Notes on the design of bioequivalence study: Nicotine Chewing Gum

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 chewing gums containing nicotine.

Pharmacokinetics of nicotine in chewing gum

When the gum is chewed, nicotine is steadily released into the mouth and is rapidly absorbed through the buccal mucosa. Nicotine is readily absorbed from the buccal mucous membranes. Demonstrable blood levels are obtained within 5 – 7 minutes and reach a maximum about 30 minutes after the start of chewing. Blood levels are roughly proportional to the amount of nicotine chewed.

A proportion, by the swallowing of nicotine containing saliva, reaches the stomach and intestine where it is inactivated.

Nicotine is eliminated mainly via hepatic metabolism; small amounts of nicotine are eliminated in unchanged form via the kidneys. The plasma half-life is approximately three hours.

Guidance for the design of bioequivalence studies:

Taking into account the pharmacokinetic properties of nicotine in chewing gums, the following guidance with regard to the study design should be taken into account:

Design: A single-dose crossover design is recommended.

Dose: As the EoI includes 2 mg and 4 mg strengths, the bioequivalence study should be conducted with the 4 mg strength. The 2 mg strength may be waived if the conditions for an additional strength biowaiver are fulfilled.

Fasting/fed: A fasted state study is recommended.

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 nicotine chewing gums.

Sample size: Information currently available in the literature suggest that the intra-subject variability is 24.9% for C_{max} and 21.5% for AUC_{0-t} (DOI: 10.1007/s12325-018-0752-7). These data may facilitate the calculation of a sufficient sample size for the bioequivalence study.

Washout: Taking into account the elimination half-life of the nicotine in healthy volunteers is reported to be approximately 3 hours, a cross-over design with a wash out period of at least 2 days could be feasible.

Blood sampling: The blood sampling should be intensive immediately after administration. It is not necessary to take samples after 12 hours. For example, blood samples might be taken at pre-dose, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 min and 1.0, 1.25 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00 and 12.00 h after drug administration.

Analytical considerations: Information currently available indicates that it is possible to measure nicotine in human plasma using LC-MS/MS analytical methodology with a LLOQ of 0.2 ng/ml. 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 nicotine 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%.