Notes on the Design of Bioequivalence Study: Cabotegravir + Rilpivirine

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Team – Medicines (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 co-package of intramuscular suspensions for injection containing cabotegravir and rilpivirine.

Pharmacokinetics of cabotegravir

Cabotegravir is rapidly absorbed following oral administration, with median Tmax at 3 hours post dose for the tablet formulation. Cabotegravir may be administered with or without food. Food increases the extent of absorption of cabotegravir. Bioavailability of cabotegravir is independent of meal content: high fat meals increased cabotegravir $AUC_{0\text{-}inf}$ by 14% and increased C_{max} by 14% relative to fasted conditions. Cabotegravir has a mean terminal half-life of 41 h.

Cabotegravir IM injection exhibits absorption-limited (flip-flop) kinetics resulting from slow absorption from the gluteal muscle into the systemic circulation resulting in sustained plasma concentrations. Following a single intramuscular dose, plasma cabotegravir concentrations are detectable on the first day and gradually rise to reach maximum plasma concentration with a median Tmax of 7 days. Cabotegravir has been detected in plasma up to 52 weeks or longer after administration of a single injection. Plasma cabotegravir exposure increases in proportion or slightly less than in proportion to dose following single doses ranging from 100 to 800 mg. Cabotegravir mean apparent terminal phase half-life is absorption-rate limited and is estimated to be 5.6 to 11.5 weeks after a single dose IM injection. The significantly longer apparent half-life compared to oral reflects elimination from the injection site into the systemic circulation.

Pharmacokinetics of rilpivirine

Rilpivirine extended-release injection exhibits absorption rate-limited kinetics (i.e., flip-flop pharmacokinetics) resulting from slow absorption from the gluteal muscle into the systemic circulation resulting in sustained rilpivirine plasma concentrations. Rilpivirine absorption was described through two parallel absorption pathways: a fast absorption route describing the initial rilpivirine peak; and a second slow absorption route determining the terminal part of the rilpivirine concentration–time curve, with the terminal elimination half-life being determined by the slowest absorption process.

Following a single intramuscular dose, rilpivirine plasma concentrations are detectable the first day and gradually rise to reach maximum plasma concentrations after a median of 3-4 days. It was also reported that after a single dose, rilpivirine concentrations peaked at days 6–8. Plasma rilpivirine exposure increases in proportion or slightly less than in proportion to dose following single and repeat IM injections of doses ranging from 300 to 1200 mg.

Rilpivirine has been detected in plasma up to 84 weeks or longer after administration of a single dose. The mean apparent half-life of rilpivirine following administration is absorption rate-limited and was estimated to be 13-28 weeks. A pop-PK analysis calculated that the estimated terminal elimination half-life of rilpivirine after IM administration was 200 days. Full steady state is expected to be achieved after 2.2 years of treatment, but most of the accumulation (80%) occurs within the first 48 weeks., with limited further accumulation thereafter (90% and 95%of steady state after 1.5 and 2 years, respectively).

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of cabotegravir and rilpivirine suspensions for injection, the following guidance with regard to the study design should be taken into account.

It is acceptable to conduct separate bioequivalence studies for each component of the co-package or to conduct a single BE study with both components of the co-package.

Study design:

For cabotegravir, a single dose with crossover or parallel design may be employed.

For rilpivirine, a single dose parallel design is recommended.

Dose: As the EoI only includes the strength 600 mg / 3 ml (200 mg/ml) intramuscular extended-release suspension for injection for cabotegravir and 900mg/3ml (300 mg/ml) intramuscular extended-release suspension for injection for rilpivirine, the bioequivalence study(ies) should be conducted with this strength.

Fasted/fed: N/A.

Subjects: Healthy adult subjects should be recruited. It is not necessary to include patients in the bioequivalence study(ies).

Parent or metabolite data for assessment of bioequivalence: The parent drugs are considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of the parent compound for cabotegravir and rilpivirine (E-isomer).

Sample size:

After intramuscular administration of cabotegravir the expected inter-subject CV is around 55-60%. These data may facilitate the calculation of a sufficient sample size for a cross-over bioequivalence study.

After intramuscular administration, rilpivirine pharmacokinetics shows a moderate to high inter- or between-subject variability (CV) in AUC, C_{max} and C_{trough} ranging from 37 - 39%, 36 - 40% and 32 - 77%, depending on the assessed study. These data may facilitate the calculation of a sufficient sample size for a bioequivalence study.

Washout: In the case that a crossover design study is conducted for cabotegravir intramuscular suspension for injection, a wash-out period of 60 weeks would be necessary, Therefore, a parallel design is an acceptable alternative. N/A for rilpivirine.

Blood sampling:

For cabotegravir, blood samples should be taken frequently around the 7th day and for at least 42 weeks. For example, samples can be taken pre-dose and at 4, 8, 16, 24, 48, 96, 120, 144, 168, 192 hours, 2, 3, 4, 6, 8, 12, 20, 28, 36, and 42 weeks after administration.

For rilpivirine, blood samples should be taken frequently around the 7th day and for at least 44 weeks. For example, samples can be taken pre-dose and at days 0.33, 0.66, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14 days and 2, 3, 4, 6, 8, 12, 20, 28, 36, 44 and 52 weeks after administration post-dose.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure cabotegravir and rilpivirine in human plasma using LC-MS/MS analytical methodology (LLOQ: 15 and 3 ng/mL, respectively, Bevers et al., 2024. Development, validation and clinical implementation of a UPLC-MS/MS bioanalytical method for simultaneous quantification of cabotegravir and rilpivirine E-isomer in human plasma. J Pharm Biomed Anal. 2024 20;238:115832. doi: 10.1016/j.jpba.2023.115832). 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). The bioanalytical method for each analyte should be validated in the presence of the other analyte if the two components are studies in a single BE study (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).

Statistical considerations: The data for cabotegravir and rilpivirine should meet the following bioequivalence standards in a single-dose parallel design study:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}t}$ of the test to comparator product should be within 80.00 – 125.00%
- The 90% confidence interval of the relative mean $AUC_{0\text{-}inf}$ 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%.
- The 90% confidence intervals of partial AUCs (e.g., $AUC_{0\text{-}8\ weeks}$ and $AUC_{8\ weeks\text{-}t}$ for cabotegravir, and $AUC_{0\text{-}6\ weeks}$ and $AUC_{6\ weeks\text{-}t}$ for rilpivirine) should also be submitted as supportive information.