MED WHOPAR for TB229.

Dose: As the EoI includes para-aminosalicylic Acid (PAS), 5.52 g powder (for oral solution) in sachet (equivalent to 4 g p-aminosalicylic acid), this strength and dose should be tested.

Fasted/fed: As PAS should be taken in fed state due to an increase in systemic exposure and better tolerability, fed state studies are recommended. The content of the sachet should be dissolved by stirring in 100 ml of water and administer immediately.

Subjects: Healthy adult subjects should be recruited. It is not necessary to include patients in the bioequivalence studies.

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 para-aminosalicylic acid.

Sample size: Very limited information is currently available on the intra-subject variability for para-aminosalicylic acid. Therefore, a pilot study is recommended to estimate the intra-subject variability of the primary pharmacokinetic parameters.

Washout: Taking into account the elimination half-life of para-aminosalicylic acid of 26 minutes to 1 hour, a washout period of 7 days is considered sufficient to prevent carry-over.

Blood sampling: The blood sampling should be intensive for the first hours after administration to properly characterize the C_{max} of para-aminosalicylic acid. For example, blood samples might be taken at pre-dose, 0.17, 0.33, 0.50, 0.67, 0,83, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.50, 4.00, 5.00, 6.00, and 8.00 hours after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure para-aminosalicylic acid 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 para-aminosalicylic acid 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 PQT/MED suggests that the comparator product might be a highly variable drug product for C_{max} and/or 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 crossover study to estimate intra-subject 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 of the acceptance limits based on the intra-subject variability of the comparator product, refer to Section 7.9.3 of Annex 8, TRS 1052 and PQT/MED guidance document "Application of reference-scaled criteria for AUC in bioequivalence studies conducted for submission to PQT/MED".