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. 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. 992, Annex 6.

Below, additional specific guidance is provided on the invited immediate release products containing para-aminosalicylic acid.

Pharmacokinetics of para-aminosalicylic acid

After two hours in simulated gastric fluid, 10% of unprotected aminosalicylic acid is decarboxylated to form meta-aminophenol, a known hepatotoxin. The acid-resistant coating of the para-aminosalicylic acid granules protects against degradation in the stomach. The small granules are designed to escape the usual restriction on gastric emptying of large particles. Under neutral conditions such as those found in the small intestine or in neutral foods, the acid-resistant coating is dissolved within one minute.

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.

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 PASER 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.

In a single 4 gram pharmacokinetic study with food in normal volunteers, the median time to peak was 6 hours with a range of 1.5 to 24 hours. The half-life of free para-aminosalicylic acid is 26.4 minutes in healthy volunteers.

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: As this is a gastro-resistant formulation, a single-dose crossover study in fasted state and another in the fed state are recommended. The fed study should be conducted with a high-fat, high-calorie meal. The products should be sprinkled in an apple sauce or yogurt to be eaten without chewing. In the fasted state study, the products should be suspended in an acidic fruit drink such as orange juice or tomato juice to protect the coating. Swirling the juice in the glass will help resuspend the granules if they sink.

Dose: As the EoI includes para-aminosalicylic Acid (PAS), 4 g, granules, sachet and PAS, 4 g (as sodium salt), granules, sachet, this strength and dose should be tested.

Fasted/fed: Two bioequivalence studies should be conducted. One in the fasting state and another in the fed state.

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 in gastro-resistant formulations (39% for C_{max}). Therefore, a pilot study is recommended to estimate the intra-subject variability of the primary pharmacokinetic parameters and to optimize the sampling times to characterise also those profiles with delayed release/absorption.

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

Blood sampling: The blood sampling should be intensive for the first three hours after administration to properly characterize the C_{max} of para-aminosalicylic acid. For example, blood samples might be taken at pre-dose, 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 14.00, 16.00, 20.00 and 24.00 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).

Statistical considerations: The data for para-aminosalicylic acid should meet the following bioequivalence standards in both single-dose crossover design studies:

- 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 AUC_{0-inf} 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 is a highly variable drug product for C_{max} and/or AUC_{0-t}. Widening of the acceptance range for AUC_{0-t} for para-aminosalicylic acid will be accepted

by PQT/MED. Therefore, the applicant may design a replicate crossover 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 of the acceptance range based on the intra-subject variability of the comparator product, refer to Section 7.9.3 of Annex 6, TRS 1003. If widening of the acceptance range is planned for the AUC_{0-t} parameter, the principles described for C_{max} in Section 7.9.3 will apply and a four period, full replicate design study should be conducted to demonstrate bioequivalence, in order to assess the variability associated with each product.