Notes on the Design of Bioequivalence Study: Norethisterone Enanthate / Estradiol Valerate

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-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 prolonged release injectable products containing norethisterone enanthate and estradiol valerate.

Pharmacokinetics of norethisterone enanthate and estradiol valerate

Norethisterone enanthate and estradiol valerate are completely absorbed after intramuscular injection. These esters are quickly and eventually completely hydrolyzed to their pharmacologically active compounds norethisterone and estradiol once they are released from the depot. Maximum levels of norethisterone and estradiol were measured about 4 – 5 days and 2 days after intramuscular administration, respectively.

Approximately 85% of the dose of both components are excreted within the injection interval of 28 days. 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. Estradiol elimination half-life was 4 – 5 days.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of norethisterone enanthate and estradiol valerate 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 norethisterone enanthate / estradiol valerate 50 mg / 5 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 parent 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. Estradiol valerate is hydrolysed to release gradually estradiol. Therefore, bioequivalence for estradiol valerate should be based on the determination of the estradiol.

Sample size: Inter-subject variability of norethisterone enanthate and estradiol C_{max} and AUC after their intramuscular injection 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 Cmax 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, norethisterone, and estradiol 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). The bioanalytical method for each analyte should be validated in the presence of the other analyte (see ICH Harmonised Guideline M10 for more information).

Statistical considerations: The data for norethisterone enanthate and estradiol 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 interval of the relative mean $AUC_{0\text{-}12\ days}$ and $AUC_{12\ days\text{-}t}$ for norethisterone enanthate and $AUC_{0\text{-}6\ days}$ and $AUC_{6\ days\text{-}t}$ of estradiol of the test to comparator product should be submitted as supportive information.
- The 90% confidence interval of the relative mean $AUC_{0\text{-}18\ days}$, $AUC_{18\ days\text{-}t}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and C_{max} of norethisterone of the test to comparator product should be submitted as supportive information.