Notes on the Design of Bioequivalence Study: Semaglutide

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-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 injectable products containing semaglutide.

Pharmacokinetics of semaglutide

Maximum concentration was reached 1 to 3 days post dose after subcutaneous administration. Semaglutide exposure increased in a dose proportional manner for doses of 0.5 mg, 1 mg and 2 mg. Similar exposure was achieved with subcutaneous administration of semaglutide in the abdomen, thigh, or upper arm. Absolute bioavailability of subcutaneous semaglutide was 89%.

Semaglutide has a half-life of around 1 week making it suitable for once weekly subcutaneous administration. With an elimination half-life of approximately 1 week, semaglutide will be present in the circulation for about 5 weeks after the last dose.

Semaglutide is a polypeptide with 31 aminoacids that is chemically modified with a large hydrophilic spacer and a C18 fatty di-acid with a terminal acidic group. Considering the small size of the semaglutide structure, it is assumed that the test and comparator semaglutide can be fully characterised and compared with currently available state-of-the-art analytical methods.

Biowaiver: A waiver of the requirement to conduct an in vivo bioequivalence study is considered a possible alternative to a bioequivalence study, provided semaglutide substance sameness is demonstrated, the formulation is qualitatively the same and quantitatively similar to that of the comparator product and in vitro comparability is demonstrated using a range of orthogonal techniques. Please refer to the PQT/MED guidance document Additional Guidance on Submission Requirements for Semaglutide (glucagon-like peptide-1 (GLP-1) receptor agonist) for subcutaneous injection for more information on the requirements for semaglutide subcutaneous injection products, which can be found on the PQT/MED information page on Semaglutide Products.

Guidance for the design of bioequivalence studies

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

Design: A single-dose cross-over is recommended if a biowaiver is not feasible.

Dose: As the EoI includes semaglutide solution for subcutaneous injection, 0.68 mg per mL; 1.34 mg per mL; and 2.68 mg per mL in vial or 3 mL cartridge, the bioequivalence study can be conducted at a dose of 0.5 mg with any

strength / concentration since AUC and C_{max} of semaglutide is not affected by the change of the product concentration in the range of 1 mg/mL to 3 mg/ml.

Fasted/fed: N/A.

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.

Sample size: Intra-subject variability of semaglutide C_{max} calculated from literature (Wang F, Luan Z, Maltesen R, Reenberg AT, Westergaard L, Liu D. Pharmacokinetic Characteristics of a Once-Weekly Combination Therapy of Insulin Icodec and Semaglutide Versus Its Separate Components in Chinese Individuals with Type 2 Diabetes. Diabetes Ther. 2025;16(11):2213-2225. doi: 10.1007/s13300-025-01803-x) is around 15%. These data may facilitate the calculation of sufficient sample size for a single-dose crossover bioequivalence study.

Washout: Taking into account the apparent elimination half-life of subcutaneous semaglutide (about 1 week), a washout period of six weeks is considered sufficient to prevent carry over.

Blood sampling: Blood sampling needs to be undertaken frequently during the first 3 days to characterise C_{max} adequately and up to 35 days after the day of injection. A possible sampling scheme could be: pre-injection, 3, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72, 84, 96, 120, 144, 168, 240, 336, 504, 672 and 840 hours after injection.

Analytical considerations: Information currently available indicates that it is possible to measure semaglutide 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 semaglutide should meet the following bioequivalence standards in a single-dose cross-over design study:

- The 90% confidence interval of the relative mean AUC_{0-t} of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean C_{max} of the test to comparator product should be within 80.00 – 125.00%.