

Notes on the Design of Bioequivalence Study: Norethisterone enanthate

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. 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. 1003, Annex 6.

Below, additional specific guidance is provided on the invited prolonged release injectable products containing norethisterone enanthate.

Pharmacokinetics of norethisterone enanthate

Norethisterone enanthate is completely absorbed after intramuscular injection. The ester is quickly and eventually completely hydrolyzed to its pharmacologically active compound norethisterone once it is released from the depot.

Maximum levels of norethisterone were measured about 3 – 20 days after intramuscular administration. They amounted on average to 13.4 ± 5.4 ng/ml and 12.2 ± 2.7 ng/ml about 7 days (median) after intramuscular administration of 200 mg norethisterone enanthate in 2 ml and 1 ml oily solution, respectively.

Plasma levels of norethisterone declined in two disposition phases with half-lives of 4-5 days and 15-20 days, respectively, which were due to a biphasic release of norethisterone enanthate from the depot.

Due to the half-life of the terminal disposition phase from plasma (about 2.5 weeks) and the initial dose regimen (one injection every 2 months), a slight accumulation of norethisterone is expected after multiple administrations. A steady state will already be reached after the second administration. Norethisterone enanthate terminal elimination half-life has been reported to be 8.1 days on average.

Guidance for the design of bioequivalence studies

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

Design: A single-dose parallel design is recommended.

Dose: As the EoI includes the 200 mg depot injection for intramuscular administration, the bioequivalence study should be conducted with this strength and route of administration.

Fasted/fed: N/A.

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

Parent or metabolite data for assessment of bioequivalence: Norethisterone enanthate was quickly and eventually completely hydrolysed to its pharmacologically active compound norethisterone once it was released from the depot. However, the plasma levels of the pro-drug are measurable. The pro-drug is considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence for norethisterone enanthate should be based on the determination of the pro-drug norethisterone enanthate and norethisterone results should be submitted as supportive information.

Sample size: Inter-subject variability of norethisterone enanthate C_{max} and AUC is not described in the scientific literature. Therefore, a pilot study is recommended to calculate the sample size for a parallel bioequivalence study.

Washout: N/A.

Blood sampling: Blood sampling needs to be undertaken frequently during the first days to characterise C_{max} adequately and up to 72 days after the day of injection. A possible sampling scheme could be: pre-injection, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 7.50, 8.00, 10.0, 12.0, 14.0, 16.0, 18.0, 21.0, 24.0, 28.0, 36.0, 48.0 and 72.0 days.

Analytical considerations: Information currently available indicates that it is possible to measure norethisterone enanthate and norethisterone 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 norethisterone enanthate should meet the following bioequivalence standards in a single-dose parallel 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 AUC_{0-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 interval of the relative mean $AUC_{0-12 \text{ days}}$ and $AUC_{12 \text{ days}-t}$ of the test to comparator product should be submitted as supportive information.

The 90% confidence interval of the relative mean $AUC_{0-18 \text{ days}}$, $AUC_{18 \text{ days}-t}$, AUC_{0-t} , AUC_{0-inf} and C_{max} of norethisterone of the test to comparator product should be submitted as supportive information.